Population structure and local adaptation in elasmobranchs: insights from the small-spotted catshark (*Scyliorhinus canicula*)

Submitted by **Gregory Wannell** to the University of Exeter
as a thesis for the degree of
Masters by Research in Biological Sciences
In November 2020

This thesis is available for Library use on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……..

Abstract

One quarter of elasmobranchs, which includes sharks and rays, are now threatened with extinction. Their unique life history traits make them particularly susceptible to anthropogenic pressures such as overfishing, habitat loss and global warming. To understand how these pressures are affecting natural shark populations we must understand their genetic diversity and how they become adapted to their local environment. Key to this is identifying discrete populations (or units for management), understanding the neutral and adaptive processes shaping population structure and identifying key genes responsible for local adaptation. In chapter one of this thesis, the importance and use of genetics and genomics in elasmobranch conservation and managements is reviewed. In chapter two, mitochondrial DNA control region sequencing of *Scyliorhinus canicula* edge populations was performed to resolve range wide population structuring. Samples from across the northeast Atlantic (NEA) were genetically homogenous. More complex structuring was seen in the Mediterranean with evidence of an isolated, genetically distinct cluster in the eastern Mediterranean, highlighting the importance of neutral processes in promoting genetic differentiation in this species. In chapter 3, double-digest restriction associated DNA (ddRAD) and seascape genomics was utilised to identify putative genomic regions under selection and to investigate the genomic basis of regional adaptation. Using a panel of 9,052 single nucleotide polymorphisms (SNPs) fine-scale structuring was revealed in both the NEA and the Mediterranean, with temperature, salinity, oxygen and depth all appearing to drive local adaptation in this small-coastal shark species. This thesis highlights the importance of studying genetic diversity and its drivers for successful future conservative of elasmobranchs.

List of Contents

List of Figures

Chapter 1

Chapter 2

Chapter 2 – Supplementary Information

Chapter 3

Chapter 3 – Supplementary Information

List of Tables

Chapter 2

Chapter 3

Chapter 3 - Supplementary Information

Acknowledgements

Firstly, I owe massive thanks to my supervisor Andrew Griffiths who has been incredibly supportive throughout and a great supervisor and friend. Thank you for your guidance, insight and for always having time to chat no matter the time of day. Your positivity and passion for learning is contagious and I will forever be appreciative of your support, particularly during the darker days of the pandemic! You are truly a great academic and I look forward to continuing to work with you.

Secondly, thanks to my second supervisor Bonnie Fraser, for your insight into (what was) the unfamiliar world of genomics and the continued support; I'm actually starting to understand some of the lab meetings! Without your knowledge and generosity many aspects of this project would have not been possible, and for that, I'll always be thankful. If I ever need to scrounge any more lab consumables or sequencing power, I know who to come to.

Further, I owe a massive thanks to the Molecular Ecology and Evolution (MEEG) Group who have become great friends. Special thanks to Charlie Ellis, Kirsty MacLeod, Tom Jenkins, Daniel Osmond, Jessica Rudd and Joanne Morten for being great office and lab buddies and making the whole experience ever more enjoyable. Thanks for the many, many coffee breaks, constant code, distractions and memories.

Thanks to all the academics and staff at the University of Exeter who have helped a various stages through the project. It's great to be part of such a leading research community full of friendly and wonderful people. Further afield, a special thanks to

Chrysa Gubili who has been an excellent unofficially ‘third’ supervisor overseas whom one day I hope to have the pleasure of meeting and thanking in person.

Finally, I would like to thank all the people close to me. I am very grateful to have such a loving family and great friends who are so supportive. A very special thanks to Jess for always being by my side, for the constant reminders of the important things in life and for your continued love. To my family for their support and love over the many years.

Chapter 1: General Introduction

1.1 Elasmobranchs: status and threats

The elasmobranchs (sharks and rays) make up the majority of biodiversity within Chondrichthyes (cartilaginous fishes). They are one of the oldest and most successful lineages of vertebrates, emerging 455 million years ago and have adapted to live in wide-ranging coastal, demersal, pelagic and freshwater habitats (Compagno, 2005; Weigmann, 2016). Now they make up one of the most specious groups of aquatic predators with large evolutionary distances between the 1,200 species of shark, skates and rays found worldwide (Compagno, 2005; Stein *et al.,* 2018; Weigmann, 2016). Such diversity of form and function suggest unique genetic properties that support their adaptation and evolutionary success, allowing them to survive mass extinction events throughout time (Grogan *et al.,* 2012). However, their ability to survive the rapid changes of the Anthropocene has been questioned, and the elasmobranchs have attracted increasing scientific concern. This has been due to large declines in abundance of many species (Dulvy *et al.,* 2014; Ferretti *et al.,* 2010; Davidson *et al.,* 2016), their vulnerability to overexploitation (Compagno, 1990; García *et al.,* 2008; Stevens *et al.,* 2000), global concern over their extinction risk (Dulvy *et al.,* 2014, 2017) and their ecological importance to many ecosystems (Myers *et al.,* 2007; Heithaus, Wirsing & Dill, 2012; Heupel *et al.,* 2014).

Many elasmobranchs are particularly vulnerable to anthropogenic pressures due to their slow growth, late sexual maturity, long life spans, long gestation and low fecundity (Compagno 1990; García *et al.,* 2008; Conrath and Musick, 2012), meaning populations can take a long time to recover from elevated levels of mortality

(Gallucci *et al.,* 2006; Domingues *et al.,* 2018). Indeed, many species now face unprecedented population declines primarily due to elevated fishing pressure and bycatch, but also from habitat destruction exaggerated by pollution and climate change (Clarke *et al.,* 2006; Dulvy *et al.,* 2014; Ward-Paige *et al.,* 2012; Wheeler *et al.,* 2020). The effects of overexploitation are becoming well documented (Ellis *et al.,* 2005; Ferretti *et al.,* 2008; MacNeil *et al.,* 2020; Roff *et al.,* 2018; Sguotti *et al.,* 2016) with evidence of local extirpation events (Dulvy & Reynolds, 2002; Lawson *et al.,* 2020; Sguotti *et al.,* 2016). For example, the common skate (*Dipturus batis*) and three angel shark species (*Squatina sp.*) have declined in abundance and have seemingly disappeared from much of their European range (Dulvy & Reynolds, 2002; Lawson *et al.,* 2020). Globally, one quarter of all elasmobranchs are threatened with extinction (Dulvy *et al.,* 2014), making them one of the most threatened groups of vertebrates on the planet (Ward-Paige *et al.,* 2012).

Climate change has begun to alter the marine environment by causing large-scale changes in ocean temperatures and acidity (Pörtner *et al*., 2014), and in turn, shifts in other environmental factors such as salinity and dissolved oxygen (Cheng *et al*., 2019; Skliris *et al*., 2018). The biology and distribution of sharks are already known to be influenced heavily by their environments (Bangley *et al*., 2018; Carlisle *et al*., 2009; Froeschke *et al*., 2010; Hyatt *et al*., 2018; Speed *et al*., 2010; Ward-Paige *et al*., 2015), yet, it is still poorly understood how populations are likely to respond to rapid change. It is only recently that laboratory studies have begun to highlight the detrimental effects ocean warming and acidification can have on elasmobranch reproduction and development (Di Santo, 2015; Gervais *et al*., 2016, 2018; Johnson *et al*., 2016; Musa *et al*., 2020; Pistevos *et al*., 2015, 2017; Rosa *et al*., 2014, 2016;

Wheeler *et al*., 2020). For example, elevated temperature has been shown to significantly shorten developmental time and growth in both the small-spotted catshark *Scyliorhinus canicula* (Musa *et al*., 2020) and the brownbanded bamboo shark *Chiloscyllium punctatum* (Rosa *et al*., 2014), with both authors also noting an increase in mortality. However, this work is extremely difficult to conduct on large growing species and such investigations have only just begun to focus on smaller, oviparous species that are more amenable to laboratory manipulations.

Elasmobranchs are ectotherms, and as such it is expected that many species will likely need to shift their geographical distribution to remain within their preferred temperature regimes, as already observed in other marine fish species (Burrows *et al*., 2011; Perry *et al*., 2005). This may be problematic for species that are highly philopatric to specific nurseries (Fieldham *et al*., 2014) or aggregation sites (Bessudo *et al*., 2011; Chapman *et al*., 2015). These may rely on the phenotypic plasticity and/or adaptation to counteract the effects of climate change. Even so, considering the long generation times typical of elasmobranchs (García *et al*., 2008; Conrath & Musick, 2012), adaption may not be able to keep pace with rapid changes of the Anthropocene. Furthermore, as abundance declines so does the genetic diversity of those populations through the effects of inbreeding and genetic drift, significantly reducing their evolutionary potential and chances of long-term survival (Frankham *et al*., 2002). The adaptive response to rapid human induced environmental change in many animals remains uncertain - this is certainly true for elasmobranchs and further study is needed to gain insight into the key environmental selection pressure/drivers and evolutionary processes in sharks.

The decline of elasmobranch populations is of grave concern. They have highly important ecological roles in coastal and oceanic ecosystems as meso or apex predators and their collapse would have ecosystem wide ramifications (Ferretti *et al*., 2010; Heupel *et al*., 2014; Roff *et al*., 2016; Stevens *et al*., 2000). Additionally, elasmobranchs have direct value in fisheries (Dulvy *et al*., 2017) and ecotourism (Huveneers *et al*., 2017) and their decline could cause substantial economic loss. Yet, an estimated 46.8 % of all species lack enough data to make an accurate assessments of their population status (Dulvy *et al*., 2014). Many landings are still unregulated, unrecorded, mislabelled and discarded at sea (Clarke *et al*., 2006; Bornatowski *et al*., 2013; Davidson *et al*., 2016; Dulvy *et al*., 2017), and fishery statistics are thought to be three to four times underestimated (Clarke *et al.*, 2006; Worm *et al*., 2013). Without the critical data needed to make population assessments and impose regulation, the successful management and conservation of elasmobranch populations will remain an overwhelming challenge. It is also clear that maintaining genetic diversity and understanding evolutionary processes should be at the forefront of conservation policy and management to allow adaptation of populations to future change (Bernatchez *et al*., 2016; Crandall *et al*., 2000; Domingues *et al*., 2018), yet such studies are lacking in elasmobranchs (Figure 1.1). Developing more affordable, accurate genetic methods to identify discrete populations, determine key genes responsible for local adaptations, and identify priority areas and stocks for conservation, will better inform future management and help toward the long-term survival of these species (Dudgeon *et al*., 2012; Domingues *et al*., 2018; Johri *et al*., 2019).

1.2 Genetic and genomic tools in elasmobranch conservation

Over the last decades, molecular genetic markers have become a powerful tool for population studies and conservation efforts (Ovenden *et al*., 2018). Such approaches have revealed cryptic species (Borsa *et al*., 2016), informed the definition of fishery management units (Braccini *et al*., 2016; von der Heyden *et al*., 2014) and identified key drivers of genetic diversity in marine taxa (Bernatchez *et al*., 2019; Diopere *et al*., 2018; Griffiths *et al*., 2011; Torrado *et al*., 2020). However, genetic and genomic research on elasmobranchs has generally lagged behind and it is estimated only ~10 % of shark and ray species have been investigated in terms of their population structure, genetic diversity and demographic history (Domingues *et al*., 2018; Figure 1.1). The majority of the studies also represent the first and only genetic assessment of a particular species (Dudgeon *et al*., 2012). It is only in the last decade that a substantial increase has been seen (Figure 1.1), triggered by rising concern over population status and the increased affordability of molecular techniques (see reviews in Domingues *et al*., 2018; Dudgeon *et al*., 2012; Johri *et al*., 2019; Ovenden *et al*., 2018). Currently, the majority of genetic studies in elasmobranchs are based on mitochondrial and/or nuclear microsatellite markers. However, advances in high-throughput sequencing now allow for more extensive molecular analysis at ever decreasing costs.

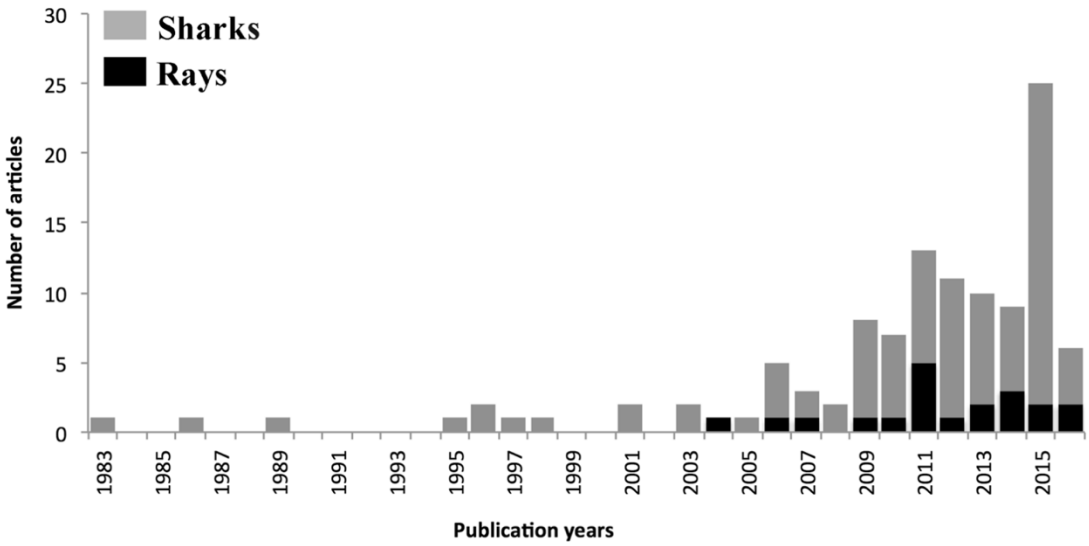


Figure 1.1. Number of articles published between 1983 and 2016 that describe genetic diversity of shark and ray species. Figure taken from Domingues et al., (2018).

1.2.1 Mitochondrial DNA

Mitochondrial DNA (mtDNA) has been widely utilised in elasmobranch population and evolutionary biology, broadening the understanding of population size (Ovenden *et al*., 2016), reproductive strategies (Portnoy and Heist, 2012), taxonomy (Griffiths *et al*., 2010; Last *et al*., 2016; Cariani *et al*., 2017), trade (Griffiths *et al.,* 2013; Hobbs *et al*., 2019; Steinke *et al*., 2017; Wannell *et al*., 2020) and species presence-absence from environmental DNA (eDNA) monitoring (Postaire *et al.*, 2020). In fact, eDNA is being increasingly adopted and proven to be a powerful method to assess the geographic distribution and habitat use of many shark species. Importantly, it has been successful in detecting endangered species such as the scalloped hammerhead (*Sphyrna lewini*, Budd *et al.*, 2021) and the largetooth sawfish (*Pristis pristis,* Simpfendorfer *et al.*, 2016), acting as a critical survey tool for conservation

efforts. But perhaps the most consistent use of mtDNA in elasmobranch studies is in the investigation of genetic diversity and population structure (Ferreri *et al*., 2018; Gubili *et al.*, 2014; Kousteni *et al*., 2015; Leone *et al.,* 2017*;* Veríssimo *et al*., 2010). Its wide application results from several favourable characteristics including, a relatively large quantity in the cell, small genome size, haploid, maternal inheritance and a higher mutation rate than nuclear DNA (Castro et al., 1999; Rubinoff & Holland, 2005). The latter is particularly useful for studying elasmobranchs which have some of the slowest evolving genomes of all vertebrates (Venkatesh *et al*., 2014).

The use of mtDNA has allowed for important insights into neutral drivers of population structure in elasmobranchs, such as historical (Catarino *et al*., 2015; Griffiths *et al*., 2011) and contemporary barriers to gene-flow (Gubili *et al*., 2016; Kousteni *et al*., 2015), alongside species-specific life-history traits such as site fidelity (Hull *et al*., 2019) and vagility (Castro *et al*., 2007; Bailleul *et al*., 2018). Indeed, the strongest signals of population structure have often been seen in smaller, demersal species of shark that have limited dispersal capabilities (Gubili *et al*., 2014; Chapman *et al*., 2015; Corrigan *et al*., 2016). Whereas structuring in larger pelagic species tends to be trans-oceanic and reflects their highly mobile lifestyles (Benavides *et al*., 2011; Bernard *et al*., 2016). This has important consequences for how these species should be managed. Nevertheless, there are some limitations to this marker. For example, significant effort is needed for the refinement of primers in understudied species, although some universal primers have been developed (Ivanova *et al*., 2007), substitution rates can vary between different loci given conflicting results, and PCR artefacts have been shown to cause issues when amplifying highly-conserved

regions of mtDNA (Gupta *et al*., 2015; Rubinoff & Holland, 2005). Furthermore, mtDNA markers also offer limited scope of non-neutral processes potentially driving adaptive divergence in natural populations (Domingues *et al*., 2018; Johri *et al*., 2019).

1.2.2 Single nucleotide polymorphism (SNPs)

As high-throughput sequencing technologies become ever cheaper, many studies now centre around quantify patterns of variation in genome-wide single nucleotide polymorphisms (SNPs) to investigate genetic diversity. This can offer many advantages in comparison to studies employing mitochondrial and microsatellite markers. Firstly, genetic differentiation studies employing thousands of SNP markers can be done with a relatively small number of samples from a given location compared to neutral markers (Jefferies *et al*., 2016; Willing *et al*., 2012). For example, Jefferies *et al.* (2016) found a large number of SNPs could obtain finer population structure than microsatellites using only 17.6 % of samples. This could prove highly beneficial in elasmobranch research given the large amount of threatened species and the difficulty in obtaining tissues samples from highly mobile, solitary shark species (Heist, 2009). Additionally, large SNP panels often provide greater resolution and have enabled researchers to resolve fine-scale structuring in marine taxa (Jenkins *et al*., 2018; Milano *et al*., 2014; Carreras *et al*., 2017). Importantly, SNPs are also found across coding and non-coding regions, which enable both neutral and adaptive genetic variation to be investigated in one marker class. Their ubiquity across the genome means they should also be present in genes under selection allowing the identification of locally adapted populations and the study of the evolutionary consequences of natural and anthropogenic pressures

such as climate change and overexploitation (Dudgeon *et al*., 2012). Finally, by integrating SNPs with the emerging fields of landscape and seascape genomic (Liggins *et al*., 2019; Selkoe *et al*., 2016), causal relationships among genetic variation and environment clines can be identified. This approach has already been successfully applied in a diverse array of marine taxa and have allowed key questions in evolutionary and ecological biology to be addressed (Bernatchez *et al*., 2019; Diopere *et al*., 2018; Torrado *et al*., 2020), but not in elasmobranchs.

The use of large SNP panels in elasmobranch research is lacking, and to date only a small number of studies have used SNPs to determine biogeography and population structure (Maisano Delser *et al*., 2016; Manuzzi *et al*., 2019; Marra *et al*., 2019; Momigliano *et al*., 2017; Pazmiño *et al*., 2017, 2018; Portney *et al*., 2015; Veríssimo *et al*., 2018). Nevertheless, this work provided additional evidence of the greater resolution attained with large SNP panels and highlights the importance of further genome-wide assessments for the future management of elasmobranchs. For example, Portnoy *et al*. (2015) identified two distinct populations of bonnethead sharks in the Atlantic and Gulf of Mexico that were previously unseen, and Pazmino *et al*. (2017) identified extensive structuring in the Galapagos shark that was absent using mitochondrial DNA. However, both studies were also restricted in their conclusions due to a lack of genomic resources available for elasmobranchs. Currently, only four shark species and one Chimaera have fully sequenced genomes. These include the elephant shark (*Callorhinchus milii*, Venkatesh *et al*., 2014), the whale shark (*Rhincodon typus*, Read *et al*., 2017), great white shark (*Carcharhodon carcharias*, Marra *et al*., 2019), brownbanded bamboo shark (*Chiloscyllium punctatum*, Hara *et al*., 2018) and cloudy catshark (*Scyliorhinus*

torazame, Hara *et al*., 2018). They have provided considerable insight into elasmobranch evolution and gene function (Marra *et al*., 2019), highlighting the exciting potential of future genomic studies to increase our understanding of elasmobranch biology, behaviour and conservation.

1.2.3 Restriction site associated DNA sequencing (RAD-seq)

The development of reduced representation genome sequencing techniques, such as restriction site associated DNA sequencing (RAD-seq, Davey *et al*., 2010), allow for the identification of thousands of SNP markers in non-model organisms (Davey *et al*., 2010; Peterson *et al*., 2012; Willing *et al*., 2011). RAD-seq uses restriction enzymes to fragment and sample a fraction of the genome (Davey *et al*., 2010). Given that Elasmobranchs have some of the largest genomes amongst all vertebrates (3.8-6.7 Gigabases) this can significantly reduce sequencing costs. Fragments are then sequenced with high-throughput sequencing and resulting reads can be aligned to a reference genome, or assembled *de novo* (without a genome) into stacks of identical reads and treated as candidate alleles. SNPs can then be called between alleles at the same locus across individuals and populations for comparative studies (Davey *et al*., 2010). Since its publication, various adaptations of the traditional RAD protocol have been developed, including ddRAD (Peterson *et al*., 2012), 2bRAD (Wang *et al*., 2012) and ezRAD (Toonen *et al*., 2013).

Double-digest RAD sequencing (ddRADseq), uses a double restriction enzyme digest that allows for improved tunability and accuracy over the size of fragments generated (Peterson *et al*., 2012). Genomic DNA is first digested with a common cutting enzyme (sequence found frequently across the genome) followed later by the

introduction of a second, rarer cutting enzyme (site occurs only rarely in the genome). With careful selection over the enzymes used specific size fragments can be selected for resulting in the need for less sequencing power (Peterson *et al*., 2012, Figure 1.2). This allows a smaller number of SNPs to be targeted in a greater number of individuals, alongside significantly reducing the costs of library preparation (Peterson *et al*., 2012). Overall, this makes genome-wide studies more accessible in labs that may have limited financial resources and more feasible for undergraduate and postgraduate research projects.

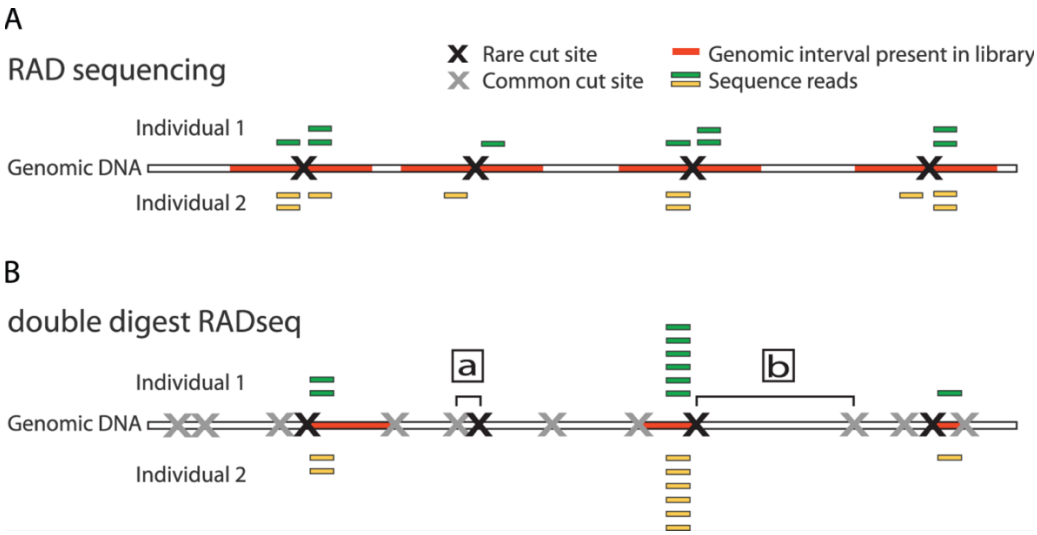


Figure 1.2. (A) Traditional Restriction-Site Associated DNA sequencing (RADseq) using a single restriction enzyme (RE). (B) Double digest RAD sequencing (ddRADseq) using a two enzyme double digest followed by precise size selection that excludes regions fla by either [a] very close or [b] very distant RE recognition sites, recovering a library consisting of only fragments close to the target size (red segments). Figure taken from Peterson *et al*., (2012).

RAD-seq approaches do also come with their limitations which are important to note. Despite generating hundreds to thousands of polymorphic markers, only a small proportion of the genome is still sequenced, meaning key areas of variation could be

missed. This is especially true when working with larges genomes, such as that of the elasmobranch (3.8-6.7 Gb), and/or low levels of linkage disequilibrium (Lowry *et al.*, 2017). The methodology of RAD-seq also raises questions over the true representation of genotypes as a consequence of genotyping errors and coverage (Fountain *et al.*, 2016), alongside the introduction of bias. For example, Arnold *et al.* (2013) reported that the choice of restriction enzyme may introduce bias in allele frequency estimations and careful consideration should be given when using this technique to make population genetic inferences. The sparseness of markers generated by RAD-seq may can also pose particular challenges when performing genome scans in studies of local adaptation, limiting the detection of signatures of selection, particularly less intense selective events (Lowry *et al.*, 2017; Benjelloun *et al.*, 2019). Often, only a small proportion of the strongest selective sweeps will be detected (Tiffin and Ross-Ibarra, 2014). On a similar note, when identifying loci involved in local adaptation, the confounding effects of population structure and demographic history must be corrected for in analytical methods used (Tiffin and Ross-Ibarra, 2014; Rellstab *et al.*, 2015; Hoban *et al.*, 2016). If this is not done correctly, neutral genetic structure can mimic patterns of non-neutral/adaptive processes resulting in the discovery of false positives (Excoffier, Hofer and Foll, 2009). With sequencing constantly improving and becoming cheaper, many studies are now adopting whole-genome sequencing (WGS) to search for signatures of selection (Hoban *et al.*, 2016; Fuentes-Pardo and Ruzzante, 2017). Although this is by far the best approach for genome scans, it is still not feasible for small budget studies on species with large genomes.

1.3 Study species: The small-spotted catshark (*Scyliorhinus canicula*)

The small-spotted catshark, *Scyliorhinus canicula* (Linnaeua, 1758) is a small but important coastal shark that has a demersal lifestyle occurring over sandy, gravel or muddy bottoms (Compagno, 1984). It is an oviparous species producing egg cases throughout the year (Kousteni *et al*., 2010) and considered an opportunistic predator preying on various benthic organisms (Kousteni *et al*., 2017). It has a wide distribution on the continental and uppermost slope throughout the Mediterranean Sea and in the northeast Atlantic (NEA), extending from Norway to Senegal (Compagno, 1984; Compagno, Dando and Fowler, 2005). It is a eurybathic species, being distributed from the first few metres to ~400 m in the NEA (Compagno, 1984), 500 m in the western Mediterranean (Bottari *et al*., 2014; Ramírez-Amaro *et al*., 2015) and reaching depths of 780 m in the eastern Mediterranean (Mytilineou *et al*., 2005).

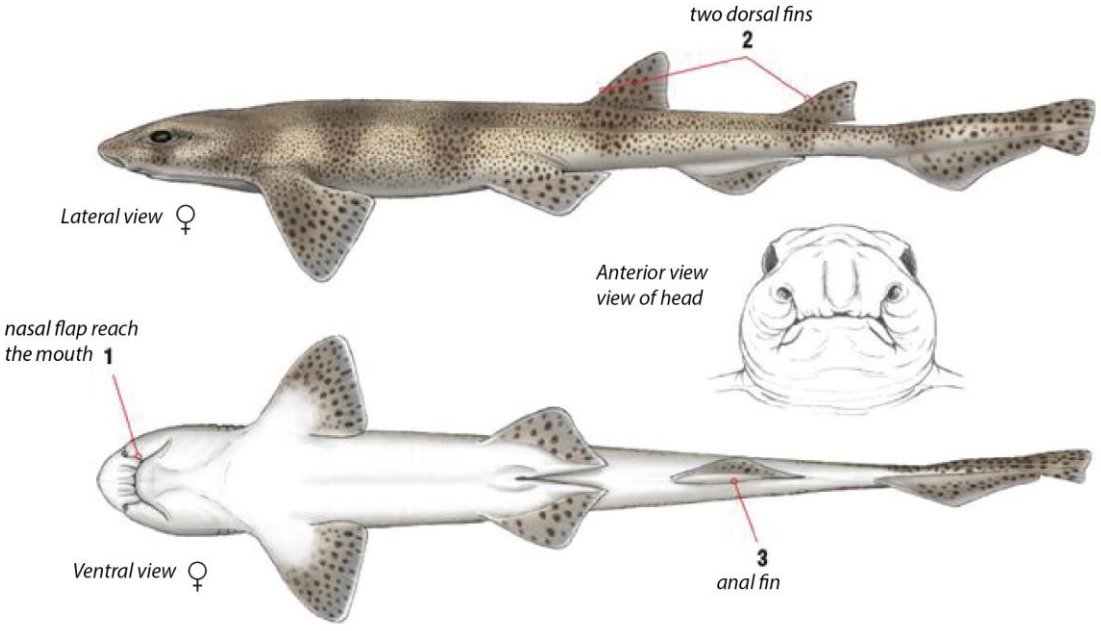


Figure 1.3. Taxonomic illustration of a female small-spotted catshark *Scyliorhinus canicula*. (Illustrated by Marc Dando).

S. canicula is a moderately important commercial species, which is primarily caught as bycatch in demersal fisheries often by trawling nets (Compagno *et al*., 2005). It is considered one of the most abundant elasmobranch species within its distribution (Compagno *et al*., 2005) and is currently listed as Least Concern on the IUCN Red List (Serena *et al*., 2016). However, like all elasmobranch species, its life history traits make it particularly vulnerable to increased fisheries pressures and environmental change (Compagno *et al*., 1990; Dulvy *et al*., 2014). Indeed, overexploitation has resulted in localised reductions in abundance in some areas, including the Adriatic Sea (Barausse *et al*., 2014), Wadden Sea (Wolff, 2000) and off the Tunisian coast (Mnasri, 2012). There is also concern about the future status of this species given its close proximity to human populations and the predicted effects of habitat destruction, coastal pollution and climate change (Dulvy *et al*., 2014).

S. canicula is developing into an excellent model species for sharks having been researched since the early 20th century (Ford, 1921). Previous studies on the reproductive biology of *S. canicula* have highlighted differences among traits between and within different seas. Geographical variation in reproductive characteristics such as maximum size, size at maturity, egg-laying rates and peak laying seasons have been well documented, particularly between the NEA and Mediterranean (Capapé *et al*., 2008, 2014; Ellis & Shackley, 1995; Henderson & Casey, 2001; Jennings *et al*., 1999; Kousteni *et al*., 2010; Rodríguez-Cabello *et al*., 2004). Growth, maximum size and size at maturity also vary with latitude in the NEA (Ellis & Shackley, 1995; Henderson & Casey, 2001; Ivory *et al*., 2005; Mendes *et al*., 2004; Rodríguez-Cabello *et al*., 2004) and longitude in the Mediterranean (Kousteni *et al*., 2010; Finotto *et al*., 2015; Capapé *et al*., 2014, 2008; Bendiab *et al*., 2012),

with individuals from cooler regions attaining larger sizes. In fact, some morphological characteristics become so apparent that small-spotted catsharks have been suggested as a separate stock in the Cantabrian Sea (Rodrıguez-Cabello *et al*., 2004) and a distinct taxon in west Africa based on unique morphological characters in this region (Litvinov, 2003).

The dispersal potential of *S. canicula* is considered low, due to traits such as oviparity (Wheeler, 1978) and high site fidelity (Sims *et al.*, 2001; Rodríguez-Cabello *et al*., 2004). According to a tagging study in the Cantabrian Sea, 70% of recaptured individuals did not move > 24 km with a maximum distance of 256 km recorded (Rodríguez-Cabello *et al.,* 2004). Following similar patterns of other demersal, coastal species of shark (Gubili *et al*., 2014; Chapman *et al*., 2015; Corrigan *et al*., 2016) its lower dispersal potential has been found to promote genetic structuring. Previous genetic studies based on mtDNA and microsatellites show clear division between the NEA and the Mediterranean (Gubili *et al.,* 2014) and further complex structuring within Mediterranean (Gubili *et al.,* 2014; Kousteni *et al.,* 2015). This has been attributed to the phylogeographic history of the Mediterranean, its complex topology and deep waters acting as a barrier to gene flow (Gubili *et al.,* 2014; Kousteni *et al.,* 2015; Ramírez-Amaro *et al*., 2018). However, traditional neutral markers have failed to reveal structuring in the NEA (Barbieri *et al.,* 2013; Gubili *et al*., 2014). It wasn't until Manuzzi *et al.* (2018) used a panel of 2674 SNPs generated with 2b-RAD that fine-scale structuring of the small-spotted catshark was revealed in the NEA, showing the first evidence of a potential north-south divide.

S. canicula has been at the forefront of genetic research in elasmobranchs. It was the first species to have its complete mitochondrial genome sequenced (Delarbre *et al.*, 1998) and also has multi-tissue transcriptomic resources (Mulley et al. 2014). Excitingly, the development of a well-annotated genome for *S. canicula* is also underway as part of the Sanger Institute 25 Genomes for 25 Years project (https://www.sanger.ac.uk/collaboration/25-genomes-25-years/), which offers exciting scope for future research. This makes *S. canicula* an excellent model system for the study of genetic diversity, local adaptation and evolution in elasmobranchs that would not be feasible in other species. Utilising emerging genomic techniques and resources, alongside an existing understanding of key life history traits, can help elucidate some of the key drivers (e.g. demographic vs. environmental vs. behavioural) responsible for genetic differentiation and phenotypic divergence observed between *S. canicula* populations. As so few genomic resources exists for elasmobranchs, *S. canicula* offers a unique opportunity to gain insight into the evolutionary processes in sharks and understand how they become adapted to their environments.

1.4 Aims of this thesis

1.4.1 Chapter 2

Utilising the mtDNA control region (CR), Chapter 2 of this thesis will aim to resolve the genetic structuring of *Scyliorhinus canicula* edge populations identified in West Africa, Northern Aegean and Cyprus, expanding on previous populations genetic studies of this species.

1.4.2 Chapter 3

Using ddRAD and seascape genomics, Chapter 3 will aim to identify genomic regions under selection in *Scyliorhinus canicula* to better describe the genomic basis and key drivers underlying regional adaptation.

Additional work

Bound at the end of my thesis is further work I undertook during the course of my Masters by Research. It included the development of a new DNA minibarcode to facilitate species identification in processed batoid samples in the hope to improve trade monitoring. This work was published in Conservation Genetics Resources:

Wannell, G., Griffiths, A. M., Spinou, A., Batista, R., Mendonça, M., Wosiacki, W., Fraser, B., Wintner, S., Papadopoulos, A., Krey, G. & Gubili, C. (2020). A new minibarcode assay to facilitate species identification from processed, degraded or historic ray (batoidea) samples. *Conserv Genet Resour*. 12, 659-668.

Whilst it lies outside the tight focus of my dissertation title on the population genetics of *S. canicula*, it still focuses on molecular tools in elasmobranchs. Therefore, I hope that you will considered it as part of submission of work towards my Masters by Research.

1.5. References

Arnold, B., Corbett-Detig, R. B., Hartl, D. & Bomblies, K. (2013). RADseq underestimates diversity and introduces genealogical biases due to nonrandom haplotype sampling. *Mol. Ecol.* 22(11):3179–3190.

Bailleul, D., Mackenzie, A., Sacchi, O., Poisson, F., Bierne, N. & Arnaud-Haond, S. (2018). Large-scale genetic panmixia in the blue shark (*Prionace glauca*): A single worldwide population, or a genetic lag-time effect of the “grey zone” of differentiation? *Evol. Appl.* 11:614-630.

Benavides M. T., Horn, R. L., Feldheim, K. A., Shivji, M. S*. et al*. (2011). Global phylogeography of the dusky shark *Carcharhinus obscurus*: implications for fisheries management and monitoring the shark fin trade. *Endang. Species. Res.* 14:13-22.

Benjelloun, B. *et al*. (2019). An evaluation of sequencing coverage and genotyping strategies to assess neutral and adaptive diversity. *Mol. Ecol. Res.* 19(6):1497–1515.

Bernard, A. M., Feldheim, K. A., Heithaus, M. R., Wintner, S. P., Wetherbee, B. M. & Shivji, M. S. (2016). Global population genetic dynamics of a highly migratory, apex predator shark. *Mol. Ecol*. 25:5312-5329.

Bernatchez, S., Xuereb, A., Laporte, M., Benestan, L., Steeves, R., Laflamme, M., *et al*. (2019). Seascape genomics of eastern oyster (*Crassostrea virginica*) along the Atlantic coast of Canada. *Evol. App.* 12(3):587–609.

Bessudo, S., Soler, G.A., Klimley, A. P., Ketchum, J. T., Hearn, A. & Arauz, R. (2011). Residency of the scalloped hammerhead shark (*Sphyrna lewini*) at Malpelo Island and evidence of migration to other islands in the Eastern Tropical Pacific. *Environ. Biol. Fishes.* 91:165–76.

Bornatowski, H., Braga, R. R. & Vitule, J. R. S. (2013). Shark mislabelling threatens biodiversity. *Science.* 340:923.

Borsa, P., Shen, K., Arlyza, I. S. & Hoareau, T. B. (2016). Multiple cryptic species in the blue-spotted maskray (Myliobatoidei: Dasyatidae: Neotrygon spp). *Comptes Rendus Biologies*, 339:417- 426.

Bottari, T., Busalacchi, B., Profeta, A., Mancuso, M., Giordano, D. & Rinelli, P. (2014). Elasmobranch distribution and assemblages in the southern Tyrrhenian Sea (central Mediterranean). *J. Aquat. Res. Dev.* 5(2):216-225.

Brumfield, R. T., Beerli, P., Nickerson, D. A. & Edwards, S. V. (2003). The utility of single nucleotide polymorphisms in inferences of population history. Trends *Ecol. Evol*. 18:249–256.

Budd, A. M., Cooper, M. K., Le Port, A., Schils, T., Mills. M. S., Deinhart, M. E., Huerlimann, R. & Strugnell, J. M. (2021). First detection of critically endangered scalloped hammerhead sharks (Sphyrna lewini) in Guam, Micronesia, in five decades using environmental DNA. *Ecol. Indic*. 127: 107649.

Burrows, M. T., Schoeman, D. S., Buckley, L. B., Moore, P., Poloczanska, E.S., Brander, K. M., Brown, C., Bruno, J. F., Duarte, C. M. & Halpern, B. S. (2011) The pace of shifting climate in marine and terrestrial ecosystems. *Science*. 334:652–655.

Cao, Y., Waddell, P. J., Okada, N. & Hasegawa, M. (1998). The complete mitochondrial DNA sequence of the shark, *Mustelus manazo*: evaluating rooting contradictions to the living vertebrates*. Mol. Biol. Evol*. 15:1637–1646.

Capapé. C., Mnasri-Sioudi. N., Kamel-Moutalibi, O. E., Boumaïza, M., Amor, M. M. B. & Reynaud, C. (2014). Production, maturity, reproductive cycle and fecundity of small-spotted catshark, *Scyliorhinus canicula* (Chondrichthyes: Scyliorhinidae) from the northern coast of Tunisia (Central Mediterranean*). J Ichthyol.* 54:111–126.

Capapé, C., Vergne, Y., Reynaud, C., Guélorget, O. & Quignard, J.P. (2008). Maturity, fecundity and occurrence of the smallspotted catshark *Scyliorhinus canicula* (Chondrichthyes: Scyliorhinidae) off the Languedocian coast (southern France, north-western Mediterranean). *Vie Milieu.* 58: 47–55.

Carreras, C., Ordóñez, V., Zane, L., Kruschel, C., Nasto, I., Macpherson, E. & Pascual, M. (2017). Population genomics of an endemic Mediterranean fish: Differentiation by fine scale dispersal and adaptation. *Sci. Rep.* 7: 43417.

Castro J, Picornell A, Ramon M (1999). Mitochondrial DNA: A tool for populational genetics studies. *Int Microbiol* **1**: 327–332.

Castro, A. L. F., Stewart, B. S., Wilson, S. G., Hueter, R. E., Meekan, M. G., Motta, P.J., Bowen, B. W. & Karl, S. A. (2007). Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). *Mol. Ecol*. 16: 5183-5192.

Catarino, D., Knutsen, H., Veríssimo, A. G. *et al.* (2015). The Pillars of Hercules as a bathymetric barrier to gene flow promoting isolation in a global deep-sea shark (*Centroscymnus coelolepis*). *Mol. Ecol.* 24:6061–6079.

Cariani, A., Messinetti, S., Ferrari, A., Arculeo, M., Bonello, J. J., Bonnici, L. *et al.* (2017). Improving the Conservation of Mediterranean Chondrichthyans: The ELASMOMED DNA Barcode Reference Library. *PLoS ONE.* 12(1):e0170244.

Chapman, D. D., Feldheim, K. A., Papastamatiou, Y. P. & Hueter, R. E. (2015). There and Back Again: A Review of Residency and Return Migrations in Sharks, with Implications for Population Structure and Management. *Ann Rev Mar. Sci*. 7(1):547–570.

Cheng, L., Abraham, J., Hausfather, Z. & Trenberth, K. E. (2019). How fast are the oceans warming. *Science*. 363(6423):128–129.

Clarke, S. C., McAllister, M. K., Milner-Gulland, E. J., Kirkwood, G .P., Michielsens, C. G. J., Agnew, D. J., Pikitch, E .K., Nakano, H. & Shivji, M. S. (2006). Global estimates of shark catches using trade records from commercial markets. *Ecol. Lett.* 9:1115-1126.

Collie, J. S., Escanero, G. A. & Valentine, P. C. (2000). Photographic evaluation of the impacts of bottom fishing on benthic epifauna. *ICES J. Mar. Sci*. 57(4):987–1001.

Compagno, L. J. V. (1990). Alternative life-history styles of cartilaginous fishes in time and space. *Environ. Biol. Fishes.* 28:33–75.

Compagno, L. J, V., Dando, M. & Fowler, S. (2005). *A field guide to the sharks of the world*. Harper Collins Publishers Ltd, London.

Conrath, C, L., Musick, J. A. (2012) Reproductive biology of elasmobranchs. In: Carrier, J. C., Musick, J. A., Heithaus, M. R. eds. *Biology of sharks and their relatives*. CRC Press, Boca Raton: 291–311.

Corrigan, S., Huveneers, C., Stow, A., Beheregaray, B. (2016). A multilocus comparative study of dispersal in three codistributed demersal sharks from eastern Australia. *Can. J. Fish. Aquat. Sci*. 73:1–10.

Crandall, K., Bininda-Emonds, O., Mace, G., Wayne, R. K. (2000). Considering evolutionary process in conservation biology. *Trends Ecol. Evol*. 15, 290–295.

Davidson, L. N. K., Krawchuk, M. A. and Dulvy, N. K. (2016). Why have global shark and ray landings declined: Improved management or overfishing? *Fish. Fish*. 17:438–458.

Delarbre, C., Spruyt, N., Delmarre, C., Gallut, C., Barriel, V., Janvier, P., Laudet, V. & Gachelin, G. (1998). The complete nucleotide sequence of the mitochondrial DNA of the dogfish, *Scyliorhinus canicula*. *Genetics.* 150:331–344.

Diopere, E., Vandamme, S. G., Hablützel, P. I., Cariani, A., van Houdt, J., Rijnsdorp, A., Tinti, F., FishPopTrace Consortium., Volckaert, F. A. M. & Maes G. E. (2018). Seascape genetics of a flatfish reveals local selection under high levels of gene flow. *ICES J. Mar. Sci.* 75(2): 675–689.

Di Santo, V. (2015) Ocean acidification exacerbates the impacts of global warming on embryonic little skate, Leucoraja erinacea (Mitchill). *J. Exp. Mar. Biol. Ecol.* 463:72–78.

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., Carlson, J. K., Davidson, L. N. K., Fordham, S. V., Francis, M. P., Pollock, C. M., Simpfendorfer, C. A., Burgess, G. H., Carpenter, K. E., Compagno, L .J .V., Ebert, D. A., Gibson, C., Heupel, M. R., Livingstone, S. R., Sanciangco, J. C., Stevens, J. D., Valenti, S. and White, W. T. (2014). Extinction risk and conservation of the world's sharks and rays. *ELife.* 3:1–34.

Dulvy, N. K., Simpfendorfer, C. A., Davidson, L. N. K., Fordham, S. V., Bräutigam, A., Sant, G. and Welch, D. J. (2017). Challenges and priorities in shark and ray conservation. *Curr. Biol.* 27:565–572.

Dulvy, N. K. & Reynolds, J. D. (2002). Predicting extinction vulnerability in skates. *Conserv. Biol.* 16, 440–450.

Ellis, J. R., Dulvy, N. K., Jennings, S., Parker-Humphreys, M. & Rogers, S.I. (2005). Assessing the status of demersal elasmobranchs in UK waters: a review. *J. Mar. Biol. Ass. U.K*. 85:1025–1047.

Excoffier, L., Hofer, T. & Foll, M. (2009). Detecting loci under selection in a hierarchically structured population. *Heredity.* 103(4):285–298.

Feldheim, K. A., Gruber, S. H., DiBattista, J. D., Babcock, E. A., Kessel, S. T., Hendry, A. P., Pikitch, E. K., Ashley, M. V. & Chapman, D. D. (2014). Two decades of genetic profiling yields first evidence of natal philopatry and long-term fidelity to parturition sites in sharks. *Mol. Ecol*. 23:110-117.

Ferrari, A., Tinti, F., Bertucci Maresca, V., Velonà, A., Cannas, R., Thasitis, I., Costa, F. O., Follesa, M. C., Golani, D., Hemida, F., Helyar, S. J., Mancusi, C., Mulas, A., Serena, F., Sion, L., Stagioni, M. & Cariani, A. (2018), Natural history and molecular evolution of demersal Mediterranean sharks and skates inferred by comparative phylogeographic and demographic analyses. *PeerJ.* 6:e5560.

Ferretti, F., Myers, R. A., Serena, F. & Lotze, H. K. (2008). Loss of large predatory sharks from the Mediterranean Sea. *Conserv Biol*, 22:952-964.

Ferretti, F., Worm, B., Britten, G. L., Heithaus, M. R., and Lotze, H. K. (2010). Patterns and ecosystem consequences of shark declines in the ocean. *Ecol. Lett*. 13(8):1055–1071.

Ford, E. (1921). A contribution to our knowledge of the life histories of the dogfishes landed at Plymouth. J*. mar biol. Ass. U.K*.12: 468-505.

Fountain, E. D., Pauli, J. N., Reid, B. N., Palsboll. P, J. & Peery. M. Z. (2016). Finding the right coverage: the impact of coverage and sequence quality on single nucleotide polymorphism genotyping error rates*. Mol. Ecol. Res*. 16(4):966–978.

Frankham, R., Ballou, J. D. & Briscoe, D. A. (2002). *Introduction to conservation genetics*. Cambridge University Press, Cambridge.

Frías-Espericueta, M. G., Cardenas-Nava, N. G., Márquez-Farías, J.F., Osuna-López, J. I., Muy-Rangel, M. D., Rubio-Carrasco, W. & Voltolina, D. (2014). Cadmium, copper, lead and zinc concentrations in female and embryonic pacific sharpnose shark (*Rhizoprionodon longurio*) tissues. *Bull. Environ. Contam. Toxicol*. 93:532–535.

Fuentes-Pardo, A. P. & Ruzzante, D. E. (2017). Whole-genome sequencing approaches for conservation biology: Advantages, limitations and practical recommendations. *Mol. Ecol*. 26(20):5369–5406.

Gallucci, V. F., Taylor, I. G. & Erzini, K. (2006). Conservation and management of exploited shark populations based on reproductive value. *Can. J. Fish. Aquat. Sci*. 63:931–942.

García, V. B., Lucifora, L. O. & Myers, R. A. (2008). The importance of habitat and life history to extinction risk in sharks, skates, rays and chimaeras. *Proc .R. Soc. Lond. B. Biol. Sci.* 275:83–89.

Gervais, C., Mourier, J. & Rummer, J. L. (2016). Developing in warm water: irregular colouration and patterns of a neonate elasmobranch. *Mar. Biodivers*. 46:743–744.

Gervais, C. R., Nay, T. J., Renshaw, G., Johansen, J. L., Steffensen, J. F. & Rummer, J. L. (2018). Too hot to handle? Using movement to alleviate effects of elevated temperatures in a benthic elasmobranch, Hemiscyllium ocellatum. *Mar. Biol.* 165:162-174.

Griffiths, A. M., Miller, D. D., Egan, A., Fox, J., Greenfield, A. & Mariani S. (2013). DNA barcoding unveils skate (Chondrichthyes: Rajidae) species diversity in 'ray' products sold across Ireland and the UK. *PeerJ,* 1:e129.

Griffiths, A. M., Sims, D. W., Cotterel, S. P., Nagar, A. E., Ellis, J. R., Lynghhhhammar, A. *et al.* (2010). Molecular markers reveal spatially segregated cryptic species in a critically endangered fish, the common skate (Dipturus batis). *Proc. R. Soc. B*. 277(1687):1497–1503.

Griffiths, A. M, Sims, D. W., Johnson, A., Lynghammar, A., McHugh, M., Bakken, T. & Genner, M. J. (2011). Levels of connectivity between longnose skate (Dipturus oxyrinchus) in the Mediterranean Sea and the north-eastern Atlantic Ocean. *Conser. Genet.* 12(2):577–582.

Gubili, C., Macleod, K., Perry, W., Hanel, P., Batzakas, I., Farrell, E. D., Griffiths A. M. (2016). Connectivity in the deep: Phylogeography of the velvet belly lanternshark. *Deep-Sea Res. Part I: Oceanogr. Res.* 115:233–239.

Gubili, C., Sims, D. W., Veríssimo, A., Domenici, P., Ellis, J., Grigoriou, P., Johnson, A. F., McHugh. M., Neat, F., Satta, A., Scarcella, G., Serra-Pereira, B., Soldo, A., Genner, M. J. & Griffiths, A. M. (2014). A tale of two seas: contrasting patterns of population structure in the small-spotted catshark across Europe. *R. Soc. Open. Sci.* 1140175.

Gupta, A., Bhardwaj, A., Supriya, S. P., Pal, Y., *et al*. (2015). Mitochondrial DNA- a Tool for Phylogenetic and Biodiversity Search in Equines. *J Biodivers. Endanger. Species S1.* S1.006.

Hara, Y., Yamaguchi, K., Onimaru, K., Kadota, M., Koyanagi, M., Keeley, S.D., Tatsumi, K., Tanaka, K., Motone, F., Kageyama, Y. *et al*. (2018). Shark genomes provide insights into elasmobranch evolution and the origin of vertebrates. *Nat. Ecol. Evol*. 2, 1761-1790.

Heupel, M. R., Knip, D. M., Simpfendorfer, C. A., & Dulvy, N. K. (2014). Sizing up the ecological role of sharks as predators. *Mar. Ecol. Prog. Ser*. 495:291–298.

von der Heyden, S., Beger, M., Toonen, R.J., van Herwerden, L., Juinio-Meñez, M.A., Ravago-Gotanco, R., Fauvelot, C. & Bernardi, G. (2014) The application of genetics to marine management and conservation: examples from the Indo-Pacific. *B. Mar. Sci*. 90, 123– 158.

Hoban, S. et al. (2016). Finding the genomic basis of local adaptation: Pitfalls, practical solutions, and future directions. *Am. Nat*. 188(4):379–397.

Hobbs, C. A. D., Potts, R. W. A., Bjerregaard Walsh, M. *et al*. (2019). Using DNA Barcoding to Investigate Patterns of Species Utilisation in UK Shark Products Reveals Threatened Species on Sale. *Sci. Rep.* 9:1028.

Hoenig, J. M. & Gruber, S. H. (1990). Life-history patterns in the elasmobranchs: implications for fisheries management. *NOAA Tech. Rep. NMFS.* 90:16.

Hull, K. L., Asbury, T. A., da Silva, C. *et al.* (2019). Strong genetic isolation despite wide distribution in a commercially exploited coastal shark. *Hydrobiologia.* 838:121–137.

Hume, J. B. (2019). Higher temperatures increase developmental rate & reduce body size at hatching in the small-eyed skate Raja microocellata: implications for exploitation of an elasmobranch in warming seas. J. Fish Biol. 95:655–658.

Huveneers, C., Meekan, M. G., Apps, K., Ferreira, L. C., Pannell, D., & Vianna, G. M. S. (2017). The economic value of shark-diving tourism in Australia. *Rev. Fish Biol. Fish.* 27:665–680.

Ivanova, N. V., Zemlak, T. S., Hanner, R. H. & Hebert, P. D. N. (2007). Universal primer cocktails for fish DNA barcoding. *Mol Ecol Notes.* 7:544-548.

Jenkins, T. L., Ellis, C. D., Triantafyllidis, A. & Stevens, J. R. (2019). Single nucleotide polymorphisms reveal a genetic cline across the north-east Atlantic and enable powerful population assignment in the European lobster. *Evol. Appl.* 12(10):1881–1899.

Jeffree, R. A., Warnau, M., Oberhansli, F., Teyssie, J. L. (2006). Bioaccumulation of heavy metals and radionuclides from seawater by encased embryos of the spotted dogfish *Scyliorhinus canicula*. *Mar. Pollut. Bull.* 52:1278–1286.

Johnson, M. S., Kraver, D. W., Renshaw, G. M. C. & Rummer, J. L. (2016). Will ocean acidification affect the early ontogeny of a tropical oviparous elasmobranch (Hemiscyllium ocellatum)? *Conserv. Physiol.* 4:1–11.

Johri, S., Doane, M., Allen, L. & Dinsdale, E. (2019). Taking Advantage of the Genomics Revolution for Monitoring and Conservation of Chondrichthyan Populations. *Diversity*. 11:49.

Karl, S., Castro, A. L. F., López, J., Charvet, P. & Burgess, G. (2011). Phylogeography and conservation of the bull shark (*Carcharhinus leucas*) inferred from mitochondrial and microsatellite DNA. *Conserv. Genet*. 12:371–382.

Kousteni, V., Karachle, P. K. & Megalofonou, P. (2017). Diet of the small-spotted catshark *Scyliorhinus canicula* in the Aegean Sea (eastern Mediterranean). *Mar. Biol. Res*. 13(2):161–173.

Kousteni, V., Kontopoulou, M. & Megalofonou P. (2010). Sexual maturity and fecundity of *Scyliorhinus canicula* (Linnaeus, 1758) in the Aegean Sea. *Mar. Biol. Res*. 6:390– 98.

Last, P. R., Naylor, G. J. P. & Manjaji-Matsumoto, B. M. (2016). A revised classification of the family Dasyatidae (Chondrichthyes: Myliobatiformes) based on new morphological and molecular insights. *Zootaxa*. 4139(3):345-368.

Lawson, J. M., Pollom, R. A., Gordon, C. A., Barker, J., Meyers, E. K. M., Zidowitz, G., Ellis, J. R., Bartolí, A., Morey, G., Fowler, S. L., Alvarado, D. J., Fordham, S. V., Sharp, R., Hood, A. R. & Dulvy, N. K. (2020). Extinction risk and conservation of critically endangered angel sharks in the Eastern Atlantic and Mediterranean Sea, ICES J. Mar. Sci. 77(1):12–29.

Leone, A., Urso, I., Damalas, D., Martinsohn, J., Zanzi, A., Mariani, S., Cariani, A. (2017). Genetic differentiation and phylogeography of Mediterranean-North Eastern Atlantic blue shark (*Prionace glauca*, L. 1758) using mitochondrial DNA: Panmixia or complex stock structure? *PeerJ.* 12.

Ligas, A., Osio, G. C., Sartor, P., Sbrana, M. & De Ranieri, S. (2013). Long-term trajectory of some elasmobranch species off the Tuscany coasts (NW Mediterranean) from 50 years of catch data. *Sci. Mar*. 77:119–127.

Liggins, L., Treml, E. A. & Riginos, C. (2019). Seascape Genomics: Contextualizing Adaptive and Neutral Genomic Variation in the Ocean Environment. In: Oleksiak M., Rajora O. eds. *Population Genomics: Marine Organisms. Population Genomics*. Springer, Cham.

Lowry, D. B., Hoban, S., Kelley, J. L., Lotterhos, K. E., Reed, L. K., Antolin, M. F. & Storfer, A. (2017). Breaking RAD: an evaluation of the utility of restriction site-associated DNA sequencing for genome scans of adaptation. *Mol. Ecol. Res.* 17(2):142–152.

MacNeil, M. A., Chapman, D. D., Heupel, M. *et al*. (2020). Global status and conservation potential of reef sharks. *Nature*. 583, 801–806.

Marra, N. J., Stanhope, M. J., Jue, N. K., Wang, M., Sun, Q., Bitar, P. P., Richards, V. P., Komissarov, A., Rayko, M., Kliver, S. *et al*. (2019). White shark genome reveals ancient elasmobranch adaptations associated with wound healing and the maintenance of genome stability. *Proc. Natl. Acad. Sci. USA*. 116:4446–4455.

Merly, L., Lange, L., Meÿer, M., Hewitt, A.M, Koen, P., Fischer, C., Muller, J., Schilack, V., Wentzel, M. & Hammerschlag, N. (2019). Blood plasma levels of heavy metals and trace elements in white sharks (*Carcharodon carcharias*) and potential health consequences. *Mar. Pollut. Bull.* 142:85–92.

Milano, I., Babbucci, M., Cariani, A., Atanassova, M., Bekkevold, D., Carvalho, G. R. *et al*. (2014). Outlier SNP markers reveal fine-scale genetic structuring across European hake populations (Merluccius merluccius). *Mol. Ecol*. 23:118–135.

Mnasri, N., El Kamel, O., Boumaïza, M., Reynaud, C. & Capapé, C. (2012). Food and feeding habits of the small-spotted catshark, *Scyliorhinus canicula* (Chondrichthyes: Scyliorhinidae) from the northern coast of Tunisia (central Mediterranean). *Cahiers de Biologie Marine.* 53(1):139–50.

Momigliano, P., Harcourt, R., Robbins, W. D., Jaiteh, V., Mahardika, G. N., Sembiring, A. & Stow, A. (2017). Genetic structure and signatures of selection in grey reef sharks (*Carcharhinus amblyrhynchos*). *Heredity*. 119, 142–153.

Mulley, J., Hargreaves, A., Hegarty, M., Heller, SR. & Swain, M. (2014). Transcriptomic analysis of the lesser spotted catshark (*Scyliorhinus canicula*) pancreas, liver and brain reveals molecular level conservation of vertebrate pancreas function. *BMC genomics* 15:1074.

Musa, S. M., Ripley, D. M., Moritz, T. & Shiels, H. A. (2020). Ocean warming and hypoxia affect embryonic growth, fitness and survival of small-spotted catsharks, *Scyliorhinus canicula*. *J Fish Biol*. 97: 257– 264.

Myers, R. A., Baum, J. K., Shepherd, T. D., Powers, S. P. & Peterson, C. H. (2007). Cascading effects of the loss of apex predatory sharks from a Coastal Ocean. *Science* 315:1846–1850.

Mytilineou, Ch., Politou, C. Y., Papaconstantinou, C., Kavadas, S., D'Onghia, G. & Sion, L. (2005). Deep-water fish fauna in the Eastern Ionian Sea. *Belgian J. Zool*. 135 (2):229–33.

Ovenden, J. R., Dudgeon, C. L., Feutry, P., Feldheim, K. A. & Maes, G. E. (2018) *Genetics and genomics for fundamental and applied research on elasmobranchs*. In: Carrier, J. C., Heithaus, M. R. & Simpfendorfer, C. A. eds. Shark research: emerging technologies and applications for the field and laboratory. CRC Press, Boca Raton: 235–253.

Ovenden, J.R., Leigh, G.M., Blower, D.C., Jones, A.T., Moore, A., Bustamante, C., Buckworth, R.C., Bennett, M.B. & Dudgeon, C.L. (2016), Can estimates of genetic effective population size contribute to fisheries stock assessments? *J Fish Biol*, 89:2505-2518.

Pazmiño, D. A., Maes, G. E., Simpfendorfer, C. A., Salinas-de-León, P., Herwerden, L. (2017). Can Genome-wide SNPs reveal low effective population size within confined management units of the highly vagile Galapagos shark (*Carcharhinus galapagensis*). *Conserv. Genet*. 18:1151–1163.

Perry, A. L., Low, P.J., Ellis, J. R. & Reynolds, J. D. (2005). Climate change and distribution shifts in marine fishes. *Science.* 308:1912–1915.

Pistevos, J. C. A., Nagelkerken, I., Rossi, T., Olmos, M. & Connell, S. D. (2015). Ocean acidification and global warming impair shark hunting behaviour and growth. *Sci. Rep.* 5:16293.

Pistevos, J. C. A., Nagelkerken, I., Rossi, T. & Connell, S. D. (2017). Antagonistic effects of ocean acidification and warming on hunting sharks. *Oikos*. 126:241–247.

Pörtner, H. O., Karl, D., Boyd, P. *et al*. (2014). *Ocean systems*. In: Field, C. B., Barros, V. R., Dokken, D. J., Mach, K. J., Mastrandrea, M. D., Bilir, T. F. *et al*. eds. Climate change 2014: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. Contribution of Working Group II to the Fifth Assessment Report of

the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge: 411–484.

Portnoy, D. S. & Heist, E. J. (2012). Molecular markers: progress and prospects for understanding reproductive ecology in elasmobranchs. *J. Fish. Biol*. 80: 1120-1140.

Portnoy, D. S., Puritz, J. B., Hollenbeck, C. M., Gelsleichter, J., Chapman, D. & Gold, J. R. (2015). Selection and sex-biased dispersal in a coastal shark: the influence of philopatry on adaptive variation. *Mol. Ecol*. 24: 5877-5885.

Postaire, B. D., Bakker, J., Gardiner, J., Wiley, T. R. & Chapman, D. D. (2020). Environmental DNA detection tracks established seasonal occurrence of blacktip sharks (Carcharhinus limbatus) in a semi-enclosed subtropical bay. *Sci. Rep*. 10: 1–8.

Powter, D. M. & Gladstone, W. (2008). The reproductive biology and ecology of the Port Jackson shark *Heterodontus portusjacksoni* in the coastal waters of eastern Australia. *J. Fish. Biol*. 72:2615-2633.

Ramírez-Amaro, S., Ordines, F., Terrasa, B., Esteban, A., García, C., Guijarro, B. & Massutí, E. (2015). Demersal chondrichthyans in the western Mediterranean: assemblages and biological parameters of their main species. *Mar. Freshw. Res.* 67:636–652.

Read, T. D., Petit, R. A., Joseph, S. J., Alam, M. T., Weil, M. R., Ahmad, M., Bhimani, R., Vuong, J. S., Haase, C. P., Webb, D. H. *et al.* (2017). Draft sequencing and assembly of the genome of the world's largest fish, the whale shark: *Rhincodon typus* Smith 1828. *BMC Genom*. 18:532-571.

Rellstab, C. *et al.* (2015). A practical guide to environmental association analysis in landscape genomics. *Mol. Ecol.* 24(17):4348–4370.

Roff, G., Brown, C.J., Priest, M.A. *et al.* (2018). Decline of coastal apex shark populations over the past half century. *Commun. Biol. 1*. 223.

Roff, G., Doropoulos, C., Rogers, A., Bozec, Y.-M., Krueck, N. C., Aurellado, E. *et al*. (2016). The ecological role of sharks on coral reefs. *Trends Ecol. Evol*. 31:395–407.

Rosa, R., Baptista, M., Lopes, V. M. *et al*. (2014). Early-life exposure to climate change impairs tropical shark survival. *Proc. R. Soc. B. Biol. Sci.* 281:20141738.

Rosa, R., Paula. J. R., Sampaio, E. *et al*. (2016) Neuro-oxidative damage and aerobic potential loss of sharks under elevated CO2. *Mar. Biol.* 163:119.

Rubinoff, D. & Holland, B. S. (2005). Between Two Extremes: Mitochondrial DNA is neither the Panacea nor the Nemesis of Phylogenetic and Taxonomic Inference. *System. Biol.* 54(6):952–961.

Selkoe, K. A., Aloia, C. C. D., Crandall, E. D., Iacchei, M., Liggins, L., Puritz, J. B. *et al.* (2016). A decade of seascape genetics: Contributions to basic and applied marine connectivity. Mar. Ecol. Pro. Ser. 554:1–19.

Simpfendorfer, C. A. Kyne, P. M., Noble, T. H., Goldsbury, J., Basiita, R. K., Lindsay, R., Shields, A., Perry, C. & Jerry, D. R. (2016). Environmental DNA detects Critically Endangered largetooth sawfish in the wild. *Endanger. Species Res*. 30(1):109–116.

Skliris, N., Zika, J. D., Herold, L. *et al*. (2018). Mediterranean Sea water budget long-term trend inferred from salinity observations. *Clim. Dyn.* 51:2857–2876.

Spaet, J. L., Jabado, R. W., Henderson, A. C., Moore, A. B. & Berumen, M. L. (2015). Population genetics of four heavily exploited shark species around the Arabian Peninsula. *Ecol. Evol.* 5(12):2317-32.

Speed, C. W., Field, I. C., Meekan, M. G. & Bradshaw, C. J. A. (2010). Complexities of coastal shark movements and their implications for management. *Mar. Ecol. Prog. Ser.* 408:275–293.

Stein, R. W., Mull, C. G., Kuhn, T. S., Aschliman, N. C., Davidson, L. N. K., Joy, J. B., Smith, G. J., Dulvy, N. K. & Mooers, A. O. (2018). Global priorities for conserving the evolutionary history of sharks, rays and chimaeras. *Nat. Ecol. Evol*. 2:288–298.

Steinke, D., Bernard, A. M., Horn, R. L., Hilton, P., Hanner and R. & Shivji, M. S. (2017). DNA analysis of traded shark fins and mobulid gill plates reveals a high proportion of species of conservation concern. *Sci. Rep.* 7:5-10.

Stevens, J. D., Bonfil, R., Dulvy, N. K. & Walker, P. A. (2000). The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES J. Mar. Sci*. 57(3):476–494.

Stevens, J. D., Walker, T. I., Cook, S. F., Fordham, S. (2005). *Threats faced by chondrichthyan fishes*. In: Fowler, S. L., Cavanagh, R., Camhi, M., Burgess, G. H., Caillet, G. M., Fordham, S., Simpfendorfer, C. A. & Musick, J.A. eds. Sharks, rays and chimaeras: the status of the Chondrichthyan fishes. Gland, Switzerland and Cambridge, UK: IUCN Species Survival Commission Shark Specialist Group: 48–57.

Sguotti, C., Lynam, C. P., García-Carreras, B., Ellis, J. R., & Engelhard, G. H. (2016). Distribution of skates and sharks in the North Sea: 112 years of change. *Glob. Change Biol*. 22:2729–2743.

Tiffin, P. and Ross-Ibarra, J. (2014). Advances and limits of using population genetics to understand local adaptation. *Trends Ecol. Evol*. 29(12):673–680.

Toonen, R. J., Puritz, J. B., Forsman, Z. H., Whitney, J. L., Fernandez-Silva, I., Andrews, K. R., Bird, C. E. (2013). ezRAD: a simplified method for genomic genotyping in non-model organisms. *PeerJ.* 1:e203.

Torrado, H., Carreras, C., Raventos, N. *et al*. (2020). Individual-based population genomics reveal different drivers of adaptation in sympatric fish. *Sci. Rep.* 10:12683.

Venkatesh, B., Lee, A. P., Ravi, V., Maurya, A. K., Lian, M. M., Swann, J. B *et al.* (2014). Elephant shark genome provides unique insights into gnathostome evolution. *Nature*. 505:174-179.

Veríssimo, A., McDowell, J. R. & Graves, J. E. (2010). Global population structure of the spiny dogfish *Squalus acanthias*, a temperate shark with an antitropical distribution. *Mol. Ecol*. 19:1651–1662.

Ward-Paige, C. A., Keith, D. M., Worm, B. & Lotze, H. K. (2012). Recovery potential and conservation options for elasmobranchs. *J. Fish. Biol.* 80:1844–1869.

Wang, S., Meyer, E., McKay, J. *et al*. (2012). 2b-RAD: a simple and flexible method for genome-wide genotyping. *Nat. Methods.* 9:808–810.

Wannell, G., Griffiths, A. M., Spinou, A., Batista, R., Mendonça, M., Wosiacki, W., Fraser, B., Wintner, S., Papadopoulos, A., Krey, G. & Gubili, C. (2020). A new minibarcode assay to facilitate species identification from processed, degraded or historic ray (batoidea) samples. *Conserv Genet Resour*. 12, 659-668.

Wheeler, C.R., Gervais, C.R., Johnson, M.S. *et al*. (2020). Anthropogenic stressors influence reproduction and development in elasmobranch fishes. *Rev. Fish. Biol. Fish.* 30:373–386.

Willing, E. M., Hoffmann, M., Klein, J.D., Weigel, D. & Dreyer, C. (2011). Paired-end RAD-seq for de novo assembly and marker design without available reference. *Bioinformatics*. 852(27):2187–2193.

Worm, B., Davis, B., Kettemer, L., Ward-Paige, C.A., Chapman, D., Heithaus, M. R., Kessel, S.T. & Gruber, S. H. (2013). Global catches, exploitation rates, and rebuilding options for sharks. *Mar. Pol.* 40:194–204.

Chapter 2: Living on the edge: Genetic diversity and population differentiation in peripheral populations of the small-spotted catshark, *Scyliorhinus canicula,* compared to central counterparts.

This chapter is written for publication in the Environmental Biology of Fishes.

2.1 Abstract

The small-spotted catshark, *Scyliorhinus canicula,* is a small benthic shark inhabiting the coastal waters of the northeast Atlantic (NEA) and Mediterranean Sea. Utilising the mitochondrial DNA control region (CR), this study aims to expand on previous investigations into the population genetic structure within and between the NEA and Mediterranean Sea. It covers the widest geographical range of *S. canicula* to date, incorporating novel samples from peripheral areas of the species range in Norway, West Africa, North Aegean and Cyprus. These could form unique stocks based on distinct life history traits and assumptions made under the central-peripheral population model (CPPM). The NEA was genetically homogenous with results suggesting a recent population expansion and connectivity with the western Mediterranean. As such, morphological and life-history differences among NEA populations do not appear to significantly influence patterns of population structure with no evidence of west Africa or Norway forming a distinct stock or taxon or undergoing edge effects. The Mediterranean exhibited pronounced structuring following a west to east pattern with a genetically distinct cluster in the eastern Mediterranean that included North Aegean, Crete and Cyprus sample collections. The strong divergence, low genetic diversity and private haplotypes of this region hint at the isolation and recent bottlenecks of this group. It is clear that historical events and bathymetry have a key role in promoting differentiation in this species but also highlight the importance of investigating peripheral marine populations and the effects of living on the edge.

Keywords: sharks; CR; genetic; peripheral; population structure

2.2 Introduction

Many studies have identified pronounced genetic structuring in a host of marine taxa which has been driven by historical events, biogeographical barriers, behavioural traits and environmental transitions (Patarnello et al. 2007; Jenkins et al. 2018). However, fewer studies have addressed the influence edge effects may have on peripheral population structure and genetic diversity (Ackiss et al. 2018; Hamabata, Kamezaki and Hikida 2014; Johannesson and André 2006; Lind et al. 2007; Nakajima et al. 2010). Under the central-peripheral population model (CPPM) many species are theorised to consist of large, central populations surrounded by numerous, smaller peripheral populations (Mayr 1963; Ecker, Samis and Lougheed 2008). These peripheral populations are more exposed to edge effects such as higher selective pressure and reduced gene flow that may result in reduced population size, low genetic diversity and high genetic divergence compared to their central counterparts (Gould 2002; Nei, Maruyama and Chakaborty 1975); a pattern that has been observed in some marine species (Ackiss et al. 2018; Ecker, Samis and Lougheed 2008; Johannesson and André 2006; Lind et al. 2007). Furthermore, peripheral populations are most likely to persist in less favourable/extreme environmental conditions that could result in strong signatures of selection and potentially host unique genotypes with adaptive characteristics not found in core populations (Macdonald et al. 2017; Volis et al. 2016). However, given that genetic diversity is a prerequisite for adaptive potential (Frankham, Ballou and Briscoe 2002; Gould 2002), the hypothesised isolation of peripheral populations also makes them particularly vulnerable to the effects of climate change and overexploitation.

Many shark species are facing large population declines and risk of extinction due to overexploitation and habitat destruction (Dulvy et al. 2014). Typical life history traits of slow growth, late sexual maturity, long life spans and low fecundity (Compagno 1990) make them particularly vulnerable to anthropogenic pressures and environmental change (Dulvy et al. 2014). In recognition of this, the importance of understanding the genetic diversity and population connectivity of shark populations is ever more critical (Domingues et al. 2018). Fortunately, over recent years there has been significant progress in our knowledge of the population structure of sharks (Dudgeon et al. 2012; Portnoy and Heist 2012). Much of this work has focused on the large pelagic sharks that are frequently considered particularly vulnerable to exploitation in fisheries (Dulvy et al. 2014). Far less focus has been placed on smaller, costal, benthic groups with differing ecologies (Domingues et al. 2018), which are perhaps more typical representatives of shark diversity. Additionally, studies of large pelagic sharks have frequently failed to demonstrate evidence of significant population structure, even over large, global scales (Castro et al. 2007; Ovenden et al. 2011; Pirog et al. 2019; Veríssimo et al. 2017). Smaller species with restricted dispersal ability could have the potential for more highly divided population structure (Chapman et al. 2015; Corrigan et al. 2016).

The small-spotted catshark, *Scyliorhinus canicula* (Linnaeus, 1758), is a small benthic shark inhabiting the coastal waters found within northeast Atlantic (NEA) extending from Norway to Senegal (Springer 1979), and throughout the Mediterranean (Compagno, Dando and Fowler 2005) except for the Black Sea (Bänarescu 1969) and the Red Sea (Gohar and Mazhar 1964). *S. canicula* covers a wide bathymetric range, but is generally most abundant at 110 m (Ebert et al. 2013;

Compagno, Dando and Fowler 2005). Although being listed as Least Concern on the IUCN Red List (Serena et al. 2016) and considered abundant in European waters (Ellis and Shackley 1997), studies have shown localised reductions in abundance and the listing of overexploited in recent stock assessments (Cardinale and Osio 2013). For example, the Adriatic Sea has seen 90% declines in stocks since the 1940s (Barausse et al. 2014) and Wolff (2000a, b) reported localised extirpation from the Wadden Sea at the edge of the species distribution. As such, further information, particularly surrounding unassessed peripheral populations, is needed to ensure no further stock declines and successful management.

Studies of the small-spotted catshark have shown marked phenotypic variation within this species (Bendiab et al. 2012; Capapé et al. 2014). This has often been reported as regional variation in key life-history traits including differences in growth rate, habitat/depth preference and reproductive biology (Kousteni et al. 2010; Finotto et al. 2015; Capapé et al. 2014, 2008; Bendiab et al. 2012; Ivory et al. 2005; Litvinov 2003; Henderson and Casey 2001; Ellis and Shackley 1995). In fact, this becomes so apparent that catsharks in the Cantabrian Sea have been suggested as a separate stock (Rodrıguez-Cabello et al. 2004) and a distinct taxon in west Africa based on unique morphological characters in this region (Litvinov, 2003). As typical of most squaloids this shark also exhibits low dispersal rates (< 300 km; Rodríguez-Cabello et al. 2004) and female philopatric behaviour (Sims et al. 2001), traits often associated with genetic structuring (Chapman et al. 2015; Ramírez-Amaro et al. 2018). Indeed, previous studies have found genetic differentiation in this shark in areas of complex geomorphology and stable habitats. Populations within the Mediterranean show divergence from those in the NEA (Gubili et al. 2014; Kousteni

et al. 2015) and further structuring within the Mediterranean using mtDNA and microsatellite data (Gubili et al. 2014; Kousteni et al. 2015; Ramírez-Amaro et al. 2018), and later in the NEA using single nucleotide polymorphisms (SNPs, Manuzzi et al. 2019). However, these studies have lacked sufficient samples from edge populations despite their potential isolation.

This study aims to expand on previous investigations into the genetic structure of the small-spotted catshark within and between the NEA and Mediterranean basin using the mitochondrial DNA control region (CR). It covers the widest geographical range of *S. canicula* to date, incorporating samples from peripheral areas of the species range in west Africa, Norway, North Aegean and Cyprus into one study. These represent regions close to the distributional limits and could form unique stocks based on distinct life history traits observed and assumptions made under the CPPM (Mayr 1963; Ecker, Samis and Lougheed 2008). The findings may provide vital insight into how edge effects could be influencing genetic variation in small coastal sharks, and whether these edge populations do require specific management efforts.

2.3 Materials and methods

2.3.1 Sample collection and DNA extraction

A total of 332 samples of adult small-spotted catsharks were analysed from 19 sampling sites covering the geographical range of *S. canicula* (Figure 2.1). This includes all 276 mitochondrial DNA (mtDNA) control region (CR) sequences (GenBank accessions: KM873790–KM874065) from Gubili *et al.* (2014). In addition, 56 new samples were collected from regions close to the distributional limits of *S.*

canicula including, the North Aegean from artisanal fisheries in 2017 (n = 20), Cyprus in 2019 during research cruises (n = 22) and west Africa (n = 14), where sampling was undertaken from Moroccan fish markets (Tangier, Casablanca, Essaouria and Agadir) in 2013 (Figure 2.1, Table 2.1). The west African samples (alongside the one Tenerife sample from Gubili et al. (2014), were pooled into one collection for subsequent analysis due to small sample sizes from each market and lack of specificity of capture locations. Fin clips were preserved in 95%-100% ethanol and stored at -20°C. Genomic DNA was extracted from ~25 ng of tissue using the QIAGEN© DNeasy Blood and Tissue kit (Qiagen, Manchester, UK) following the manufacturers protocol with minor modifications (overnight digest with proteinase K at 37 °C followed by an RNase treatment). The concentration and purity of all DNA extractions were quantified by spectrophotometry using a NanoDrop™ One (Thermo Scientific).

2.3.2 Mitochondrial DNA sequencing

Amplification of ~900 base pair (bp) section of then mtDNA CR was performed using the primers ScyD1pF (ATGACATGGCCCACATATCC) and Scan2R (TTCTCTTCTCAAGACCGGGTA) from Gubili et al. (2014). All polymerase chain reactions (PCRs) were performed in 20 µL reaction including 10.5 µL nuclease free H_2O, 4 µl 10x PCR Buffer, 0.4 µl dNTPs (0.8 mM), 0.1 µl Taq (New England BioLabs®, UK), 2 µl of each forward and reverse primers (10 mM stock; EuroFins, Germany) and 1 µL of genomic DNA (approximately 10 mg). Thermal cycling conditions consisted of an initial 3 min step at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 53 °C and 1 min at 72 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 53 °C and 1 min at 72 °C, followed by a final extensions step of 72 °C for 7 mins

on a BioRad T100™ thermocycler. Resulting amplicons were purified and sequenced by Genewiz UK Ltd. (Takeley, UK) using the forward primer. Each electropherogram was manually checked and sequences were trimmed for quality and primer presence using BioEdit v7.2.6 (Hall 1999).

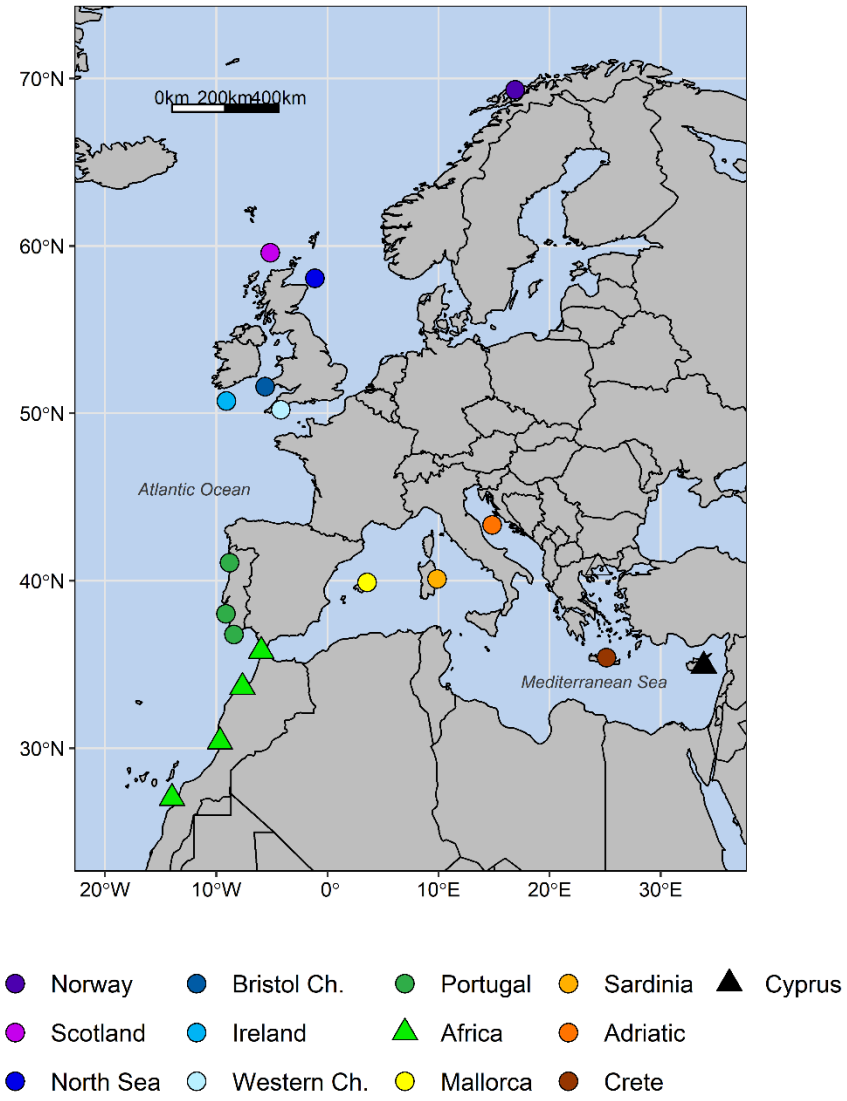


Figure 2.1. Sample sites across the north east Atlantic and Mediterranean. Samples from Gubili *et al.* (2014) are shown as circles, with newly collected samples as triangles. Portugal and Africa both originate from multiple landing locations/fish markets and have been pooled in subsequent figures to facilitate their identification. Map produced using the *rworldmap* and *ggplot2* functions in R v3.6.2 (R Core Development Team 2019).

2.3.3 Summary statistics, genetic differentiation and population structure

Sequences were collapsed into haplotypes and number of haplotypes identified using FaBox v1.5 (Villesen 2007). Nucleotide (π) and haplotype (h) diversities, alongside number of polymorphic sites (S) were calculated using ARLEQUIN v3.5.2.2 (Excoffier et al. 2005). Rarefield haplotype richness corrected for sample size (n = 22) was calculated using CONTRIB 1.02 (Petit et al. 1998). To determine effective population size (N_e) over time Bayesian skyline plots (BSPs) were generated in the software package BEAST v. 2.6.6 (Bouckaert et al. 2014) and plotted using the upper 95% highest posterior density. Generally the programme defaults were used, except the *HKY+I* mutation model was selected. Each populations was run twice for 50 to 200 million Markov chain Monte Carlo (MCMC) chains depending on length required for convergence, and sampled every 1000 steps. A fixed clock was set using a divergence rate of 0.361% as reported in Gubili et al. 2014 and imputed as 3.61×10^{-9} to get substitutions per site per year. The BSP reconstructions were conducted in R using the function *plotBSP* (Bouckaert et al. 2014).

Analyses of genetic differentiation between sampling sites were estimated by calculating pairwise values in ARLEQUIN v3.5.2.2, using both the conventional genetic distance among alleles (Φ_{st}) and the haplotype frequency-based mtF_{st}. ARLEQUIN v.3.5.2.2 was further used to test for significance between groups of sample collections. Data was grouped on the basis of geographical location, with the following hierarchy: North NEA (Norway, North Sea, Scotland, Ireland, Western Channel and Bristol Channel), South NEA (Portugal, Africa), western Mediterranean (Mallorca, Sardinia and Adriatic) and eastern Mediterranean (North Aegean, Crete and Cyprus). Significance for all tests performed in ARLEQUIN v3.5.2.2 was

determined by 10,000 permutations and significance values were adjusted to account for type I errors using the False Discovery Rate (FDR) method with the function *p.adjust* in R v3.6.2 (R Core Development Team 2019). A heat map of each measure was visualised in R v3.6.2 using the *ggplot2* function. In addition, to explore patterns of isolation by distance (IBD), Mantel tests were conducted on genetic distances (mtF_{st}) and geographical distances (km) using *the mantel.rtest* function from the R package ade4 v1.7.11 (Dray & Dufour, 2007) with significance assessed using 1,000 permutations. The geographical distance matrices were created by calculating least-cost distances via seas (avoiding landmasses) between sampling sites using the *lc.dist* function from the R package marmap v1.0 (Pante & Simon-Bouhet, 2013).

Genetic structuring was visualised between all sample sites and within individual basins (NEA and Mediterranean) using Principal Coordinates Analysis (PCoA) plots constructed using *adegenet* (Jombart 2008) and *ape* (Paradis et al. 2004) packages implemented in R v3.6.2 (R Development Core Team, 2019). Cavalli-Sforza and Edwards Chord (Cavalli-Sforza and Edward 1967) distance was used since Takezaki and Nei (1996) found it to generally show higher principal component (PC) values to best retrieve the relations among samples.

2.3.4 Haplotype network and demographic analysis

To reconstruct genealogical relationships between haplotypes, a median joining network (Bandelt et al. 1999) was constructed in POPART v1.7 (Leigh and Bryant 2015) with epsilon set to 10. To infer demographic history of populations Tajima's *D* (Tajima 1989) and Fu's F_S (Fu 1997) neutrality tests were calculated in Arlequin with

significance values determined by generating 1,000 random samples. Negative values of *D* and F_S are generally indicative of recent population expansion.

2.4 Results

2.4.1 Summary statistics

A final alignment of 412 bp mtDNA CR gene fragments from 332 individuals was obtained. A total of 20 polymorphic nucleotides and 28 haplotypes were identified (Table 2.1). Global haplotype and nucleotide diversity were 0.829 ± 0.013 and 0.004 ± 0.003, respectively. Haplotype diversity within populations ranged from 0.589 (Crete) to 0.815 (Western Channel), whilst haplotype richness ranged from 2.417 (Crete) to 4.646 (Portugal). Nucleotide diversity was low across all localities, ranging from 0.002 (Norway, Scotland, Bristol Channel, Cyprus) to 0.004 (Western Channel, Portugal, Africa, Sardinia; Table 2.1). Demographic analysis showed patterns of recent population expansion with negative values of both Tajima's *D* and Fu's F_S (Table 2.1), although only *F* values relating to Portugal and southern Ireland were significant. Samples from Sardinia and Crete had positive *D* and *F* values indicating demographic stability. This is evident in the BSP analysis (Figure S2.1) where the N_e remains relatively stable for both of these localities. For all other populations, the BSP analysis indicates a slow population increase, with the majority of samples from the NEA generally demonstrating a more sustained period of much greater population increase, particularly evident in Portugal. In most populations there is evidence of increased population growth in the past 100 000 – 500 000 years, supporting the negative Tajima's *D* and Fu's F_S, with the exception of Sardinia which shows a recent decline in N_e.

Table 2.1. Summary statistics. n, number of individuals; H_n, number of haplotypes; H_r, haplotype richness; *h*, haplotype diversity; π, nucleotide diversity; s.d., standard deviation is in brackets; *D*, Tajima's D value; *F*, Fu's F_S value; *S*, number of polymorphic sites. P <0.005, **p <0.001.

Sample site	code	*n*	H_n	H_r	*h* (±s.d.)	π (±s.d.)	*D*	*F*	*S*
Norway	NOR	4	2	-	0.667 (±0.204)	0.002 (±0.002)	1.633	0.54	1
Scotland	SCO	23	5	3.194	0.676 (±0.062)	0.002 (±0.002)	-0.897	-1.096	5
North Sea	NSE	25	6	3.900	0.703 (±0.071)	0.003 (±0.002)	-0.920	-1.619	6
South Ireland	IRE	18	6	3.036	0.680 (±0.109)	0.003 (±0.002)	-0.917	-2.350*	4
Bristol Channel	BRI	27	5	4.479	0.678 (±0.054)	0.002 (±0.002)	-0.760	-0.893	5
Western Channel	WES	24	7	4.821	0.815 (±0.045)	0.004 (±0.003)	-0.310	-1.37	7
Portugal	POR	30	10	5.646	0.805 (±0.050)	0.004 (±0.003)	-1.157	-4.237**	10
Africa	AFR	15	6	5.000	0.705 (±0.114)	0.004 (±0.001)	-1.737	-1.635	9
Mallorca	MAL	40	8	3.963	0.659 (±0.070)	0.003 (±0.002)	-0.528	-2.235	7
Sardinia	SAR	22	5	3.585	0.732 (±0.068)	0.004 (±0.003)	1.247	0.2	4
Adriatic	ADR	27	5	3.101	0.715 (±0.047)	0.003 (±0.002)	-0.563	-0.653	5
North Aegean	GRE	20	5	3.010	0.653 (±0.076)	0.003 (±0.002)	-0.272	-0.477	5
Crete	CRE	29	4	2.417	0.589 (±0.075)	0.003 (±0.002)	0.780	0.408	3
Cyprus	CYP	28	4	2.334	0.632 (±0.066)	0.003 (±0.002)	0.367	-0.738	4
All	14	332	28		0.829 (±0.016)	0.004 (±0.003)	-1.185	-16.515***	19

2.4.2 Population differentiation

Both pairwise differentiation measures showed comparable patterns between sampling sites (Figure 2.2). Pairwise values of mtF_{st} and Φ_{st} ranged from zero (e.g. Norway-Scotland) to 0.390 (Norway-Crete) and from zero (e.g. Africa-Ireland) to 0.597 (Bristol-Crete), respectively. Following similar patterns shown in Gubili et al. (2014), the highest levels of significant differentiation were evident between the NEA and the Mediterranean Sea. Mallorca was an exception in that Φ_{st} pairwise values were low

and showed no significant results when compare to all NEA populations apart from the Western Channel (0.0790), however, more significance difference was seen when comparing mtF_{st} values (Figure 2.2). Within the Mediterranean, the western and central populations (Mallorca, Sardinia, Adriatic) showed significant differentiation, even between geographically neighbouring sites. However, no evidence of genetic differentiation was found between sampling locations in the eastern Mediterranean basin (North Aegean, Crete, Cyprus). Within the NEA, pairwise values of genetic differentiation were low suggesting homogeneity across this region and no evidence of isolated populations towards the northern or southern limits.

AMOVA revealed significant genetic structuring in the hypothesised grouping scenario, with significant amount of variation seen between the four defined groups (31.12%; Table 2.2), while within-groups variation was low (4.5%; Table 2.2). F_{ST} values due primarily to differences among groups (FCT), rather than within-groups (FSC).

Table 1.2. Hierarchical AMOVA showing levels of genetic structuring among groups, among sample collection within groups and within sample collections.

Source of variation	Total variation	% of variation	F_{ct}	F_{sc}	F_{st}
Among groups	0.299	31.12	0.311 $p = 0.001$		
Among sample collections in groups	0.043	4.50		0.065 $p < 0.000$	
Within sample collections	0.620	64.38			0.356 $p < 0.000$

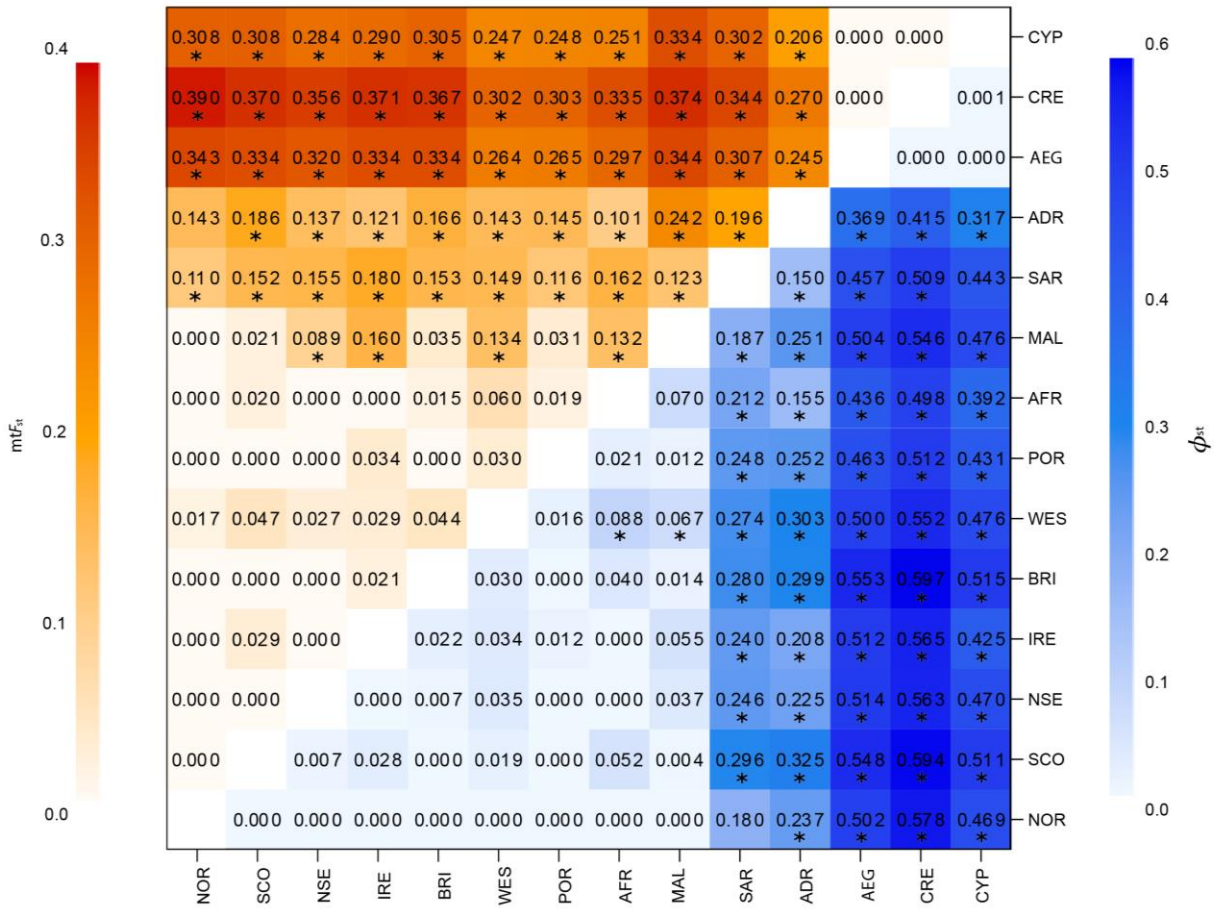


Figure 2.2. Pairwise differentiation among 14 sampling localities. Below diagonal, pairwise Φ_{st}. Above diagonal pairwise mtF_{st} values. Values in bold were significant at 95 % CI, and those marked with asterisks (*) remained significant after adjustments for type I error using the False Discovery Rate (FDR) method. See Table 2.1 for location codes.

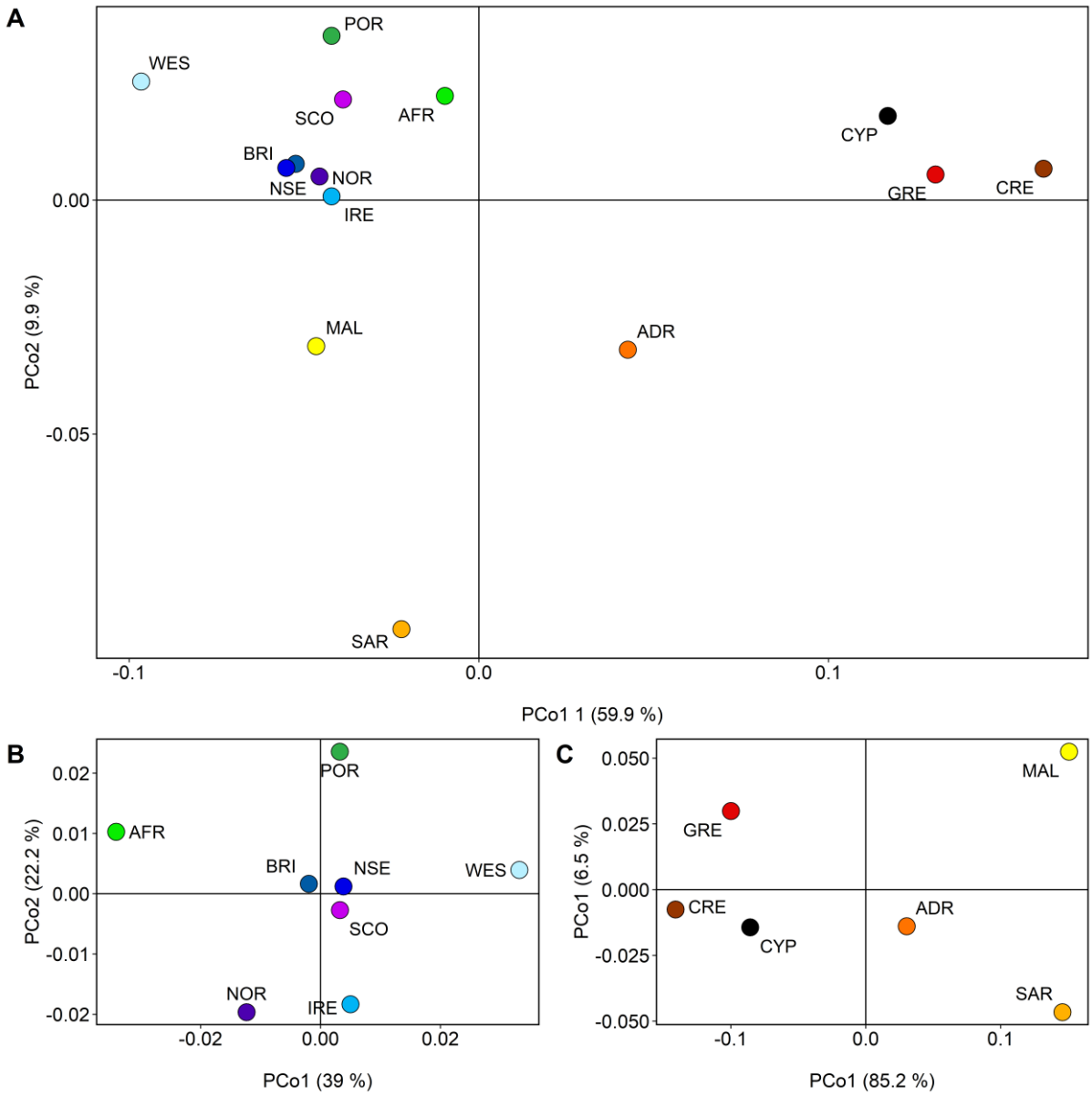


Figure 2.3. Principal component analysis (PCoA) of genetic distance between sample collections: (a) all localities within both the northeast Atlantic (NEA) and Mediterranean; (b) only NEA localities; (c) only Mediterranean localities. The first principal component (PCo1) is plotted along the x-axis and the second component (PCo2) on the y-axis. The percentage of genetic variation explained by each PC is also included. See Table 2.1 for location codes.

2.4.3 Population structure and genetic clustering

In total, both axes of the global PCoA plot explained 69.8% of variation in the dataset and showed that catsharks originating from the NEA and the Mediterranean were genetically distinct (Figure 2.3a). Mallorca however, clustered more closely with NEA sites than other Mediterranean sites. New sample collections from peripheral populations in North Aegean and Cyprus show clear clustering with the Crete collection to form a distinct eastern Mediterranean group, also supported by other pairwise differentiation values (Figure 2.2, 2.3c). There was also clear separation of western and central Mediterranean catsharks (Mallorca, Sardinia and Adriatic) from each other and the rest of the sample sites (Figure 2.3a, c) with some potential evidence of an east to west pattern of differentiation (Figure 2.3c). However, despite a clear linear association between genetic and geographical distances within the Mediterranean (Figure 2.1c) IBD was not found to be significant. The NEA sites however, showed less separation and tended to cluster much more closely together, including the peripheral populations in Norway and Africa (Figure 2.3a). When comparing only NEA sample sites perhaps there are indications of some weak geographic structuring. Central samples sites surrounding the United Kingdom (Scotland, North Sea and Bristol Channel) form a small cluster with all other, more remote sites, seemingly isolated (Figure 2.3b).

2.4.4 Haplotype network

In total, 28 haplotypes were found across all sample collections (Figure 2.4), of which, 14 were private and only found in one location. There were two common, central haplotypes which dominate across the NEA but which are also present at a much

lower frequency in the Mediterranean (except the North Aegean and Crete sample collections that completely lack hap 1 and 2, Figure 2.4). Within the NEA there was no discernible geographic pattern in haplotype distribution. The majority of African catsharks shared haplotypes with the rest of NEA with only one unique haplotype identified (hap 19). Within the Mediterranean, three dominant haplotypes (hap 18 25, and 26) were found in the eastern Mediterranean sample collections (North Aegean, Crete and Cyprus) with two haplotypes exclusive to that region (hap 25 and 26).

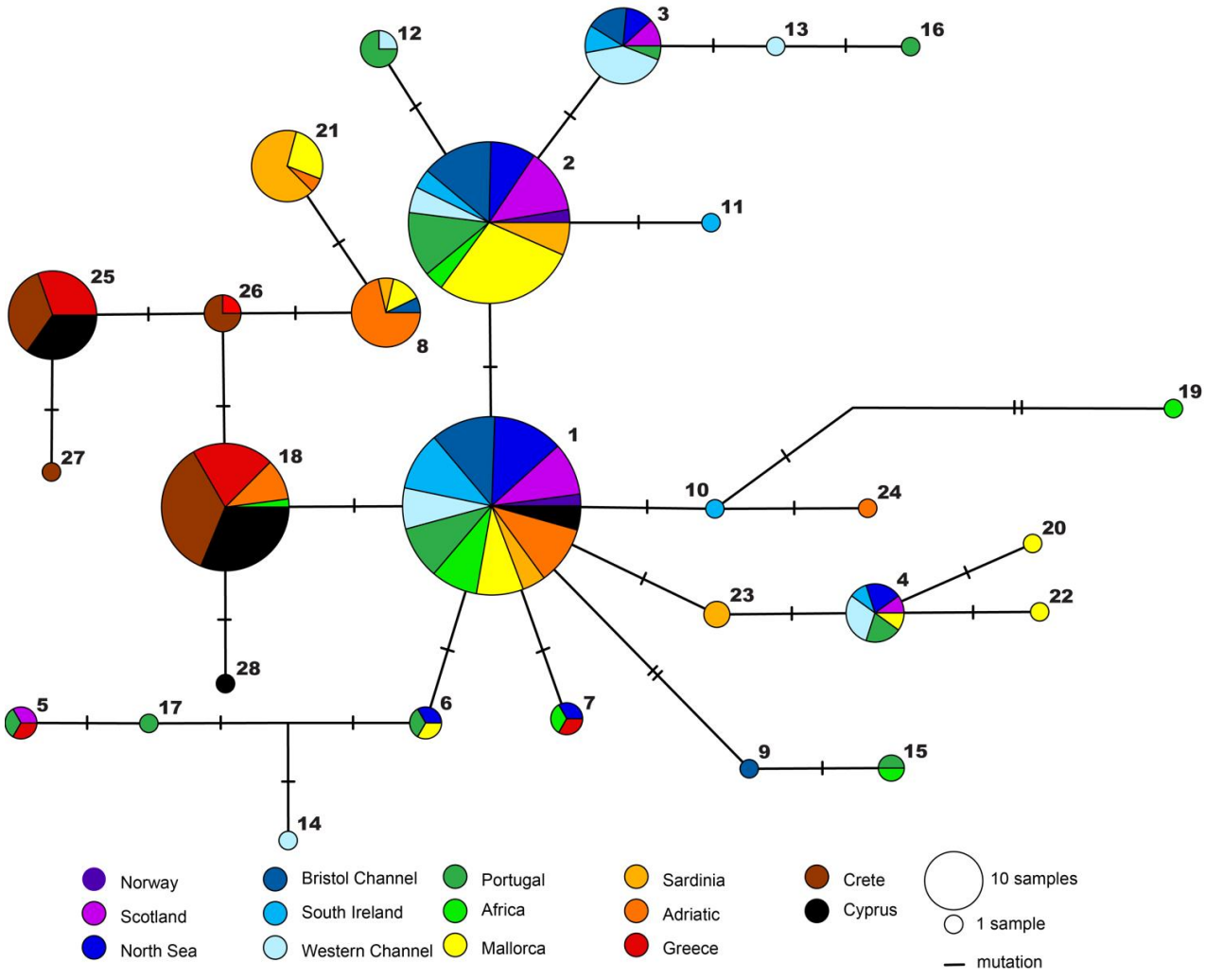


Figure 2.4. Haplotype network of CR sequences identified throughout *S. canicula* distributional range. Each node represents a unique haplotype (numbered 1-28) with the colours indicating the locality where it was found, size of the circle proportionate to the frequency and dashes indicative of inferred mutational steps.

2.5 Discussion

This study represents efforts to gather samples from peripheral regions in the geographical range of the small spotted catshark to examine patterns of genetic variation in these crucial edge populations. Moreover, this is the first genetic analysis of west African catsharks that have previously been highlighted as having unique morphological characteristics compared to the rest of the NEA and Mediterranean (Litvinov, 2003), to investigate their potential genetic distinctiveness. The results are consistent with patterns observed in previous range-wide studies of *S. canicula* using both microsatellites and mtDNA, showing a pronounced phylogeographic separation between the NEA and Mediterranean basins (Barbieri *et al.* 2014; Gubili *et al.* 2014). Similarly, findings are consistent with the mtDNA genetic differentiation found between western, central and eastern Mediterranean populations (Gubili *et al.* 2014; Kousteni *et al.* 2015), with the North Aegean and Cypriot individuals providing greater resolution into the eastern Mediterranean structure. Furthermore, the results are harmonious with patterns seen within the NEA, showing basin-wide gene flow and a homogenous population that includes individuals from west Africa and Norway (Barbieri et al. 2014; Gubili et al. 2014).

2.5.1 Between region/basin population structure

Across all localities, both the pairwise values (Figure 2.2) and the PCoA (Figure 2.3a) show strong support of significant differentiation between all NEA and Mediterranean localities, excluding Mallorca, indicating that traits associated with limited dispersal potential (Compagno, Dando and Fowler 2005; Rodríguez-Cabello et al. 2004; Sims et al. 2001) likely have an important role in limiting gene flow in this species. This is

supported by the significant association of genetic and geographical distances providing evidence of IBD across the catsharks range (Figure S2.2a). This pattern is likely exaggerated by the Atlantic-Mediterranean front which marks a major genetic boundary between populations for numerous marine taxa (Patarnello et al. 2007), including other species of elasmobranch (Leone et al. 2017; Gubili et al. 2016; Catarino et al. 2015; Griffiths et al. 2011; Chevolot et al. 2006), fish (Fruciano et al. 2011; Maggio et al. 2019) and invertebrate (Palero et al. 2008; Roman and Palumbi 2004). These patterns are often attributed to present day isolation, biological characteristics and past biogeographic events (Patarnello et al. 2007).

The point at which this transition occurs for the small-spotted catshark remains controversial and has been largely associated with restricted gene flow at the Strait of Gibraltar or the Almeria-Oran Front in other species (Galarza et al. 2009; Patarnello et al. 2007). However, the close similarity and number of shared haplotypes between Mallorca and the NEA group in this study (Figure 2.2; Figure 2.4) implies a region of connectivity further into the western Mediterranean basin (although a lack of samples in this region does limit the interpretation of the data). In agreement with Gubili et al. (2014) and Kousteni et al. (2014), it is likely that the Balearic Islands are acting as a point of secondary contact and the intrusion of Atlantic migrants following late Pleistocene recolonization and population growth; a scenario proposed by Patarnello et al. (2007). This would correspond with the increased N_e observed during this time in the some of the BSP analysis (Figure S2.1). It is possible that recolonization might have been from putative southern refugia during the late Pleistocene, towards the Last Glacial Maximum (LGM), that have been proposed in south-west Ireland (Hoarau et al. 2007) and the Iberian Peninsula (Maggs et al. 2008) as these locations showed

evidence of significant population expansion, with Portugal yielding among the highest level of genetic diversity (Table 2.1). If this is true, movement could be attributed to the species shallower depth range across the Alboran sub-basin (42–637 m; Ramírez-Amaro et al. 2015), increasing its dispersal ability, and why similar patterns were not observed for deeper water sharks (Catarino et al. 2015; Gubili et al. 2016). This would oppose the Atlantic-transition zone (the Portugal and Alboran sub-basins) hypothesised by Ramírez-Amaro et al. (2018) following a detailed study of the western Mediterranean. Their results clearly show differentiation across the area, however, limited sampling in the NEA and low F_{st} values between Balearic Islands and Spain reported in that study make it difficult to conclude the significance of this zone. Further clarification with more markers (i.e. nuclear) and sampling along the NEA coast is needed.

2.5.2 Mediterranean population structure

The clear separation of sites from the Balearic Sea (Mallorca), Tyrrhenian Sea (Sardinia), Adriatic and those in the eastern Mediterranean (North Aegean, Crete and Cyprus) support similar findings in previous studies on the small-spotted catshark (Gubili et al. 2014; Kousteni et al. 2015). This is a sea that appears to promote genetic structuring with complex patterns observed in other marine species, including invertebrates (Palero et al. 2008), fish (Fruciano et al. 2011; Maggie 2019) and other elasmobranch species (Raja *miraletus*, Ferreri et al. 2018; *Prionace glauca,* Leone et al. 2017), and frequently attributed to the unique geomorphology and/or paleogeographic history the Mediterranean Sea (Patarnell et al. 2007). Furthermore, genetic divergence also seems to correspond with the varying life-history traits of elasmobranch species inhabiting the Mediterranean Sea. For example, species with

greater dispersal capabilities instead show a lack of genetic structuring in the Mediterranean and a region of panmixia (Chevolot et al, 2006; Ferrari et al. 2018; Kousteni et al. 2016; Ramírez-Amaro et al. 2018; Gubili et al. 2016), including the closely related blackmouth catshark, *Galeus melastomus* (Ferrari et al. 2018; Ramírez-Amaro et al. 2018). Ramírez-Amaro et al. (2018) notes this difference to be a result of contrasting life-history traits, with *G. melastomus* deeper depth range (Ramírez-Amaro et al. 2015) and lack of philopatry that could promote gene flow, as seen in other deep-water sharks (Kousteni et al. 2016; Veríssimo, McDowell and Graves, 2011; Gubili et al. 2016). As such, a reasonable conclusion is that the complex geomorphology of the Mediterranean basin, alongside the limited dispersal capacity of the catshark (Compagno, Dando and Fowler 2005; Rodríguez-Cabello et al. 2004; Sims et al. 2001) could be significantly restricting gene flow, even on a small spatial scale in the Mediterranean Sea.

The greatest level of genetic differentiation was that of the eastern Mediterranean sites from the rest of the Mediterranean, with the North Aegean samples providing greater certainty to the isolation of this region than previously observed (Gubili *et al*. 2014; Kousteni *et al.* 2015). This conflicts findings by Barbieri et al. (2013) and Ferrari et al. (2018) where no genetic difference between the eastern and western Mediterranean was found. Kousteni et al. (2015) did note the weak differentiation of the Aegean population when looking at microsatellites only, however, this is likely to be reflective of the microsatellites suite used in that study and the differential patterns of inheritance between microsatellites and mtDNA. Nevertheless, the genetic isolation of this region is clear and could be a consequence of the Hellenic Trench (> 4000 m) preventing dispersal particularly across the Ionian Sea (Kousteni et al. 2015).

2.5.3 North-east Atlantic genetic structure

The genetic homogeneity found amongst all NEA small-spotted catsharks and a distinct lack of any evidence of IBD (Figure S2.2b) implies a region of high gene flow between all sampled sites (Figure 2.2). This is consistent with previous studies of *S. canicula* within the NEA using neutral markers (Barbieri et al. 2013; Gubili et al. 2014), those in other species of elasmobranch (Gubili et al. 2016; Veríssimo, McDowell and Graves 2011) and many other benthic fish (Correia et al. 2012; Cuveliers et al. 2012). Rapid recolonization of the NEA after the last glaciation and lack of gepgraphical barriers to gene flow when compared to the Mediterranean could explain the genetic similarity found in the region. Nevertheless, the NEA does show clear evidence of latitudinal morphological differences (Ivory et al. 2005; Rodrıguez-Cabello et al. 2004; Litvinov 2003; Henderson and Casey 2001; Ellis and Shackley 1995). Given that the Mediterranean structure appears to coincide with the morphological variation observed between regions, it is surprising not to find any genetic divergence within the NEA. Perhaps most unexpected was the genetic similarity of African catsharks despite the highly unique dental form of African catsharks (Litvinov 2003), thought to reflect differing patterns of trophic and sexual selection - factors known to drive adaptation and genetic differentiation. It may be that such morphological variations are environmentally driven. It was not until Manuzzi et al. (2018) utilised genomic SNP data, that a genetic separation between southern Iberia and the British Isles was revealed, and the genetic divergence of southern Portugal. It was suggested this divergence may be a consequence of the historical isolations of northern and southern populations during the last glaciation (Maggs et al. 2008).

2.5.4 Consideration of edge effects

Peripheral populations in the NEA (Norway, Africa) do not appear to conform to the predictions of the CCPM (Mayr 1963). Homogenising gene flow and population expansion within the NEA appear to be buffering any potential edge effects, with no evidence of divergence, genetic drift or decreased genetic diversity (Figure 2.2; 2.3; Table 2.1). However, it is difficult to make a clear judgement on Norwegian catsharks given the small sample size (Table 2.1). Unexpectedly, catsharks from west African waters showed some of the higher levels of haplotype richness (5.000) and haplotype diversity (0.705) more similar to those of more central NEA populations (Portugal, Western Channel, Bristol Channel; Table 2.1). The high haplotype diversity in Portugal and west Africa are consistent with the existence of a refugia along the Iberian Peninsula as previously identified for the thornback ray (Chevolot et al. 2006). It may also be that edge effects are more dominant much closer to Senegal, at the far limit of *S. canicula*s range and/or gene flow from the Mediterranean through the Strait of Gibraltar is further maintaining diversity. North Aegean, Crete and Cyprus had the lowest levels of haplotype richness and haplotype diversity across all populations (Table 2.1). This coupled with the low nucleotide diversity (Table 2.1) and genetic similarity of populations within the eastern Mediterranean (Figure 2.2; Figure 2.3c) may hint at a possible population bottleneck and/or selection to suboptimal conditions of the eastern Mediterranean removing variation; although this was not tested. Nevertheless, temperature is known to be a key driver of population change at the eastern edge with a rising trend linked to abundance declines in species of invertebrate (Rilov 2016) and fish (Given 2018). These patterns indicate populations in the eastern Mediterranean are at the edge of optimal habitat conditions, as predicted under CCPM (Mayr 1963).

2.5.5 Limitations and future work

In order to reliably define patterns of connectivity between the NEA and western Mediterranean further sampling at a finer geographic scale along the north-western African coastline, the Atlantic Portuguese and Spanish shores, the Alboran Sea and the Strait of Gibraltar is needed. Previous work also highlights differences between mtDNA and nuclear DNA markers that need further investigation. Next generation sequencing techniques such as Restriction Site Associated DNA (RAD) and Whole Genome Sequencing (WGS) may improve the resolution of this transition. They hold further promise in providing insight into identifying population structure within the NEA and the potential identification of local adaptation in the catshark. Additional samples from Norway could not be sourced for inclusion in this study, and the localities of capture for each African sample could not be obtained from a market survey. To better understand the connectivity of this region more detailed sampling will be needed and should include catsharks closer to the distributional limit to Senegal. The eastern Mediterranean peripheral populations did show signs of reduced genetic diversity most likely due to their isolation and potential selection pressures to the extreme environmental conditions. Future research should aim to understand which environmental factors (e.g. temperature, salinity, depth) are likely influencing selection and adaptation in small-spotted catsharks, providing further insight into patterns of genetic diversity and the evolution of sharks.

2.5.6 Management and wider implications

The results of this study indicate that the small-spotted catshark has the potential to form multiple genetic stocks within its distributional range which has important

implications for future management of the species. The delineating of conservation units should closely follow the overarching structure observed in this study and others (Gubili et al. 2014; Kousteni et al. 2015; Ramírez-Amaro et al. 2018), particularly surrounding the Mediterranean where multiple, differentiated stocks were observed. If not properly addressed, overexploitation could lead to the collapse of localised stocks as seen in the Adriatic (Barausse et al. 2014) and Wadden Sea (Wolff 2000a, b). The vulnerability of *S. canicula* to the effects of overexploitation (Dulvy et al. 2014) could also be exaggerated at the range edge. The reduced genetic diversity in peripheral populations of the eastern Mediterranean make this region of particularly concern. These populations could also be at most risk of climate change which has already begun to shift sea temperature in the Mediterranean (Polyakov et al. 2010; Rohling et al. 2014; Shaltout and Omsted 2014).

2.6 References

Ackiss AS, Bird CE, Akita Y, Santos MD, Tachihara K, Carpenter KE (2018) Genetic patterns in peripheral marine populations of the fusilier fish Caesio cuning within the Kuroshio Current. Ecol Evol 8:11875–11886. https://doi.org/10.1002/ece3.4644

Bänarescu P (1969) Cyclostomata si Chondrichthyes. Fauna Repub Soc România 12:106

Bandelt H, Forster P, Röhl A (1999). Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16(1):37–48

Barausse A, Correale V, Curkovic A, Finotto L, Riginella E, Visentin E, Mazzoldi C (2014) The role of fisheries and the environment in driving the decline of elasmobranchs in the northern Adriatic Sea. ICES J Mar Sci 71:1593–1603. https://doi:10.1093/icesjms/fst222

Bendiab AAT, Mouffok S, Boutiba Z (2012) Reproductive biology and growth of Lesser Spotted Dogfish *Scyliorhinus canicula* (Linnaeus, 1758) in Western Algerian coasts (Chondrichthyes, Scyliorhinidae). Biodivers J 3(1): 41-48

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ (2014) BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLoS Comput. Biol 10(4), e1003537. doi:10.1371/journal.pcbi.1003537

Capapé C, Mnasri-Sioudi N, Kamel-Moutalibi OE, Boumaïza M, Amor, MMB, Reynaud C (2014) Production, maturity, reproductive cycle and fecundity of small-spotted catshark, *Scyliorhinus canicula* (Chondrichthyes: Scyliorhinidae) from the northern coast of Tunisia (Central Mediterranean). J Ichthyol 54:111–126. https://doi.org/10.1134/S0032945214010020

Capapé C, Reynaud C, Vergne Y, Quignard JP (2008) Biological observations on the smallspotted catshark *Scyliorhinus canicula* (Chondrichthyes: Scyliorhinidae) off the Languedocian coast (southern France, northern Mediterranean). Pan-Am. J Aquat Sci 3:282–289.

Castro, ALF, Stewart, BS, Wilson, SG, Hueter, RE, Meekan, MG, Motta, PJ, Bowen BW, Karl, SA (2007) Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). Mol Ecol 16: 5183-5192. https://doi.org/10.1111/j.1365-294X.2007.03597.x

Catarino D, Knutsen H Veríssimo AG et al (2015) The Pillars of Hercules as a bathymetric barrier to gene flow promoting isolation in a global deep-sea shark (*Centroscymnus coelolepis*). Mol Ecol 24(24):6061–6079. https://doi.org/10.1111/mec.13453

Cavalli-Sforza, LL and Edwards, AWF (1976) Phylogenetic analysis: Models and estimation procedures. Am J Hum Genet 19: 233–257.

Chapman DD, Feldheim KA, Papastamatiou YP, Hueter RE (2015) There and Back Again: A Review of Residency and Return Migrations in Sharks, with Implications for Population Structure and Management. *Ann Rev Mar. Sci*. 7(1):547–570. 10.1146/annurev-marine-010814-015730

Chevolot M, Hoarau G, Rijnsdorp AD, Stam WT, Olsen, JL (2006) Phylogeography and population structure of thornback rays (Raja clavata L., Rajidae). Mol Ecol 15(12): 3693–3705. https://doi.org/10.1111/j.1365-294X.2006.03043.x

Compagno LJV (1990) Alternative life-history styles of cartilaginous fishes in time and space. Environ Biol Fishes 28:33–75

Compagno LJV, Dando M, Fowler S (2005) Sharks of the world. HarperCollins, London

Corrales X, Coll M, Ofir E et al (2018) Future scenarios of marine resources and ecosystem conditions in the Eastern Mediterranean under the impacts of fishing, alien species and sea warming. Sci Rep 8:14284. https://doi.org/10.1038/s41598-018-32666-x

Correia AT, Ramos AA, Barros F, Silva G, Hamer P, Morais P, Cunha RL, Castilho R (2012) Population structure and connectivity of the European conger eel (Conger conger) across the north-eastern Atlantic and western Mediterranean: integrating molecular and otolith elemental approaches. Mar Biol 159:1509–1525. https://doi.org/10.1007/s00227-012-1936-3

Corrigan, S., Huveneers, C., Stow, A., Beheregaray, B. (2016). A multilocus comparative study of dispersal in three codistributed demersal sharks from eastern Australia. Can J Fish Aquat Sci 73:1–10.

Cuveliers EL, Larmuseau MHD, Hellemans B, Verherstraeten SLNA, Volckaert FAM, Maes, GE (2012) Multi-marker estimate of genetic connectivity of sole (Solea solea) in the North-East Atlantic Ocean. Mar Biol 159:1239–1253. https://doi.org/10.1007/s00227-012-1905-x

Domingues RR, Hilsdorf, AWS, Gadig OBF (2018) The importance of considering genetic diversity in shark and ray conservation policies. Conserv Genet 19(3):501–525. https://doi.org/10.1007/s10592-017-1038-3

Dudgeon CL, Blower DC, Broderick D, Giles JL, Holmes BJ, Kashiwagi T, Krück NC, Morgan JA, Tillett BJ, Ovenden JR (2012) A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. J Fish Biol 80:1789–1843

Dulvy NK, Fowler SL, Musick JA, Cavanagh RD et al. (2014) Extinction risk and conservation of the world's sharks and rays. eLife 3:e00590

Ebert D, Fowler S, Compagno L (2013) Sharks of the world: a fully illustrated guide. Wild Nature Press, Plymouth, UK

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: The central–marginal hypothesis and beyond. Mol Ecol 17:1170–1188. https://doi. org/10.1111/j.1365-294X.2007.03659.x

Ellis J, Mancusi C, Serena F, Haka F, Guallart J, Ungaro N, Coelho R, Schembri T, MacKenzie K (2009) *Scyliorhinus canicula*. The IUCN Red List of Threatened Species. https://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161399A5415204.en. Accessed 09 August 2020

Ellis JR, Shackley SE (1997) The reproductive biology of *Scyliorhinus canicula* in the Bristol Channel, UK. J Fish Biol 51:361–372. https://dx.doi.org/10.1111/j.10958649.1997.tb01672.x

Excoffier L, Laval G. Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge

Fruciano C, Hanel R, Debes PV, Tigano C, Ferrito, V (2011) Atlantic-Mediterranean and within-Mediterranean molecular variation in Coris julis (L. 1758) (Teleostei, Labridae). Mar Biol 158(6):1271–1286. https://doi.org/10.1007/s00227-011-1647-1

Finotto L, Correale V, Riginella E, Mazzoldi C (2015) Contrasting life history and reproductive traits in two populations of *Scyliorhinus canicula*. Mar Biol 162:1175–1186. 10.1007/s00227-015-2659-z.

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.

Galarza JA, Carreras-Carbonell J, Macpherson E, Pascual M, Roques S, Turner GF, Rico C (2009) The influence of oceanographic fronts and early-life-history traits on connectivity among littoral fish species. Proc Natl Acad Sci USA. https://doi.org/106: 1473–1478

Given O, Edelist D, Sonin O, Belmaker, J (2018) Thermal affinity as the dominant factor changing Mediterranean fish abundance. Glob Change Biol. 24:80–89. https://doi.org/10.1111/gcb.13835

Gohar, HAF and Mazhar FM (1964) Elasmobranchs of the northwestern Red Sea. Publ Mar Biol Stn Al-Ghardaqa 13:1-144. https://doi.org/10.1111/j.1095-8649.2011.03178.x

Gould SJ (2002) The structure of evolutionary theory. Belknap Press of Harvard University Press, Cambridge, MA

Griffiths AM, Sims DW, Cotterell SP, El Nagar A, Ellis JR, Lynghammar A, McHugh M, Neat FC, Pade NG, Queiroz N, Serra-Pereira B, Rapp T, Wearmouth VJ, Genner

MJ (2010) Molecular markers reveal spatially segregated cryptic species in a critically endangered fish, the common skate (Dipturus batis). Proc Royal Soc B 277:1497–1503. https://doi.org/ https://doi.org/10.1098/rspb.2009.2111

Griffiths AM, Sims DW, Johnson A, Lynghammar A, McHugh M, Bakken T, and Genner MJ (2011) Levels of connectivity between longnose skate (Dipturus oxyrinchus) in the Mediterranean Sea and the north-eastern Atlantic Ocean. Conser Genet 12(2):577–582. https://doi.org/10.1007/s10592-010-0127-3

Gubili C, Macleod K, Perry W, Hanel P, Batzakas I, Farrell ED, Griffiths AM (2016) Connectivity in the deep: Phylogeography of the velvet belly lanternshark. Deep-Sea Res Part I: Oceanogr Res 115:233–239. https://doi.org/10.1016/j.dsr.2016.07.002

Gubili C, Sims DW, Veríssimo A, Domenici P, Ellis J, Grigoriou P, Johnson AF., McHugh M, Neat F, Satta A, Scarcella G, Serra-Pereira B, Soldo A, Genner MJ and Griffiths AM (2014) A tale of two seas: contrasting patterns of population structure in the small-spotted catshark across Europe. R Soc open sci 1140175. https://doi.org/10.1098/rsos.140175

Hale SS, Buffum HW, Kiddon JA, Hughes MM (2017) Subtidal benthic invertebrates shifting northward along the US Atlantic Coast. Estuaries Coasts 1:13. https://doi.org/10.1007/s12237-017-0236-z

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95-98. https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29

Hamabata T, Kamezaki N and Hikida T (2014) Genetic structure of green turtle (Chelonia mydas) peripheral populations nesting in the northwestern Pacific rookeries: evidence for northern refugia and postglacial colonization. Mar Biol 161:495–507. https://doi.org/10.1007/s00227-013-2352-z

Harley CDG, Randall Hughes A, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF, Tomanek L, Williams SL (2006) The impacts of climate change in coastal marine systems. Ecol Lett 9:228-241. https://doi.org/10.1111/j.1461-0248.2005.00871.x

Hauser L, Adcock GJ, Smith PJ, Ramírez JHB, Carvalho GR (2002). Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (Pagrus auratus). PNAS USA 99(18):11742–11747. https://doi.org/10.1073/pnas.172242899

Henderson AC, Casey A (2001) Reproduction and growth in the lesser-spotted dogfish *Scyliorhinus canicula* (Elasmobranchii; Scyliorhinidae), from the west coast of Ireland. Cah Biol Mar 42:397–405. https://doi.org/0.2960/J.v35.m504

Hoarau G, Coyer JA, Veldsink JH, Stam WT, Olsen JL (2007). Glacial refugia and recolonization pathways in the brown seaweed Fucus serratus. Mol Eco 16:3606–3616. https://doi.org/10.1111/j.1365-294X.2007.03408.x

Jenkins TL, Castilho R, Stevens JR (2018). Meta-analysis of northeast Atlantic marine taxa shows contrasting phylogeographic patterns following post-LGM expansions. PeerJ 6:e5684 https://doi.org/10.7717/peerj.5684

Johannesson K and Andre C (2006). Life on the margin: genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. Mol Ecol 15:2013-2029. doi:10.1111/j.1365-294X.2006.02919.x

Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA (2013) diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. Ecol and Evol 4:782-788. https://doi.org/10.1111/2041-210X.12067

Kleisner KM, Fogarty MJ, McGee S, Barnett A, Fratantoni P, Greene J, Pinsky ML (2016) The effects of sub-regional climate velocity on the distribution and spatial extent of marine species assemblages. PLoS ONE 11(2):e0149220. https://doi.org/10.1371/journal.pone.0149220.

Kousteni V, Kontopoulou M, Megalofonou P (2010) Sexual maturity and fecundity of *Scyliorhinus canicula* (Linnaeus, 1758) in the Aegean Sea. Mar Biol Res 6:390–398. https://doi.org/10.1080/17451000903233771

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23(21):2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Leigh JW, Bryant D (2015) PopART: Full-feature software for haplotype network construction. Methods Ecol Evol 6(9):1110–1116. https://doi.org/10.1111/2041-210X.12410

Leone A, Urso I, Damalas D, Martinsohn J, Zanzi A, Mariani S, Sperone E, Micarelli P, Garibaldi F, Megalofonou P, Bargelloni L, Franch R, Macias D, Prodöhl P, Fitzpatrick S, Stagioni M, Tinti F, Cariani A (2017) Genetic differentiation and phylogeography of Mediterranean-North Eastern Atlantic blue shark (*Prionace glauca*, L. 1758) using mitochondrial DNA: panmixia or complex stock structure?. PeerJ 5:e4112. https://doi.org/10.7717/peerj.4112

Lind CE, Evans BS, Taylor JJU, Jerry DR (2007) Population genetics of a marine bivalve, Pinctada maxima, throughout the Indo-Australian Archipelago shows differentiation and decreased diversity at range limits. Mol Ecol 16:5193-5203. doi:10.1111/j.1365-294X.2007.03598.x

Litvinov FF (2003) Sexual dimorphism as an index of the isolation of West African populations of the catshark *S. canicula*. J Ichthyol 43:81–85. https://doi.org/10.1111/jfb.14011

Macdonald SL, Llewelyn J, Moritz C, Phillips BL (2017) Peripheral isolates as sources of adaptive diversity under climate change. Front Ecol Evol 5:1–10. https://doi.org/10.3389/fevo.2017.00088

Maggio T, Allegra A, Andaloro F et al (2019). Historical separation and present-day structure of common dolphinfish (Coryphaena hippurus) populations in the Atlantic Ocean and Mediterranean Sea. ICES J Mar Sci 76(4):1028–1038. https://doi.org/10.1093/icesjms/fsy174

Maggs CA, Castilho R, Foltz D, Henzler C, Jolly MT, Kelly J, Olsen J, Perez KE, Stam W, Väinölä R, Viard F, Wares J (2008) Evaluating signatures of glacial refugia for North Atlantic benthic marine tax. Ecol 89:108-122. https://doi.org/10.1890/08-0257.1

Manuzzi A, Zane L, Munoz-Merida A, Griffiths A, Veríssimo A (2019) Population genomics and phylogeography of a benthic coastal shark (*Scyliorhinus canicula*) using 2b-RAD single nucleotide polymorphisms. Biological Journal of the Linnean Society. 126. 289-303. 10.1093/biolinnean/bly185.

Mayr E (1963) Animal species and evolution. Havard University Press, Havard

Nakajima Y, Nishikawa A, Iguchi A, Sakai K (2010) Gene Flow and Genetic Diversity of a Broadcast-Spawning Coral in Northern Peripheral Populations. PLOS ONE 5(6):e11149. https://doi.org/10.1371/journal.pone.0011149

Nei M, Maruyama T, Chakaborty R (1975) The bottleneck effect and genetic variability in populations. Evol 29:1–10. https://doi. org/10.1111/j.1558-5646.1975.tb00807.x

Ovenden, J.R., Morgan, J.A.T., Street, R. et al (2011) Negligible evidence for regional genetic population structure for two shark species *Rhizoprionodon acutus* (Rüppell, 1837) and *Sphyrna lewini* (Griffith & Smith, 1834) with contrasting biology. Mar Biol 158:1497–1509. https://doi.org/10.1007/s00227-011-1666-y

Palero F, Abelló P, Macpherson E, Gristina M, Pascual M (2008) Phylogeography of the European spiny lobster (Palinurus elephas): Influence of current oceanographical features and historical processes. Mol Phylogenet Evol 48(2):708–717. https://doi.org/10.1016/j.ympev.2008.04.022

Patarnello T, Volckaert FAMJ, Castilho R (2007) Pillars of Hercules: Is the Atlantic-Mediterranean transition a phylogeographical break*?* Mol Ecol 16(21):4426–4444. https://doi.org/10.1111/j.1365-294X.2007.03477.x

Pawson MG, Ellis JR (2005) Stock identity of elasmobranchs in the Northeast Atlantic in relation to assessment and management. J Northwest Atl Fish Sci 35:173–193. https://doi.org/10.2960/J.v35.m480

Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in marine fishes. Science 308:1912–1915. https://doi.org/10.1126/science.1111322

Petit RJ, Mousadik AE, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. Conserv Biol 12:844–855. https://doi.org/10.1046/j.1523-1739.1998.96489.x

Pirog A, Jaquemet S, Ravigné V et al (2019) Genetic population structure and demography of an apex predator, the tiger shark *Galeocerdo cuvier*. Ecol Evol. 9:5551– 5571. https://doi.org/10.1002/ece3.5111

Portnoy DS and Heist EJ (2012) Molecular markers: progress and prospects for understanding reproductive ecology in elasmobranchs. J Fish Biol 80:1120-1140. doi:10.1111/j.1095-8649.2011.03206.x

Ramírez-Amaro S, Picornell A, Arenas M, Castro JA, Massutí E, Ramon MM, and Terrasa B (2018) Contrasting evolutionary patterns in populations of demersal sharks throughout the western Mediterranean. Mar Biol 165:3. https://doi.org/10.1007/s00227-017-3254-2

Rilov G (2016). Multi-species collapses at the warm edge of a warming sea. Sci Rep 6:10.1038/srep36897. https://doi.org/10.1038/srep36897

Rodríguez-Cabello C, Velasco F, Olaso I (1998) Reproductive biology of lesser spotted dogfish *Scyliorhinus canicula* (L., 1758) in the Cantabrian Sea. Sci Mar, 62: 187-191. https://doi.org/10.3989/scimar.1998.62n3187

Rohling EJ, Foster GL, Grant KM, Marino G, Roberts AP, Tamisiea ME, Williams F (2014) Sea-level and deep-sea temperature variability over the past 5.3 million years. Nature 508:432–477. https://doi.org/10.1038/nature13230

Roman J, Palumbi SR (2004) A global invader at home: Population structure of the green crab, Carcinus maenas, in Europe. Mol Ecol 13(10):2891–2898. https://doi.org/10.1111/j.1365-294X.2004.02255.x

Serena F, Ellis JR, Abella A, Mancusi C, Haka F, Guallart J, Ungaro N, Coelho RP, Polyakov IV, Alexeev VA, Bhatt US, Polyakova EI, Zhang X (2010) North Atlantic warming: Patterns of long-term trend and multidecadal variability. Clim Dyn 34(2):439–457. https://doi.org/10.1007/s00382-008-0522-3

Shaltout M, Omstedt A (2014) Recent sea surface temperature trends and future scenarios for the Mediterranean Sea. Oceanologia 56:411–443. https://doi.org/10.5697/oc.56-3.411

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595.

Veríssimo A, McDowell JR, Graves JE (2011) Population structure of a deep-water squaloid shark, the Portuguese dogfish (*Centroscymnus coelolepis*). ICES J Mar Sci 68:555–563. https://doi.org/10.1093/icesjms/fsr003

Veríssimo A, Sampaio Í, McDowell JR et al (2017) World without borders—genetic population structure of a highly migratory marine predator, the blue shark (*Prionace glauca*). Ecol Evol. 7:4768– 4781. https://doi.org/10.1002/ece3.2987

Villesen P (2007) FaBox: an online toolbox for fasta sequences. Mol Ecol 7(6):965-968. doi:10.1111/j.1471-8286.2007.01821

Volis S, Ormanbekova D, Yermekbayev K, Song M, Shulgina, I (2016) The Conservation Value of Peripheral Populations and a Relationship Between Quantitative Trait and Molecular Variation. Evol Biol 43(1):26–36. https://doi.org/10.1007/s11692-015-9346-3

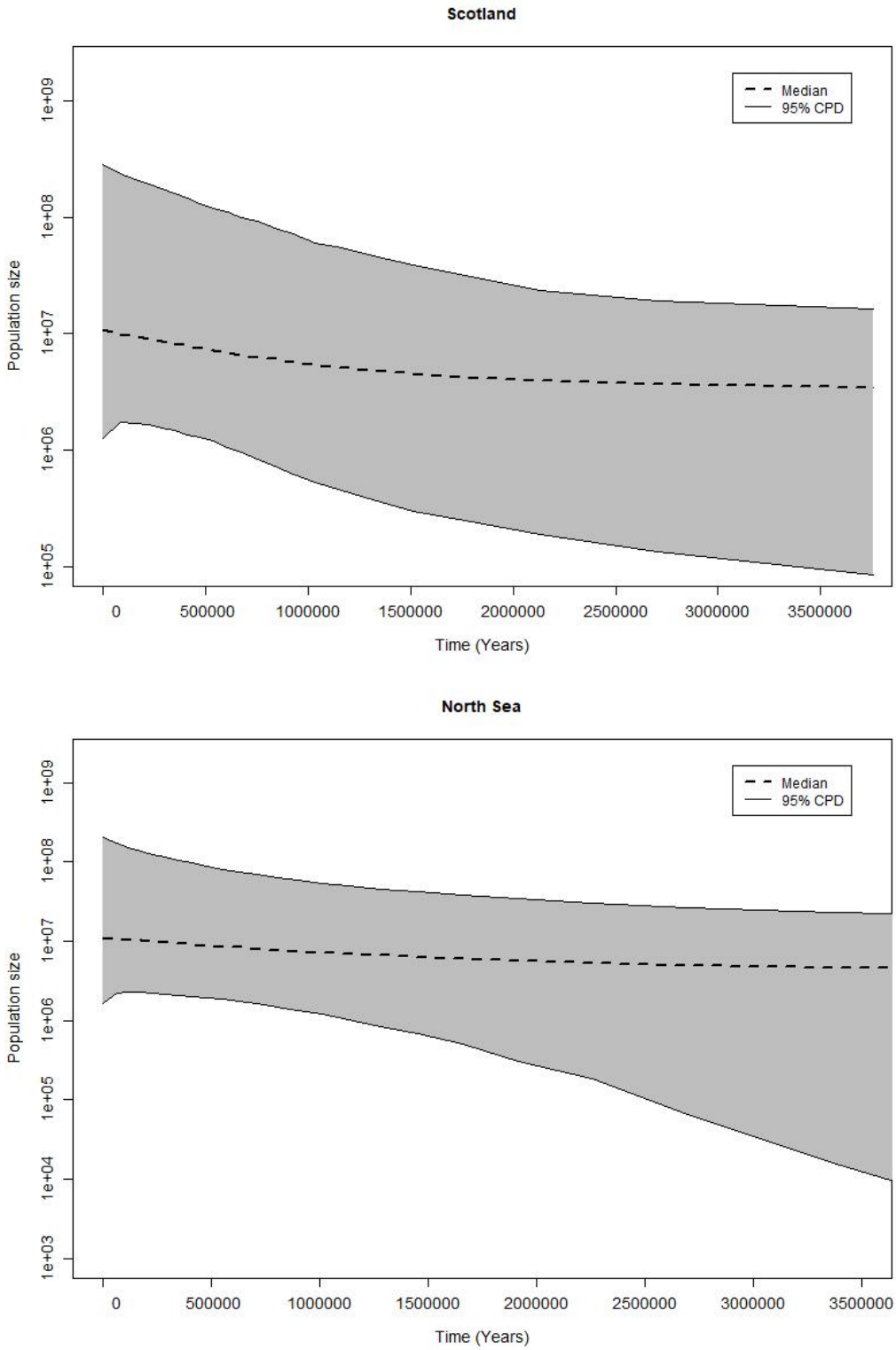
Wheeler A (1978) Key to the fishes of northern Europe. Publ. Frederick Warne. Ltd. London. 350.

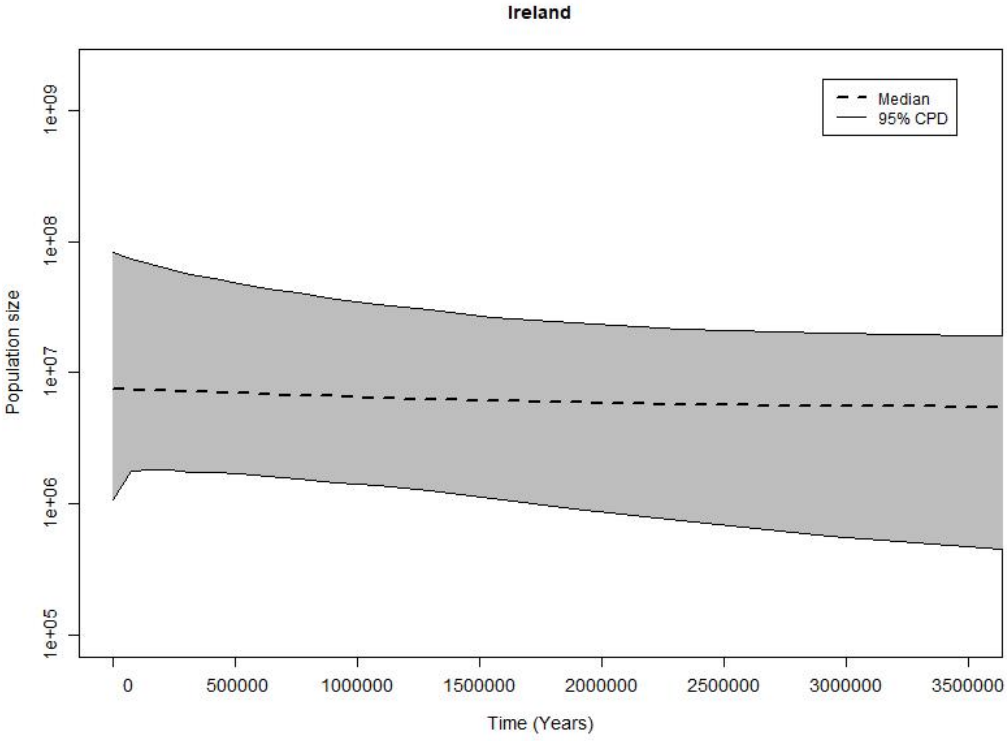
Wolff WJ (2000a) Causes of extirpations in the Wadden Sea, an estuarine area in the Netherlands. Conser Biol 14:876-885. https://doi.org/10.1046/j.1523-1739.2000.98203.x

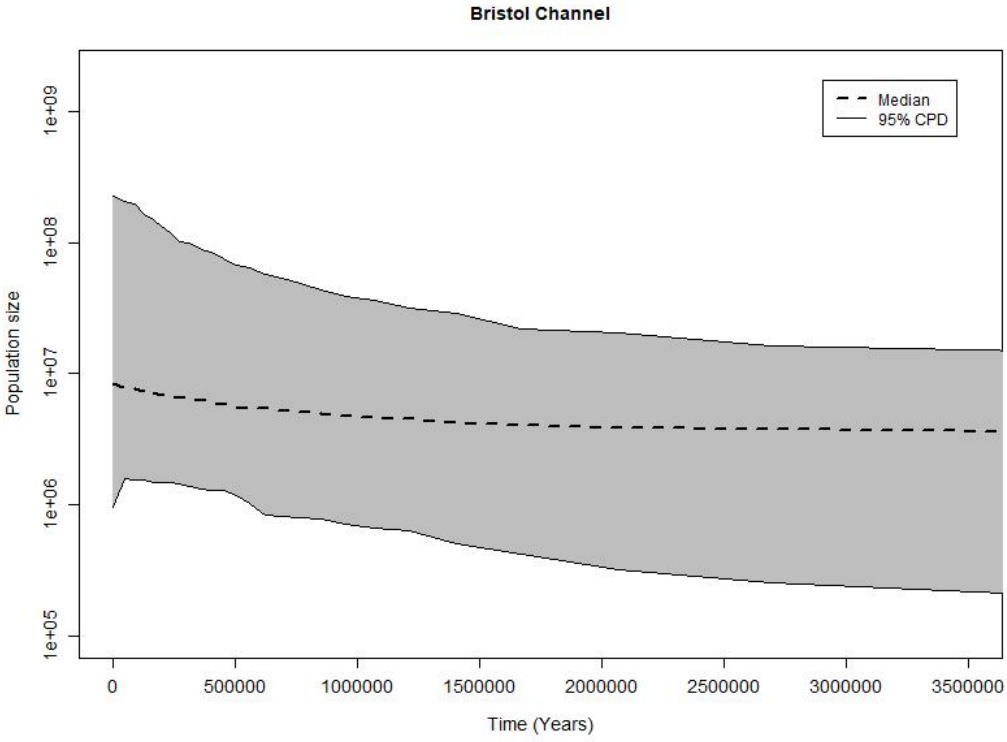
Wolff WJ (2000b) The south-eastern North Sea: losses of vertebrate fauna during the past 2000 years. Biol Conserv 95:209-217. https://doi.org/10.1016/S0006-3207(00)00035-5

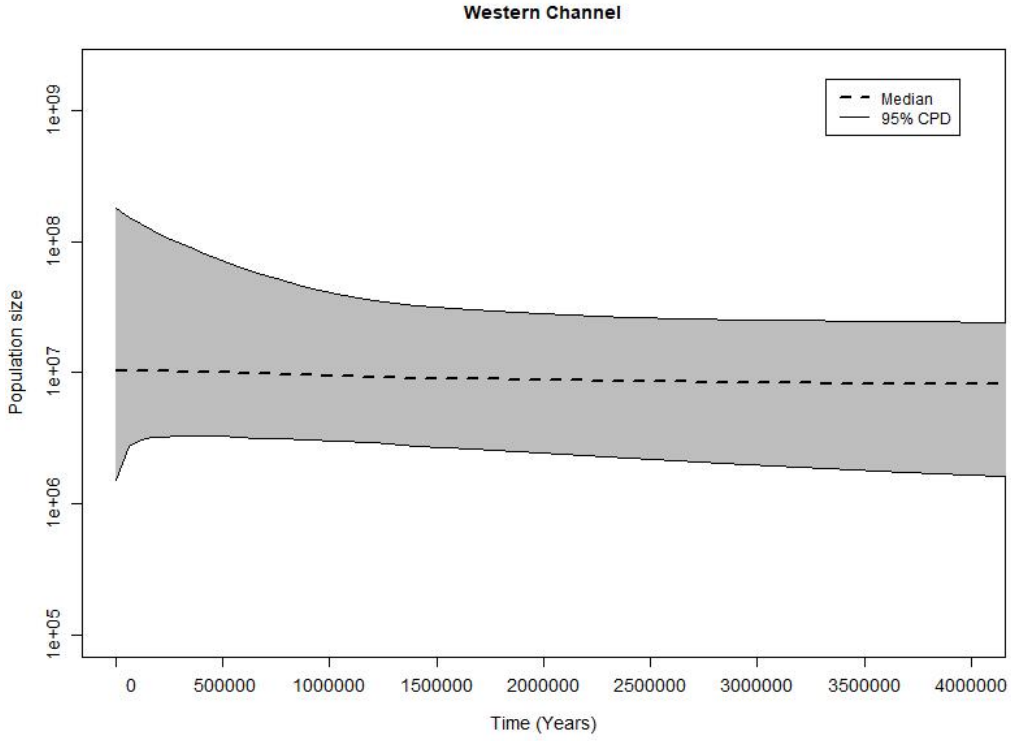
2.7. Supplementary information

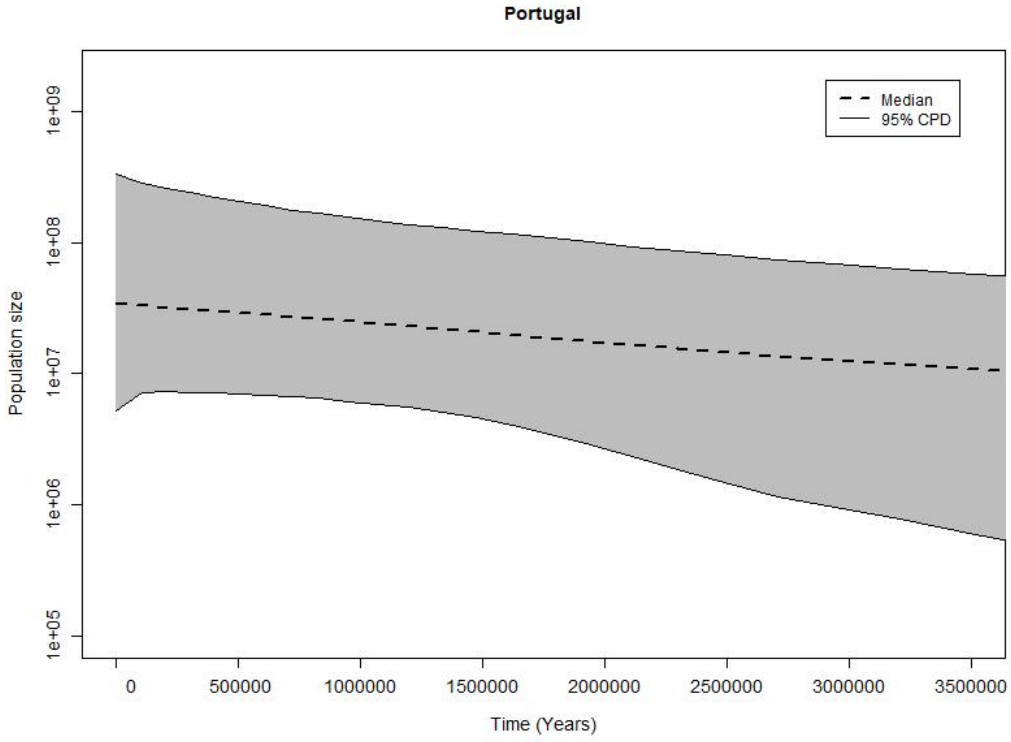
Figure S2.1 Bayesian skyline plots derived from CR sequences (mtDNA) for each sample collection showing the maternal effective population size (median and 95% confidence interval), back in time (years) since present day.

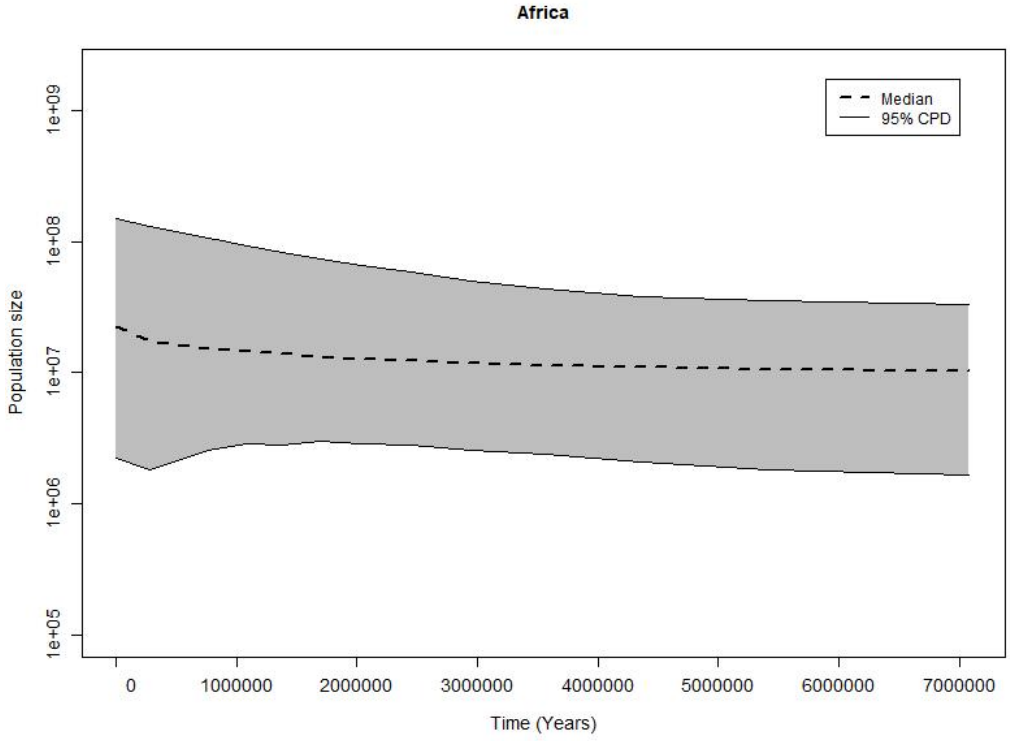


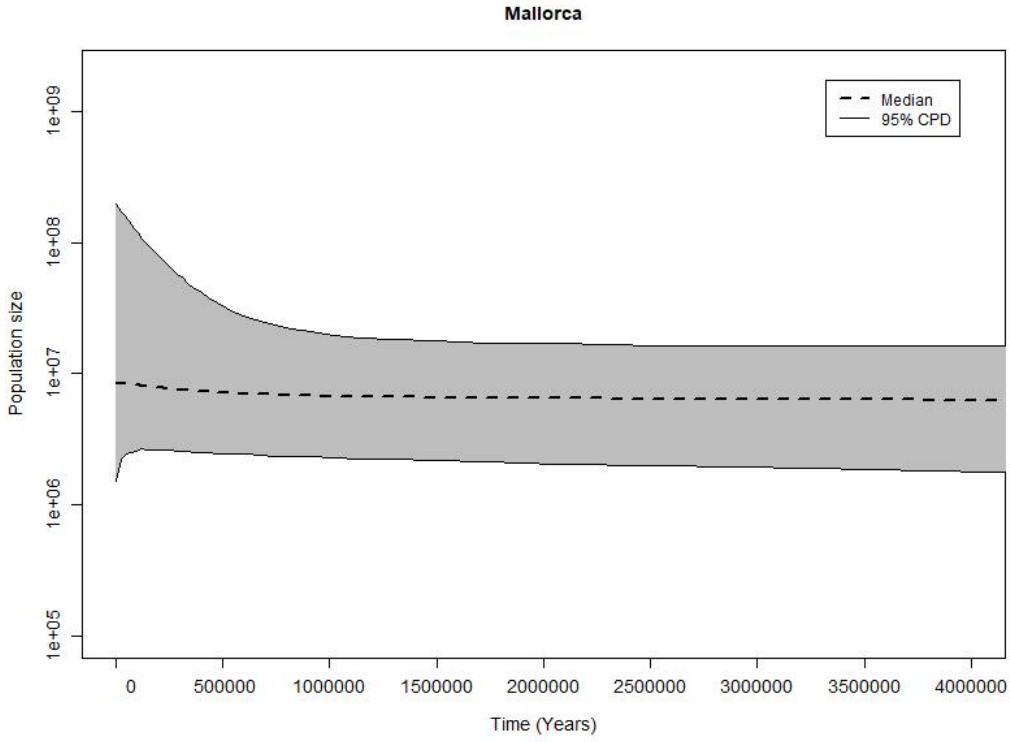

Ireland
Median
95% CPD
Population size
1e+09
1e+08
1e+07
1e+06
1e+05
0
500000
1000000
1500000
2000000
2500000
3000000
3500000
Time (Years)

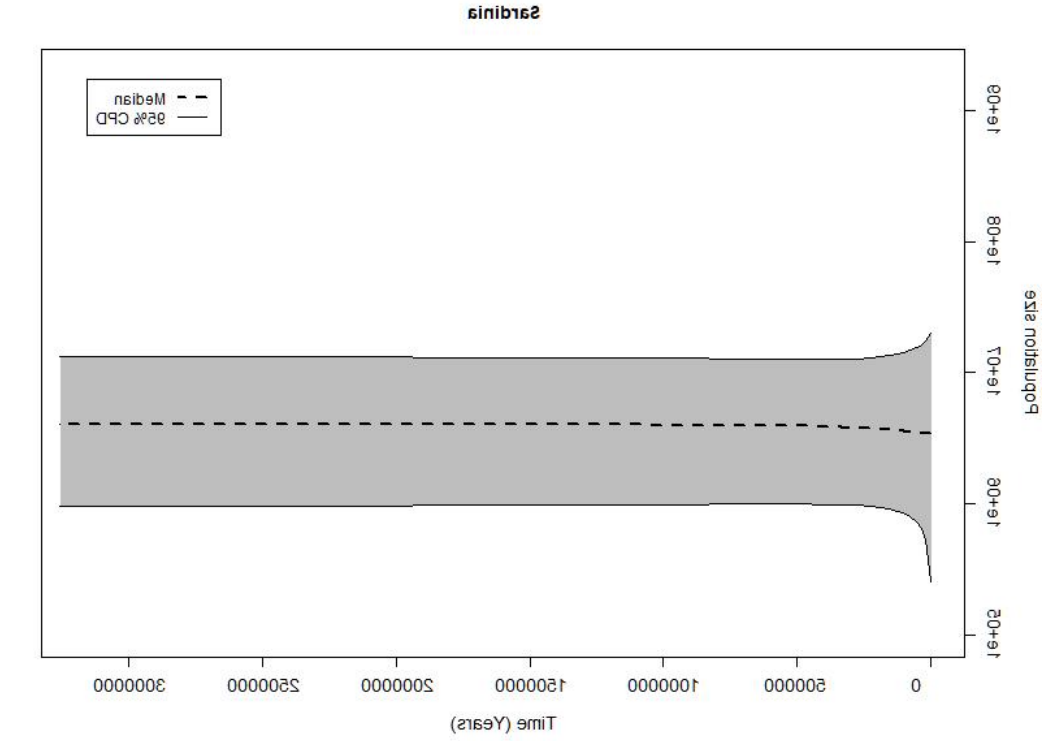

Bristol Channel
Median
95% CPD
Population size
1e+09
1e+08
1e+07
1e+06
1e+05
0
500000
1000000
1500000
2000000
2500000
3000000
3500000
Time (Years)

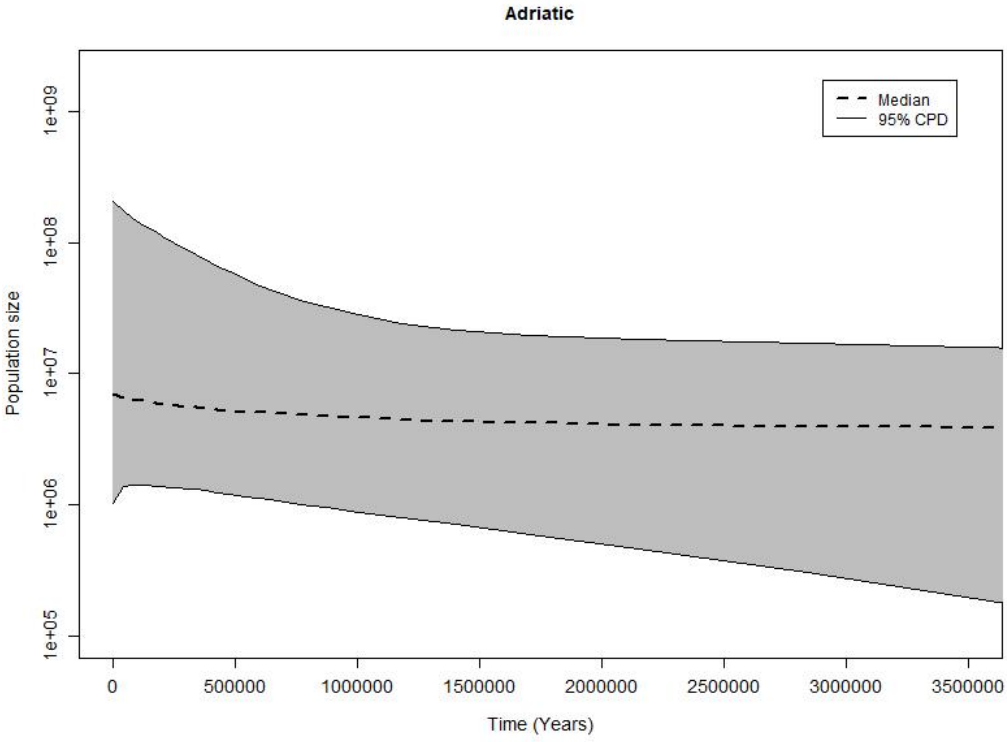

Western Channel
Median
95% CPD
Population size
1e+09
1e+08
1e+07
1e+06
1e+05
0
500000
1000000
1500000
2000000
2500000
3000000
3500000
4000000
Time (Years)

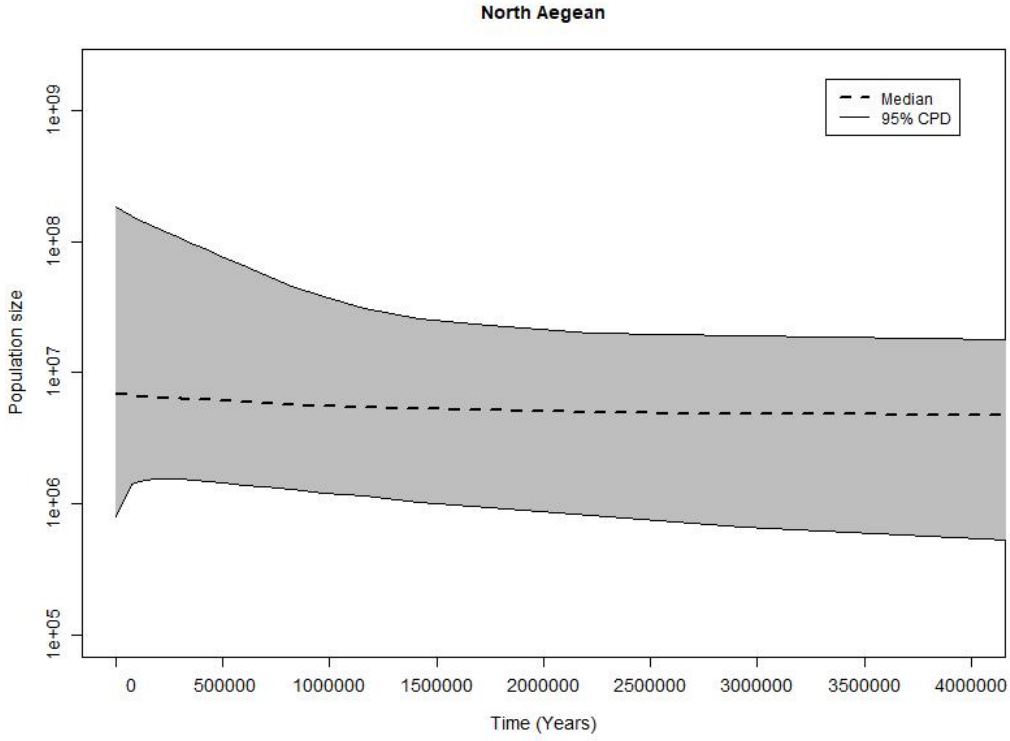

Portugal
Median
95% CPD
Population size
1e+09
1e+08
1e+07
1e+06
1e+05
0
500000
1000000
1500000
2000000
2500000
3000000
3500000
Time (Years)

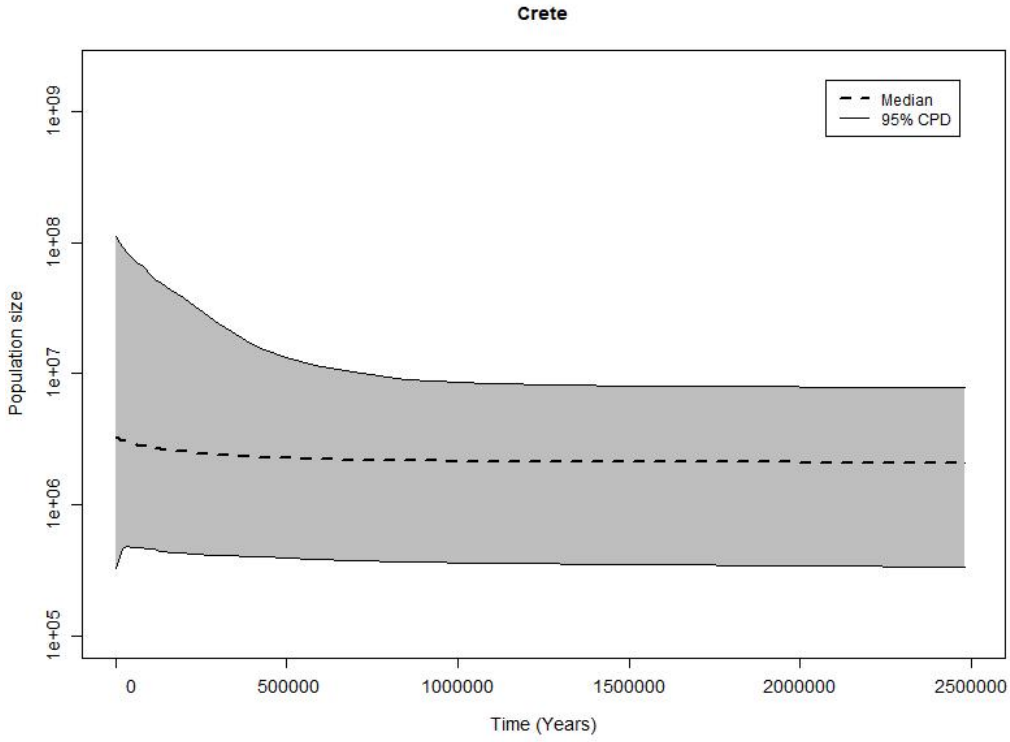

Africa
Median
95% CPD
Population size
1e+05
1e+06
1e+07
1e+08
1e+09
0
1000000
2000000
3000000
4000000
5000000
6000000
7000000
Time (Years)

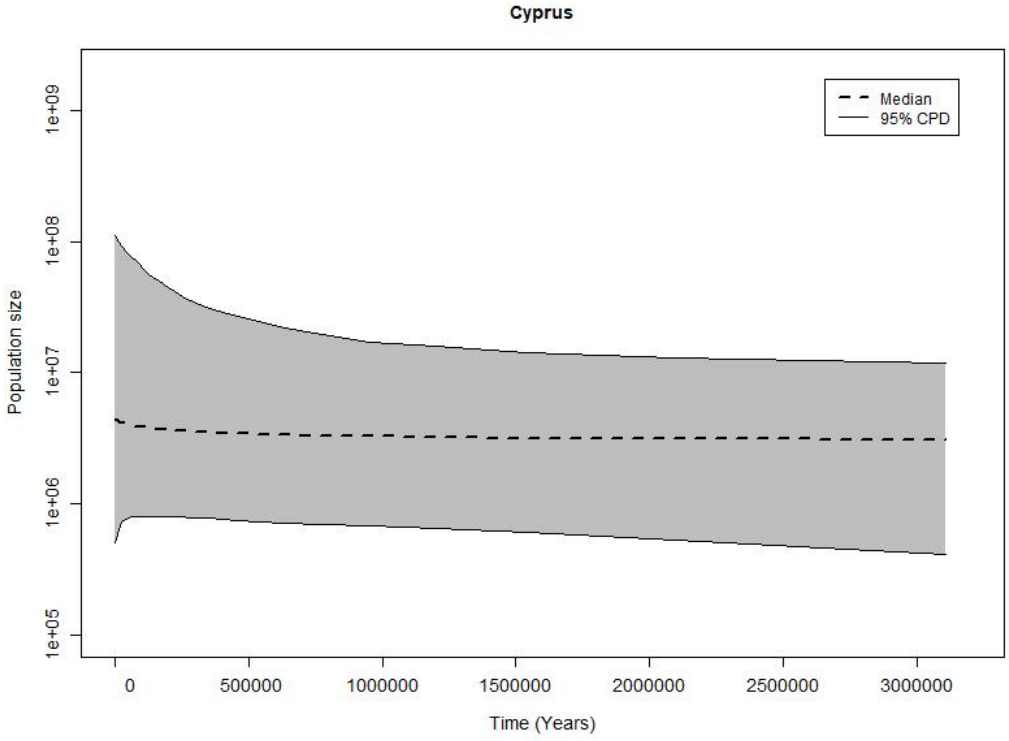

Mallorca
Median
95% CPD
Population size
1e+05
1e+06
1e+07
1e+08
1e+09
0
500000
1000000
1500000
2000000
2500000
3000000
3500000
4000000
Time (Years)


Sardinia
Median
95% CPD
Population size
1e+09
1e+08
1e+07
1e+06
1e+05
0
500000
1000000
1500000
2000000
2500000
3000000
Time (Years)


Adriatic
Median
95% CPD
Population size
1e+09
1e+08
1e+07
1e+06
1e+05
0
500000
1000000
1500000
2000000
2500000
3000000
3500000
Time (Years)


North Aegean
Median
95% CPD
Population size
1e+09
1e+08
1e+07
1e+06
1e+05
0
500000
1000000
1500000
2000000
2500000
3000000
3500000
4000000
Time (Years)


Crete
Median
95% CPD
Population size
1e+09
1e+08
1e+07
1e+06
1e+05
0
500000
1000000
1500000
2000000
2500000
Time (Years)


Cyprus
Median
95% CPD
Population size
1e+09
1e+08
1e+07
1e+06
1e+05
0
500000
1000000
1500000
2000000
2500000
3000000
Time (Years)

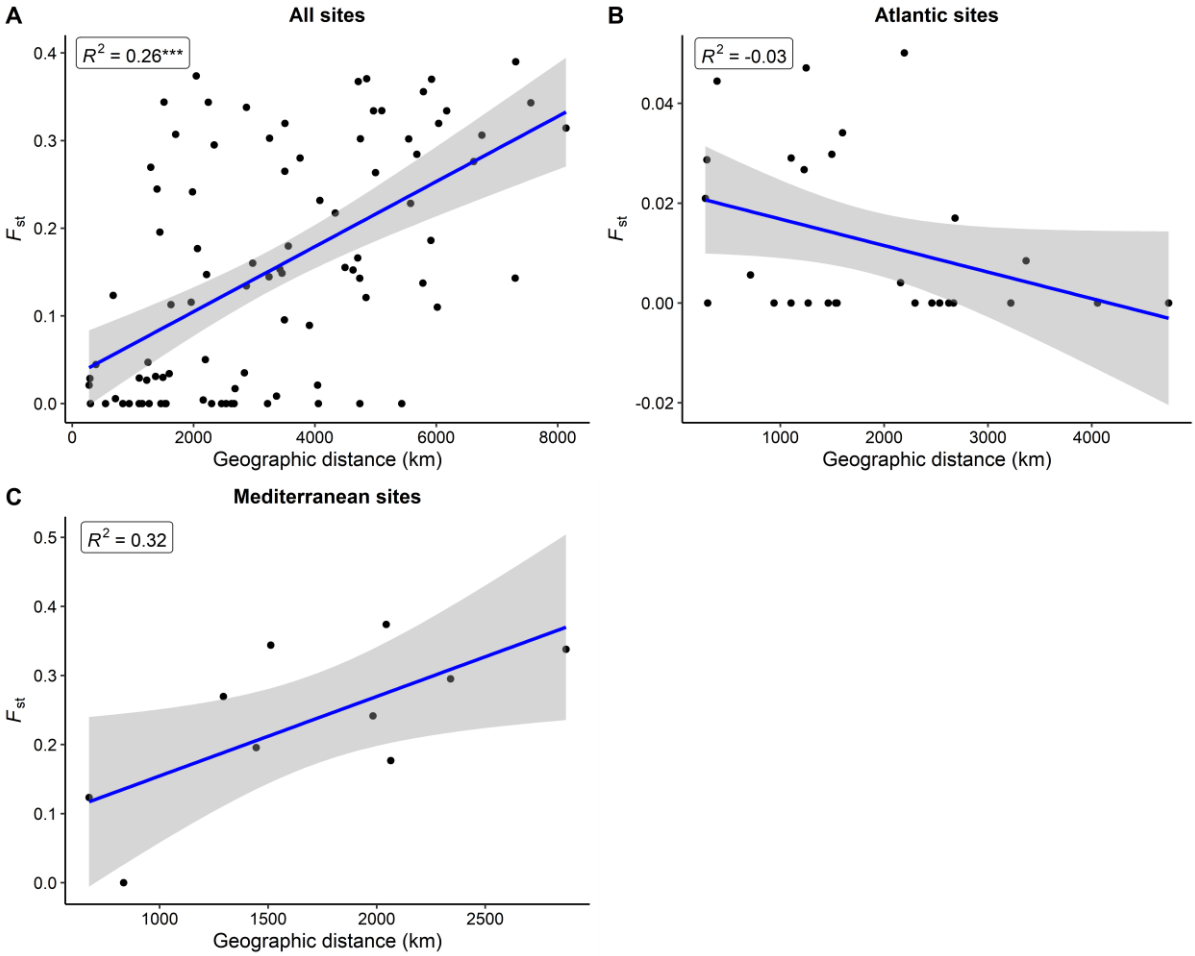


Figure SS2.2 Pairwise comparisons of geographic distances (km) and F_{st} between sampling localities of the small-spotted catshark, Scyliorhinus canicula. Mantel test were conducted using all sites (A), using only Atlantic sites (B) and using only Mediterranean sites (C). For each plot, a linear regression line (in blue) is fitted with 95 % confidence intervals (in grey). Asterisks denote significance levels: *<0.05, **<0.01, ***<0.001.

Chapter 3: ddRAD reveals genomic population structure and evidence for local adaptation in the small-spotted catshark, *Scyliorhinus canicula,* from across its range.

This chapter is written for publication in the Heredity. Supplementary materials for this chapter are available at the end of the chapter.

3.1 Abstract

Sharks are one of the most threatened groups of vertebrate on the planet today, yet their capacity to adapt to environmental change is poorly understood. Following developments in genomics, high-throughput sequencing now allows for the detection of genetic structuring shaped by selection, elucidating environmentally driven adaptation. We used double-digest restriction site association DNA (ddRAD) to investigate 38 individuals of the small-spotted catshark (*Scyliorhinus canicula*) from 7 locations in the northeast Atlantic (NEA) and Mediterranean to search for signatures of local adaptation. Using a panel of 9,052 single nucleotide polymorphisms (SNPs) we revealed fine-scale structuring in the NEA and eastern Mediterranean. This was further emphasised when only analysing loci identified as candidates of selection by combining outlier analysis with environmental data. Adaptation to temperature appeared as an important potential driver of genetic structure alongside salinity, oxygen, and depth. This is the first time such an approach has been employed for an elasmobranch species and offers a unique opportunity to gain biological insight into the evolutionary processes in sharks and understand how they become adapted to their local environments.

Keywords: shark; elasmobranch; genomic; local adaptation; selection; environment; temperature; evolution

3.2 Introduction

Local adaptation is the evolutionary process whereby populations become specifically adapted to the characteristics of their local environment resulting in a higher fitness than other members of same species from elsewhere (Kawecki and Ebert, 2004). The capacity for such local adaptation is a result of selection from environmental differences across a species' range, but the homogenising effects of gene flow between populations can curtail this process (Kawecki and Ebert, 2004). Historically, marine populations were thought to lack the potential for local adaptation given the apparent absence of geographical barriers, large population sizes, and high gene flow they frequently show (Bohonak, 1999; Allendorf *et al*., 2010). However, recent advances suggests local adaptation may instead be common in marine species as the vast and changing marine environment provides more chances for natural selection (Benestan *et al.*, 2016; Carreras *et al.*, 2017, 2020; Bernatchez *et al.*, 2019; Vendrami *et al.*, 2019; Torrado *et al.*, 2020), even in the presence of high gene flow (Limborg *et al.*, 2012; Milano *et al.*, 2014; Katherine Cure *et al.*, 2017; Diopere *et al.*, 2018). Much focus is now being placed in the field of seascape genomics (the integration of genomic and environmental data) to measure local adaptation among marine populations and identify the genomic basis of biologically relevant traits under environmental selection (Selkoe *et al.*, 2016; Liggins, Treml and Riginos, 2020).

The development of genotype-by-sequencing (GBS) techniques, such as restriction site associated DNA (RAD; Davey and Blaxter, 2010) and double-digest RAD (ddRAD) (Peterson *et al.*, 2012) has revolutionised the field of seascape genomics, providing high-resolution genomic data for non-model organisms (Helyar *et al.*,

2011). Importantly, this has allowed for the discovery of outlier loci (i.e. loci with high F_{ST} relative to neutral expectations) presumed to be indicative of local adaptation (Limborg *et al.*, 2012) and has revealed fine-scale genetic structuring in many marine species (Nielsen *et al.*, 2009; D. S. Portnoy *et al.*, 2015; Carreras *et al.*, 2017; Momigliano *et al.*, 2017; Veríssimo *et al.*, 2018; Jenkins *et al.*, 2019; Vendrami *et al.*, 2019). Many techniques are now available to identify these outlier loci including outlier analysis (OA, Foll and Gaggiotti, 2008; Prive *et al.*, 2020) and environmental association analysis (EAA, Frichot and Francois, 2015; Gautier, 2015), with the latter identifying associations between outlier loci and environmental variation (Selkoe *et al.*, 2016). For example, using EAA Diopere *et al.* (2018) identified loci associated with winter sea temperature, food availability, and coastal currents in sole (*Solea solea*), and Berg *et al.* (2015) found loci strongly correlated with habitat differences in salinity, oxygen and temperature in Atlantic cod (*Gadus morhua L.*). Gaining greater insight into processes of adaptation and the underlying genetic architecture are important steps in determining a species' response to over exploitation and environmental change (Waldvogel *et al.*, 2020) – perhaps one the most critical challenges in biology today.

Shark populations around the world are experiencing severe declines due to overexploitation and habitat destruction exaggerated by pollution and climate change (Field *et al.,* 2009; Dulvy *et al.*, 2014; Wheeler *et al.*, 2020), and are now regarded as one of the most threatened groups of vertebrate (Dulvy *et al.*, 2014). This may have grave consequences on the genetic diversity and adaptive potential of shark and ray populations (Rodrigo Rodrigues Domingues, Hilsdorf and Gadig, 2018). Yet, little is known of the genetic and phenotypic nature of local adaptation in this vulnerable

group, clouding predictions on how sharks might respond to environmental change. To date there have been limited genomic studies of sharks (Delser *et al.*, 2016; Pazmino *et al.*, 2017; Veríssimo *et al.*, 2018; Yuichiro Hara *et al.*, 2018; Manuzzi *et al.*, 2019; Marra *et al.*, 2019) with even fewer searching for adaptive divergence (D. S. Portnoy *et al.*, 2015; Momigliano *et al.*, 2017; Junge *et al.*, 2019). This study focuses on a small demersal species of shark, the small-spotted catshark (*Scyliorhinus canicula*), and for the first time utilises a seascape genomic approach to begin to address the genetic architecture of adaptation in an elasmobranch species.

The small-spotted catshark has a wide distribution in the northeast Atlantic (NEA), extending from Norway to Senegal and throughout the Mediterranean (Compagno, Dando and Fowler, 2005). This encompasses diverse physical and biological environments varying in temperature, oxygen, salinity and depth; known drivers of adaptation in other marine fish (Angilletta and Dunham, 2003; Gaggiotti *et al.*, 2009; White *et al.*, 2010; Berg *et al.*, 2015; Diopere *et al.*, 2018; Torrado *et al.*, 2020). The catshark is also developing into an excellent model species for sharks having been researched since the early 20th century (Ford, 1921; Eales, 1949). It has well-documented geographical variation in reproductive characteristics such as maximum size, size at maturity, egg-laying rates and peak laying seasons in the NEA (Ellis and Shackley., 1997; Henderson and Casey, 2001; Litvinov, 2003; Rodriguez-Cabello *et al.*, 2004, 2007; Ivory *et al.*, 2005) and Mediterranean (Capape *et al.*, 2008, 2014; Kousteni, Kontopoulou and Megalofonou, 2010; Aat, Mouffok and Boutiba, 2012; Finotto *et al.*, 2015). In many cases, these were thought to be a response to differing environmental pressures throughout the catsharks range, particularly temperature.

However, whether this is a response of phenotypic plasticity, local adaptation or both remains unknown. Some traits appear to coincide with population structuring identified using microsatellite and mitochondrial, showing clear separation of the NEA and the Mediterranean (Gubili *et al.*, 2014) with further complex structuring within the Mediterranean (Gubili *et al.*, 2014; V. Kousteni *et al.*, 2015) and the NEA (Manuzzi *et al.*, 2019). Although alluded at, no study to date has addressed the potential role of selection in driving these patterns of divergence, and highlight the need for more detailed genomic studies to elucidate both neutral and adaptive genetic differentiation in this species.

This study compares environmental and genome-wide data for *S. canicula* throughout its distributional range. Using ddRAD combined with OA and EEA, this study aims to reveal regions of the genome under section to understand the extent to which populations might specialise to their local environment. Furthermore, both the NEA and Mediterranean offer independent clines in key environmental variables, especially temperature, a critical evolutionary driver (Rohde, 1992; Currie *et al.*, 2004). If independent shark populations that occupy environments with similar conditions showed common regions of their genomes under section, these would be strong candidates for genes under selection and indicate convergent patterns of evolution. As so few genomic resources for sharks exist, this study offers a unique opportunity to gain biological insight into the evolutionary processes in sharks and understand how they become adapted to their local environments.

3.3 Materials and methods

3.3.1 Sample selection

A total of 40 individual fin clips of adult small-spotted catsharks were selected for the construction of RAD libraries. This includes six individuals from seven different localities; four from the North Atlantic Ocean (NEA), and three from the Mediterranean (Figure 3.1; Table 3.1). Due to the rarity and difficulty of obtaining Norwegian samples, only four samples were available from this region (Table 3.1). Localities were selected based on their climatic profile in both the NEA and Mediterranean (Table 3.1) alongside inferences of population structure from studies (Barbieri *et al.*, 2014; Gubili *et al.*, 2014; Kousteni *et al.*, 2015; Ramirez-Amaro *et al.*, 2018; Manuzzi *et al.*, 2019; Chapter 2).

3.3.1 ddRAD library preparation and sequencing

All samples had been stored in 95-100 % ethanol at -20 °C prior to extraction. Genomic DNA was extracted from ~25 ng of tissue using the QIAGEN© DNeasy Blood and Tissue kit (Qiagen, Manchester, UK) following the manufacturers protocol with minor modifications; overnight digest with proteinase K at 37 °C followed by an RNase treatment (2 µl of 100mg/ml). Each extraction was checked on a 1% agarose gel and via spectrophotometry using the Nanodrop One (Thermo Scientific™) to assess the quality, concentration and contamination. The concentration of double stranded DNA (dsDNA) was further quantified using the QuantiFluor® dsDNA System and the GloMax® Discover Microplate Fluorometer following the manufacturers' protocol. All samples were normalised to a concentration of 20 ng/µL using nuclease-free water.

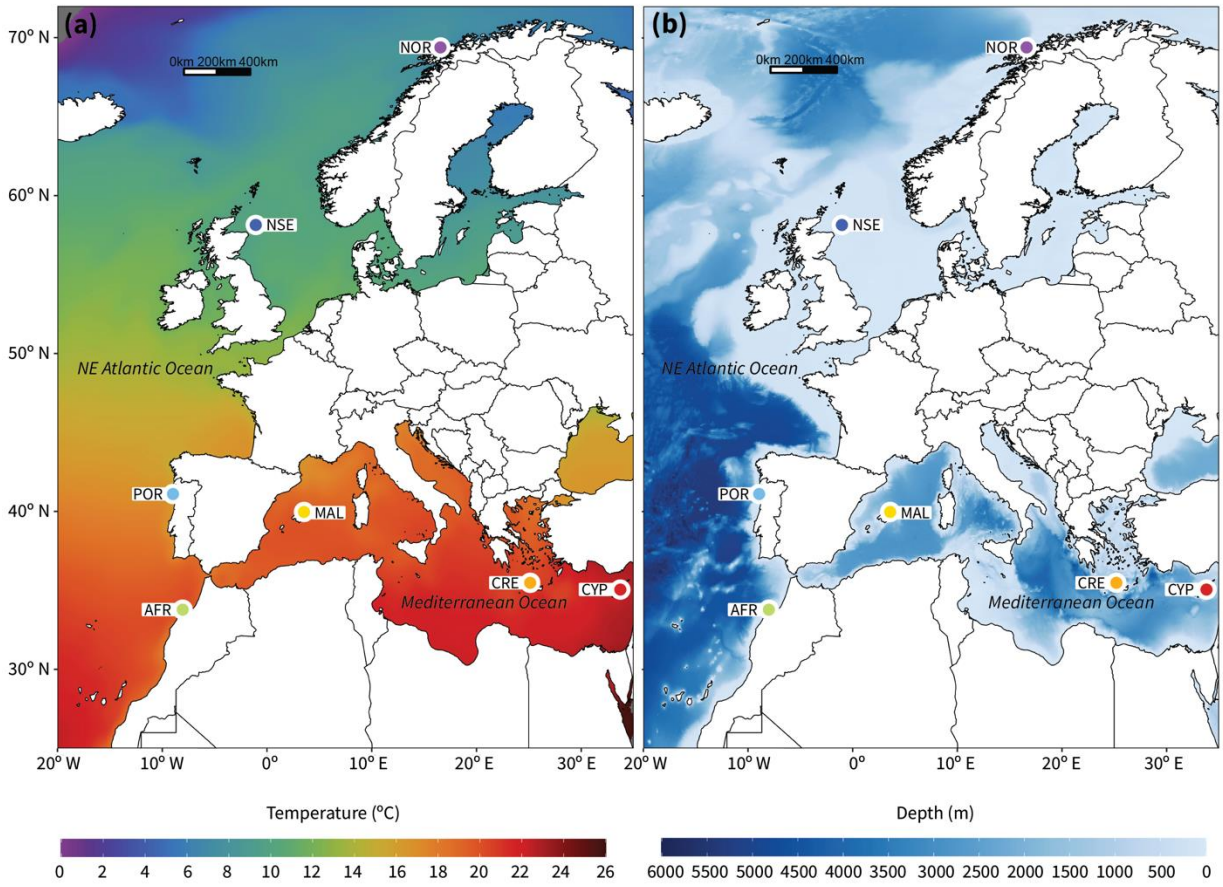


Figure 3.1. Sample localities of *Scyliorhinus canicula* overlaid on: (a) mean annual sea surface temperature at the present day, (b) depth. Data obtained from the Bio-ORACLE repository (Assis et al., 2017). Colours used to denote each sample site are consistent throughout all subsequent figures to facilitate their identification.

The ddRAD libraries were constructed following Poland et al. (2012) with slight modifications. Briefly, 200 ng of genomic DNA per sample was digested with restriction enzymes *PstI-HF* (NEB) and *MspI* (NEB) at 37 °C for 2 h followed by 65 °C for 20 min. Restriction fragments of each sample were ligated to a unique barcoded *PstI* adaptor (Table S3.1) at 25 °C for 2 h followed by 65 °C for 20 min. 5 µL of each sample ligate were pooled and purified using an NEB Monarch® PCR & DNA clean-up kit split over two columns and following the manufacturers protocol. PCR amplification was performed in eight 25 µL reactions, consisting of 2 µL of DNA ligate and 23 µL of PCR master mix per reaction using the following recipe: 45 µL of

5x Phusion® HF buffer, 27 µL of dNTPs (2mM), 9 µL of each forward pand reverse Illumina sequencing primers, 2.7 µL of Phusion DNA-polymerase and 125 µL of nuclease free water. PCR conditions were as follows: initial denaturing at 98 °C for 30 s, followed by 15 cycles of 95 °C for 10 s, 62 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min. All eight PCR products were pooled before a final clean up step using the NEB Monarch® PCR & DNA clean-up kit spit over two columns. DNA was eluted in 17 µL of nuclease free water and pooled to form the final library. Fragment size and quantification (ng/µL) was checked on an Agilent® 4200 TapeStation (Figure S3.1). The final library comprising of 40 individuals each with unique barcodes was sequenced on half a lane of an Illuminia NoveSeq 6000 (paired end, 2 x 150 bp) at the Exeter Sequencing Service, UK.

3.3.3 Data filtering and SNP calling

Forward and reverse raw reads were screened for adapters, a phred-type quality cut-off of Q22 and a minimum read length of 25 bp in Fastp v 0.20.0 (Chen et al. 2018) before processing in STACKS v 2.0 (Catchen *et al.*, 2011; Rochette, River-Colon and Catchen, 2019). Using the *process_RADtags* pipeline sequences were demultiplexed based on individually assigned barcodes and any reads shorter than 50 bp, with ambiguous barcodes or no RAD cut-site were removed*. De novo* assembly of the paired-end reads was performed using the wrapper script *denovo_map.pl* in STACKS v 2.0. Values of the three main parameters (*-m, -M* and *-n*) were chosen following the optimisation procedures described by Paris *et al.* (2017) and (Rochette and Catchen, 2017). Briefly, the *denovo_map* pipeline was run several times with varying increments of *M* = *n* with each parse (Figure S3.2) keeping *m* set to 3. The combination of parameters yielding the highest number of

polymorphic loci present in at least 80 % of the individuals was determined as optimal (*r80 loci*; Paris *et al.*, 2017). Due to the high levels of differentiation between populations of the species (Barbieri *et al.*, 2014; Gubili *et al.*, 2014; Kousteni *et al.*, 2015; Ramírez-Amaro *et al.*, 2018; Manuzzi *et al.*, 2019; Chapter 2) *denovo_map* was also run at *n = M – 1* to account for the high potential of fixed SNPs. This resulted in the highest number of polymorphic *r80* loci and the final optimisation parameters of *m*3 *M*1 *n*2 (Figure S3.2).

Following *de novo* assembly, additional filtering was performed in the *populations* program of STACKS v 2.0. Putative loci were only retained if present in ≥ 95% of individuals across populations (*-R*), in ≥ 50% of individuals within a populations (*-r*), had a minor allele count (MAC) of 3 and maximum observed heterozygosity (H_o) of 0.5. A maximum threshold of 0.5 H_o was used as this is maximum frequency true variants are often considered to have (Dufresne *et al.*, 2013). Only the first SNP per locus was included in the analysis to minimise the likelihood of linkage between SNPs. VCFTools (Danecek *et al.*, 2011) was used to remove loci with a minimum depth of coverage ≤ 5 and maximum depth of coverage ≥ 87.5 (mean read depth plus two times standard deviation) to remove potential multicopy loci. Loci showing significant evidence of linkage disequilibrium (LD) above an r2 threshold of 0.5 were further removed in PLINK v 1.9 (Purcell *et al.*, 2007). Number of SNPs retained after each filtering step are show in Table S3.2.

3.3.4 Population structure analysis

Genetic differentiation between sample localities were estimated by calculating pairwise values of F_{ST} (Weir and Cockerham, 1984) and G'_{ST} (Hedrick, 2005) using

the *diffCalc* function of the R package *diveRsity* (Keenan *et al.*, 2013). Significance was determined by calculating bias-corrected 95% confidence intervals (1,000 replicates) and testing whether values were significantly different from zero. A heat map of both pairwise values was visualised in R using the *ggplot2* function (R Development Core Team, 2019). Population structuring was visualised between all sample sites and within individual basins (NEA and Mediterranean) using Principal Coordinates Analysis (PCoA) plots constructed using the R packages *adegenet* (Jombart, 2008) and *ape* (Paradis, Claude and Strimmer, 2004). The Bayesian clustering package STRUCTURE v.2.3.4 (Pritchard, Stephens and Donnelly, 2000) was used to infer the admixture ancestry of individuals. A 'hierarchical' approach (Vaha *et al.*, 2007), with two rounds of analysis was employed to capture major structure within the RAD data and was run under the admixture model with a burn-in of 50,000 and 100,000 Markov chain-Monte Carlo (MCMC) for five repetitions. CLUMPAK (Kopelman *et al.*, 2015) was used to visualise the results and the optimal value of *K* for each round was determined by examining the delta *K* method of Evanno *et al.* (2005) in the R package *pophelper* v 2.2.5.1 (Francis, 2017, Figure S3.4).

3.3.5 Outlier analysis and environmental association

A combination of one OA based on genetic differentiation and two EAA methods were employed: PCAdapt v4.0.3 (Prive *et al.*, 2020), latent factor mixed model (LFMM, Frichot and Francois, 2015) and BayPass (Gautier, 2015). All approaches account for underlying population structure and perform well under scenarios of high differentiation, similar to that of the small-spotted catshark (Barbieri *et al.*, 2014; Gubili *et al.*, 2014; V. Kousteni *et al.*, 2015; Manuzzi *et al.*, 2019). PCAdapt uses

principal components analysis (PCA) to detect loci under selection and assumes that markers highly discriminatory for population differentiation are candidates for local adaptation. It was run with $K = 3$ as this was the optimal number of principal components (PCs) that best explained the percentage of variance within the SNP data. Additional PCs did not ascertain population structure anymore. *p*-values were corrected for false discovery rate (FDR) according to Benjamini and Hochberg (1995) using the R package *qvalue* (R Core Development Team, 2019) and SNPs with an alpha level < 0.01 were considered outliers. By default, alleles displaying a minimum allele frequency (MAF) < 5 % were removed in the PCAdapt analyses.

The two EAA methods, LFMM and BayPass, identify individual SNPs in strong association with environmental variables. As such, data on seven environmental variables for each sample locality were selected from the Bio-ORACLE repository (Assis *et al.*, 2017): mean annual sea surface temperature (SST), mean annual sea bottom temperature (SBT), SST range (difference between max and min SST), SBT range, mean sea bottom (SB) salinity (hereon salinity), mean SB dissolved oxygen (hereon oxygen) and mean depth (Table 3.2). These variables were selected to capture the key environmental gradients in both the Mediterranean and NEA (Figure 3.1; Table 3.1) which show very similar, yet independent clines particularly for temperature and salinity enabling questions of convergent evolution to be asked. Initially, 11 variables were considered but three were dropped due to a strong correlation > 0.70 with another covariable (Table S3.3). The Pearsons correlation was calculated in R using the package *sdmpredictors* (Assis *et al.*, 2017).

LFMM detects association of allele frequencies differences with population-specific covariables while accounting for population structure via the so-called latent factors. LFMM was run within the R package *LEA* (Frichot and Francois, 2015) with 10,000 interactions following a 5000 burn-in. Z-scores from 10 independent runs were combined and the resulting *p*-values were corrected for false discovery rate (FDR) according to Benjamini and Hochberg (1995) with an alpha level < 0.01 (-log10(*p*-value) of 2). BayPass has been shown to be among the most efficient at identifying true positives under high population structure (Gautier, 2015). It uses a Bayesian hierarchical model approach to detect loci associated with environmental covariables whilst accounting for any background population structure by incorporating a covariance matrix of population allele frequencies (Gautier, 2015). The covariance matrix was estimated under the core model and checked to resemble the F_{ST} matrix calculated in this study (Figure 3.2; Figure S3.3). BayPass v 2.0 was run separately for each covariable under the standard covariate model with 25,000 iterations following a 5000 burn-in and thinning by a factor of 25. Simulated pseudo-observed data sets (PODs), created using the *simulate.baypass* R function (Gautier, 2015), were used to calculate the 99% quantile and 1% thresholds for each covariable as suggested by (Gautier, 2015). SNPs with an empirical Bayesian *p*-value (eBPmc) above the 1% threshold were considered significantly associated. Loci identified as outliers in multiple methods were visualised in a Venn diagram constructed in R using the package *VennDiagram* (Chen and Boutros, 2011). An outlier dataset was created representing only outlier SNPs identified in all three analysis, and a neutral SNP dataset representing all SNPs not identified as outliers.

3.3.6 Functional annotation

To identify the functional significance of the all outlier SNPs, ddRAD tags were first mapped against the small-spotted catshark transcriptome (Mulley *et al.*, 2014) using a *blastn* search in NCBI with an e-value threshold of ≤ 1e-3 and ≥ 80% sequence homology. Significant reference transcripts were then further annotated against the UniProtKB reference proteomes plus Swiss_Prot database (*blastx,* e-value ≤ 1e-3) noting significant gene ontology terms (GO-terms). A summary of GO-terms and their descriptions were obtained in REVIGO (Supek *et al.*, 2011).

3.4 Results

3.4.1 Genome-wide data

ddRAD sequencing generated a total of 739,547,448 raw reads from 40 individuals, with 317,336,574 (43%) reads retained following quality filtering. Due to poor read recovery (< 1,500,000) and poor depth of coverage (< 10) Mallorca38 and Por13 were removed from further analysis (Table S3.1). This resulted in a final dataset of 38 individuals (Table 3.1) with an average of 8,350,962 reads per individual and a mean coverage of 16.43 (Table S3.1). Following the filtering pipeline a total of 9,052 SNPs were retained with a mean read length of 61.67 bp and mean read depth per locus of 37. All populations had a called genotype for > 97% of SNPs (Table 3.2).

3.4.2 Genetic structure and diversity

Observed heterozygosity ($H_{o)}$ ranged from 0.191 (Cyprus) to 0.231 (Crete) and were higher than expected heterozygosity (H_e) values in all populations. Nucleotide diversity (P_i) was higher in NEA populations (0.221 – 0.225) than Mediterranean

(0.182 – 0.191) with Crete having the lowest value (0.182). Wrights inbreeding coefficient (F_{is}; Wright, 1951) was low across all populations (-0.067 – 0.041). The negative value observed in Crete (-0.067) is likely due to the low nucleotide diversity but high H_o observed in this population and could be evidence of a recent population expansion. Africa had the highest percentage of polymorphic loci (PPL) and Norway the lowest with 71.3% and 59.6%, respectively (Table 3.2), although this could be a result of Norway smaller population size. The number of private alleles ranged from 7 (Portugal) to 30 (Crete), with far more private alleles found in Crete and Cyprus (23) than any other populations (Table 3.2), suggesting genetic drift due to isolation.

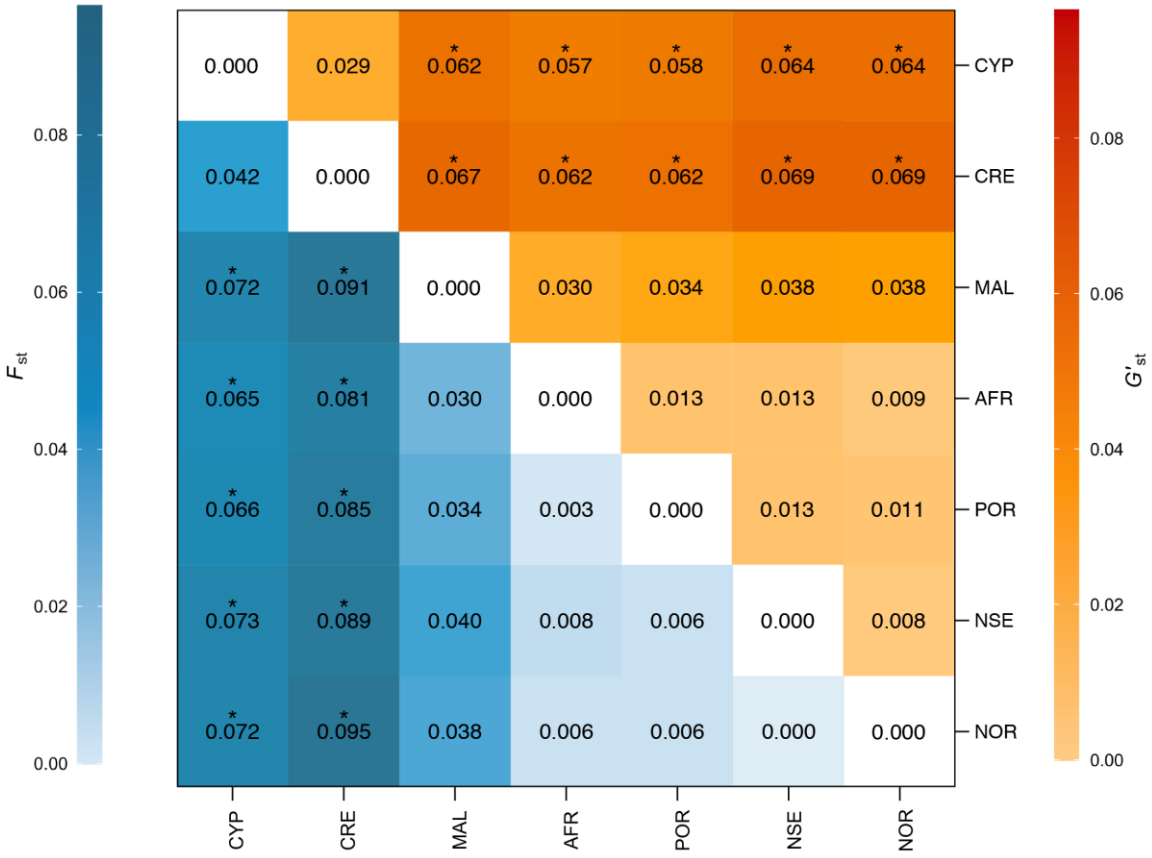


Figure 3.2. Pairwise differentiation among all seven sampling localities of *Scyliorhinus canicula* using a panel of 9,052 SNPs. Below diagonal, pairwise F_{st}. Above diagonal, pairwise G'ST. Values marked with asterisks (*) were significant at 95 % confidence interval. See Table 3.2 for location codes.

Table 3.1. Environmental variables of each *Scyliorhinus canicula* sample sites. Variables extracted from the Bio-ORACLE repository (Assis et al. 2017) using the geographical coordinates of each sample site. SST, sea surface temperature; SBT, sea bottom temperature; SB, sea bottom.

				Coordinates		Environmental variables						
Sea	**Sample location**	**Code**	**Sample size**	**Lat**	**Long**	**Mean SST**	**SST range**	**Mean SBT**	**SBT range**	**SB salinity**	**Depth**	**SB Dissolved O2**
Norwegian sea	Norway	NOR	4	69.33	16.88	7.3	10.1	6.5	4.1	35.1	153	288.9
North Sea	North Sea	NSE	6	58.07	-1.14	10.2	8.6	7.7	4.1	35.2	107	265.8
North Atlantic	Portugal	POR	5	38.04	-9.14	17.3	6.9	12.4	1.5	35.7	359	201
North Atlantic	Morocco	AFR	6	33.66	-7.65	19.5	8.5	18.6	6.1	36.5	27	245.8
Balearic Sea	Mallorca	MAL	5	39.91	3.57	19.7	13.4	14.8	5.3	38.1	68	253
Sea of Crete	Crete	CRE	6	35.43	25.11	20.7	12	15.4	1.2	39	227	207.3
Levantine Sea	Cyprus	CYP	6	34.93	33.86	22.4	13	17.1	2	39.1	123	233.2

Table 3.2. Summary of sample size and genetic diversity of seven *Scyliorhinus canicula* sample site using a panel of 9,052 SNPs. *n*, number of individuals; Depth, mean loci read depth; GCR, genotype call rate; NPL, number of polymorphic loci, H_o, overserved heterozygosity; Pi, nucleotide diversity; F_{is}, Wrights inbreeding coefficient.

Code	***n***	**Depth**	**GCR %**	**NPL**	**PPL %**	**Private alleles**	**H_o**	**H_e**	**Pi**	**F_{is}**
NOR	4	43.3	99.6	5392	59.6	8	0.204	0.194	0.221	0.036
NSE	6	40.5	99.3	6394	70.6	15	0.208	0.206	0.225	0.041
POR	5	40.3	99.0	5991	66.2	7	0.208	0.200	0.222	0.032
AFR	6	33.0	98.8	6456	71.3	16	0.207	0.205	0.224	0.039
MAL	5	33.6	99.0	5547	61.3	17	0.199	0.191	0.213	0.030
CRE	6	40.1	97.8	5404	59.7	30	0.231	0.182	0.200	-0.067
CYP	6	24.3	97.8	5601	61.9	23	0.191	0.187	0.204	0.032

Global F_{ST} for the 9,052 SNP panel was 0.045, while global F_{ST} for the neutral and outlier SNP datasets were 0.035 and 0.170, respectively. Overall, marked population structure was evident (Figure 3.2; Figure 3.3a) and is best represented by three population clusters: the NEA (Norway, North Sea, Portugal, Africa), western Mediterranean (Mallorca) and eastern Mediterranean (Crete, Cyprus) when using the full panel of 9052 SNPs. The highest levels of differentiation were observed between the NEA and eastern Mediterranean populations in both 9,052 SNP (Figure 3.2) and 8,622 neutral SNP datasets (Figure S3.6), with Mallorca showing more genetic similarity to the NEA population (Figure 3.2; Figure 3.3a; Figure S3.6). Despite no significant differentiation between Crete and Cyprus using F_{ST} (Figure 3.2) there was evidence of divergence with clear separation in the Mediterranean PCA (Figure 3.3c) and the structure analysis (Figure S3.4). This was further exaggerated when only using outlier SNPs identified in all three outlier analysis (Figure S3.5c).

Intraspecific variation within the NEA using all 9052 SNPs showed some evidence of a north to south genetic cline (Figure 3.3b), although pairwise F_{ST} values observed between localities were low (Figure 3.2; Figure S3.6). The genetic clustering of samples sites despite low or insignificant pairwise F_{ST} values suggests that a small number of SNPs could be responsible for the pattern and/or gene flow between neighbouring sites is diluting the signature. This is evident in the structure analysis which shows some admixture between neighbouring sites in the NEA (Figure S3.4). However, when only using a panel of the 430 outlier SNPs distinct clustering in the NEA was seen, separating Portugal from the cline with Africa having an intermediate position. This suggest that selection to local environments could be driving some of the differentiation seen in the NEA, particular in Portugal**.**

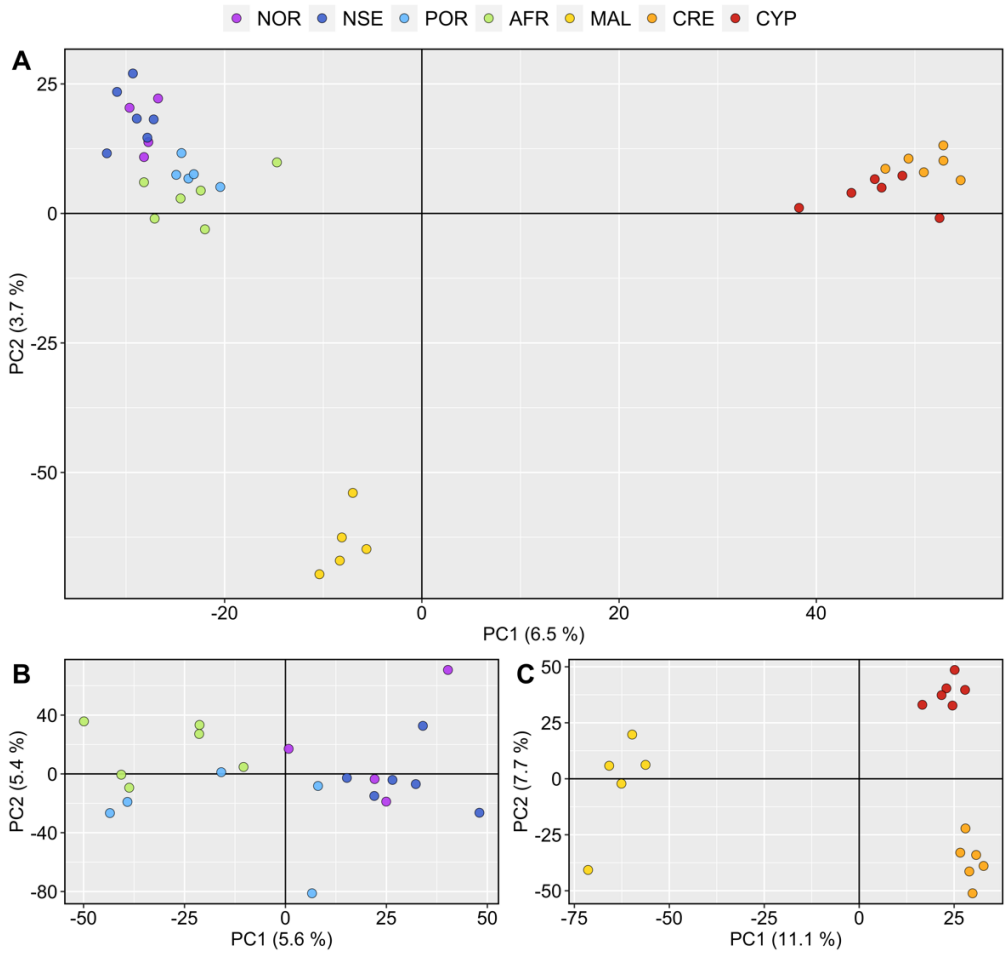


Figure 3.3. Principal component analysis (PCA) of genetic diversity between sample localities using 9052 SNPs: (a) all samples within both the Atlantic and Mediterranean; (b) only Atlantic; (c) only Mediterranean. The first principal component (PC1) and second component (PC2) explain percentage of genetic variation. See Table 3.2 for location codes.

3.4.3 Outlier detection and environmental association analysis

148 SNPs were identified in PCAdapt as outliers. Significant association with at least one environmental variable was detected in 173 SNP loci in LFMM (Figure 3.5b) and 138 in BayPass (Figure 3.5c), of which 18 SNPs were identified in both approaches (Figure 3.5d). The number and patterns of SNPs correlated to each environmental variable varied between each EAA (Figure 3.5b, c). LFMM showed most SNPs to be

significantly correlated with mean SST (45), mean SBT (63) and depth (89), whereas in BayPass most were correlated with SST range (55), SB salinity (39) and oxygen (40). Some SNPs were found to be associated with two or more environmental variables in both LFMM and BayPass, with 56 and 18, respectively (Table S3.4). Of the 56 shared SNPs identified in LFMM, the majority (62%) where associated with both mean SST and mean SBT, 12 (21%) with SST mean and oxygen and 11 (20%) with both oxygen and depth (Table S3.4). In BayPass, the majority were shared between SBT range and oxygen (39%), mean SST and mean SBT (28%) and SST range and salinity (22%; Table S3.4). When accounting for duplications of the same SNP in different environmental factors, 287 unique SNPs were found across both EAAs (Figure 3.5d). Out of the 148 SNPs identified in PCAdapt, 10 overlapped with LFMM and 16 with BayPass. Overall, the three analyses identified a total of 430 unique SNPs putatively under selection and considered herein as outlier loci (5.17% of total SNPs; Figure 3.5d). Of these SNPs, 34 were identified in at least two outlier methods (Figure 3.5d). Of these, 14 were strongly associated with SST range, 10 with SBT mean, 10 with oxygen, nine with SST mean, nine with salinity, seven with depth and three with SBT range (Table S3.4).

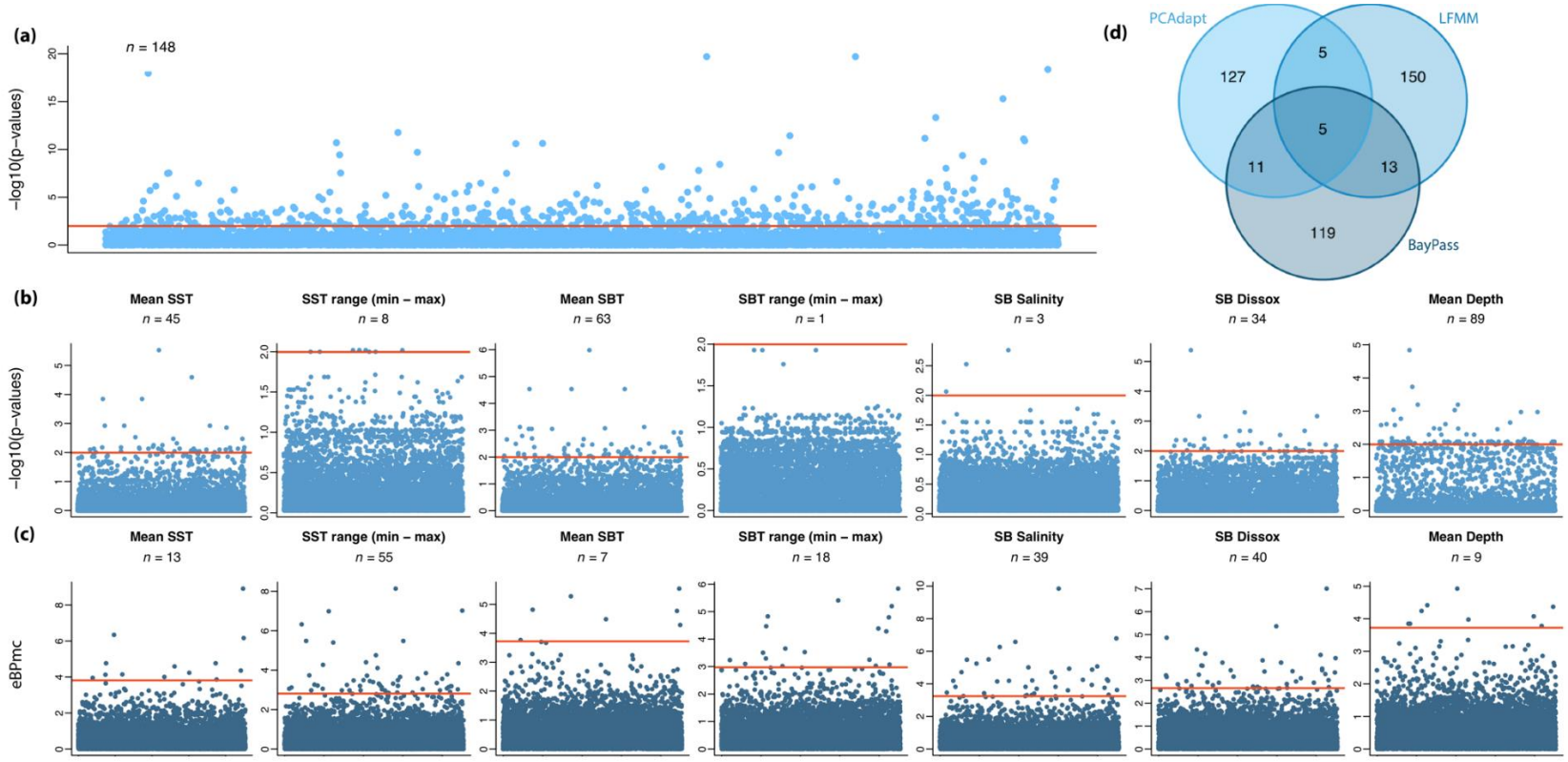


Figure 3.4. SNPs associated with adaptive divergence in the small-spotted catshark. (a) -log10(p-value) from PCAdapt for each locus, (b) -log10(p-value) from LFMM for each locus-environmental pair, (c) empirical Bayesian p-value (eBPmc) for each locus-environmental pair, (c) empirical Bayesian *p*-value (eBPmc) for each locus-environmental pair. Each point represent one of the 9,052 SNPs analysed. The solid line indicated the significant threshold for each method. SNPs with an -log10(*p*-value) > 2 or eBPmc > 1% significance threshold were considered significant signatures of adaptive divergence. *n* corresponds to the number of significant SNPs identified. (d) Venn diagram showing the total amount of SNPs identified in each method and their overlap between methods.

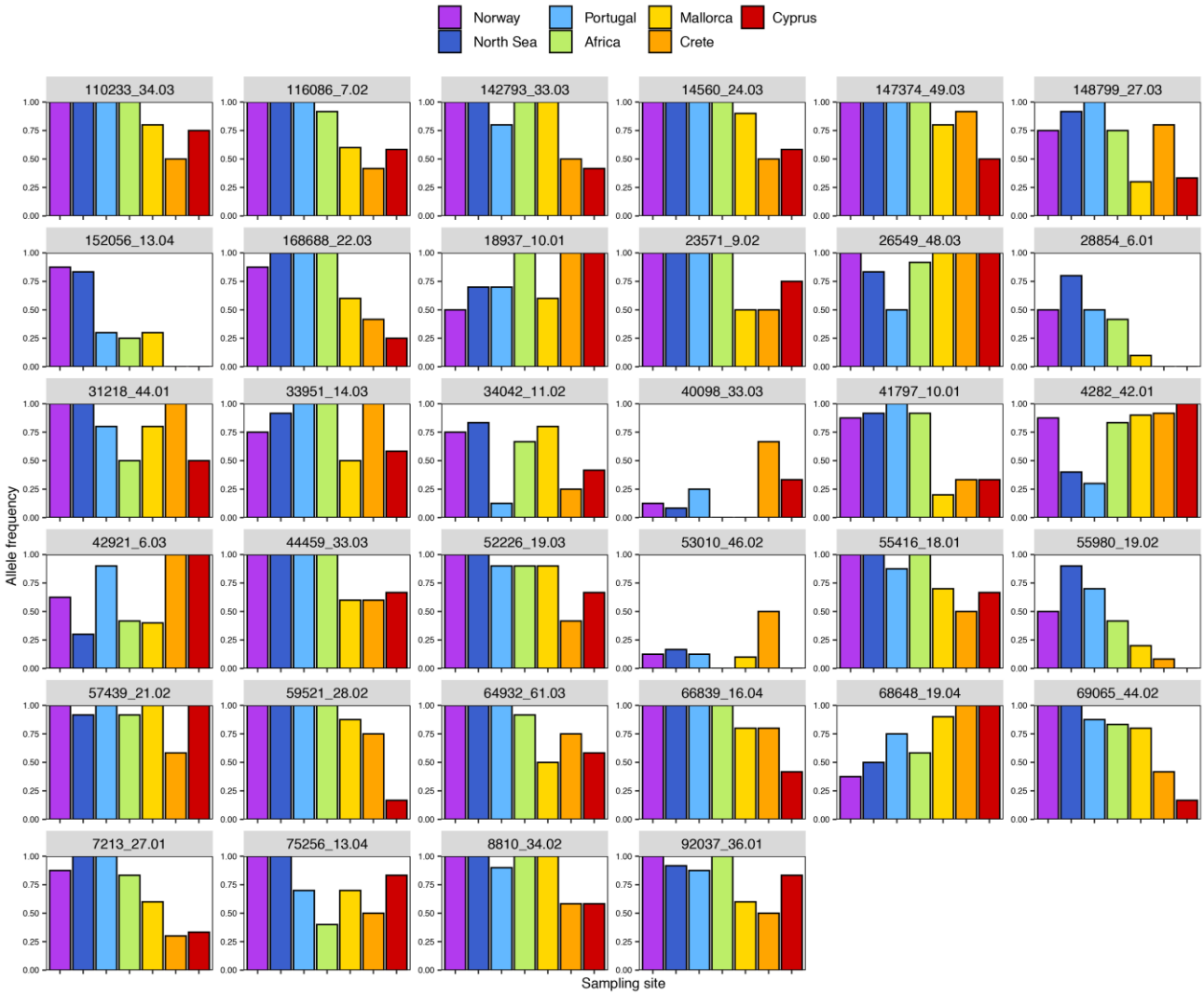


Figure 3.5. Population allele frequency of one allele for each of the 34 outlier SNPs identified in at least two outlier methods. Colours denote each population.

The population allele frequency of one allele for each of the 34 outlier SNPs identified in two or more outlier methods were visualised (Figure 3.5). This showed both clear allele frequency differences between sites from the NEA and the Mediterranean but also potential signatures of convergent evolution between these regions. Alleles frequency for SNPs 110233, 44459, 55416, 14560, 115086 and 66939 were generally high in NEA populations with some SNPs showing clinal patterns (31216, 152056, 75256; Figure 3.5) supporting the genetic cline observed in the PCA analysis (Figure 3.3; Figure S3.5). These SNPs were related to temperature

in both LFMM and BayPass (Table S3.4) and suggest its importance in driving this cline. Putative evidence of parallel genetic divergence (i.e. convergence of allele frequency pattern) can be seen in SNP18937 where allele frequency were highest in the warmest regions of both the NEA (Africa) and the Mediterranean (Crete, Cyprus) and strongly associated with temperature in both LFMM and BayPass (Table S3.4).

3.4.4 Functional annotation

RAD-tags of the 430 outlier loci had a mean sequence length of 63 bp (50 bp – 121 bp). A total of 66 (15.4 %) tags yielding unique matches (e-value < 1e-3) to small-spotted catshark transcripts (Mulley *et al.*, 2014), of which 22 could be assigned functional proteins from other organisms in the UniProtKB reference proteomes/Swiss-Prot database and eight of which had corresponding GO-terms (Table 3.3). The compilation of the biological functions using GO terms in REVIGO (Supek *et al.,* 2011) showed that the three loci were associated to DNA transcription, cytoskeletal organisation and cellular iron ion homeostasis, and eight had chemical functions associated to catalytic activity, cell growth, oxygen reduction (redox) reaction, metal ion binding and nucleic acid binding. Three of these were loci identified in two or more outlier analysis (IDs: 7213, 55980, 44459). Loci 7213 was associated with salinity in both EAA whose function appears to be related to extracellular matrix (ECM) components such as glycosaminoglycans (GAGs), loci 55980 was mapped to with the protein Ferritin linked to cellular iron ion homeostasis, and loci 44459 was associated with temperature and attributed to the zinc finger protein involved in zinc iron binding.

Table 3.3. Outlier loci identified in each analysis (P = PCAdapt, L = LFMM, B = BayPass) which had significant corresponding functions in the UniProtKB reference proteomes/Swiss_Prot database. The associated environmental variable of each SNP is also reported. GO-terms obtained from the UniProt website. **SNPs identified in all three analysis. *SNPs identified in two of analysis.

			Environmental variables							UniProtKB/Swiss_Prot description			
SNP	Length	Outlier	SST mean	SST range	SBT mean	SBT range	SB salinity	SB dissox	Depth	Protein	E-value	% ID	GO-term
7213**	69	P		B			BL			Collagen-containing extracellular matrix	8.30E-03	56%	GO:006202
55980*	72		L	BL	L		L	B		Ferritin – cellular iron ion homeostasis	1.90E-11	96.40%	GO:0006879
44459*	66	P		B						Zinc Finger, CCHC-type	3.80E-10	72.70%	GO:0008270
38377	50			B				B		LINE-1 retrotransposable element ORF1 protein	7.50E-04	50%	N/A
63563	53			B						ANK_REP_REGION domain-containing protein	1.50E-10	73%	N/A
91669	57							B		DUF1891 domain-containing protein	2.23E-11	74.40%	GO:0000226
95652	63							B		Zinc finger protein 1110	6.00E-10	75.80%	GO:0046872
136254	51			B						Glia maturation factor	1.00E-03	100%	GO:0008083
61411	57								L	Reverse transcriptase domain-containing protein	1.00E-08	78.80%	GO:0016706
62644	70								L	Reverse transcriptase domain-containing protein	5.43E-03	54.50%	GO:0003824
65041	54							L		RNA-directed DNA polymerase	2.70E-12	87.90%	N/A
65623	50		L		L					5'-nucleotidase domain-containing protein 2	7.93E-12	81.80%	N/A
80766	51							L	L	Reverse transcriptase domain-containing protein	2.00E-03	72.70%	N/A
107174	59								L	PHD-type domain-containing protein	4.00E-06	76.90%	GO:0046872
12701	64	P								Integrase catalytic domain-containing protein	1.74E-04	72%	GO:0003676
72364	110	P								Alpha-2B adrenergic receptor	2.20E-08	93.90%	N/A
84415	53	P								Kit ligand – stem cell factor	9.30E-04	54.80%	N/A
106960	51	P								Harbinger transposase-derived nuclease	9.40E-07	53.70%	N/A
114685	60	P								LINE-1 type transposase domain-containing protein 1	7.50E-03	48.50%	N/A
123215	56	P								Reverse transcriptase domain-containing protein	9.20E-07	65.60%	GO:0016706
252246	71	P								Rab-GAP TBC domain-containing protein	4.90E-05	72.70%	N/A

3.5. Discussion

Using a panel of 9052 SNPs and a seascape genomics approach this study identified a set of putatively selected loci with allele frequencies strongly correlated with habitat differences in temperature, salinity, oxygen, and depth in the small-spotted catshark. Furthermore, fine-scale population structuring was revealed in both the Mediterranean and NEA that was emphasised when only using a panel of SNP identified in the outlier analyses. These results suggest that differences in environmental factors across the small-spotted catsharks range could be driving selective divergence. Despite concerns of how shark populations might response to climate change, this is one of the first studies addressing the genomic basis of adaptation in an elasmobranch species and offers important scope for future research.

The results confirmed and provided greater resolution into the spatial population structure of the small-spotted catshark. Using a full panel of 9052 SNPs, estimates of overall genetic structure were consistent with previous genetic population studies of the small-spotted catshark, with clear separation of the NEA and Mediterranean regions (Barbieri *et al.*, 2014; Gubili *et al.*, 2014) and distinct clusters of western and eastern Mediterranean groups (Gubili *et al.*, 2014; V. Kousteni *et al.*, 2015). These are patterns shared by many marine taxa, including elasmobranchs, and thought to be the footprint of historical isolation (Patarnello, Volckaert and Castilho, 2007; Griffiths *et al.*, 2011; Leone *et al.*, 2017; Maggio *et al.*, 2019). However, results also suggest a genetic cline in the NEA and further fine-scale structuring in the eastern particularly when analysing only outlier SNPs.

The genetic cline seen in NEA suggests this region is more structured than originally proposed using mtDNA and microsatellite data (Gubili et al 2014). Although much weaker when only analysing neutral SNPs, a genetic cline was still evident in the PCA analysis (Figure S3.5b) and could be explained by isolation by distance and/or recolonization from northern and/or southern glacial refugium following the last glacial maximum (LGM) as proposed by Manuzzi et al. (2019). This is a common biogeographic history for other marine species in the NEA (Maggs *et al.*, 2008; Diopere *et al.*, 2018; Jenkins *et al.*, 2019). However, results suggests that local adaptation could be a causal factor for the genetic structuring observed in the NEA, with the cline becoming more apparent when analysing only outlier SNPs (Figure S3.5) and clinal patterns seen in some allele frequencies linked to temperature (Figure 3.5). Furthermore, overall pairwise values for the 8622 neutral SNPs dataset (Figure S3.6) were lower, suggesting SNPs identified as putative outliers significantly contribute to the overall signal of genetic differentiation seen in the 9052 SNP dataset. Selection also seems to explain some of the differentiation seen in the Mediterranean with outlier loci further separating Crete and Cyprus populations. Evidence of local adaptation in both these regions is not unprecedented, being reported in other demersal species of fish (Milano *et al.*, 2014; Berg *et al.*, 2015; Diopere *et al.*, 2018) and invertebrates (Vendrami *et al.*, 2019) in the NEA, and in the Mediterranean (Milano *et al.*, 2014; Carlos Carreras *et al.*, 2020; Torrado *et al.*, 2020) the Mediterranean, of which sea temperature and salinity were predominant drivers. The fact that outlier loci were informative for both the NEA and Mediterranean further suggests some loci may have undergone convergent genetic divergence (Bierne, Gagnaire and David, 2013). This was best demonstrated in SNP 18937 (Figure 3.5) which suggests independently evolved

traits in response to the higher temperatures in Africa and the eastern Mediterranean.

3.5.1 Genomic evidence of local adaptation

A growing number of studies employing landscape/seascape genomics continue to demonstrate that variation in natural selection across a species' range can maintain fitness-related diversity in populations (Berg *et al.*, 2015; Selkoe *et al.*, 2016; Diopere *et al.*, 2018; Simon Bernatchez *et al.*, 2019; Torrado *et al.*, 2020). Furthermore, OA and EAA are becoming increasingly popular methods for identifying putative directional selection (Rellstab *et al.*, 2015; Hoban *et al.*, 2016) and offers particular promise for this field (Selkoe *et al.*, 2016; Liggins, Treml and Riginos, 2020). However, one notable aspect of this study was that only five overlapping SNPs identified were found between all methods. This does not necessarily indicate a lack of power in the analysis but the different sensitivities of each approach and the ability to detect weak selection signatures (e.g. polygenic traits; Frichot and Francois, 2015; Gautier, 2015; Ahrens *et al.*, 2018). Similar discrepancies have been observed in other seascape genomic studies (Vendrami *et al.*, 2019) and highlights the importance of implementing several methods to capture true signatures of adaptation (Hoban *et al.*, 2016; Ahrens *et al.*, 2018; Liggins, Treml and Riginos, 2020). Nonetheless, 430 outlier loci associated with environmental variation were identified, and a further 34 strong candidates of selection identified in two or more outlier analysis. This conservative strategy might increase the risk of removing true candidate SNPs from the dataset, but it better accounts for the potential of false-positives (Hoban *et al.*, 2016; Ahrens *et al.*, 2018).

The biology and distribution of coastal shark species are known to be influenced by environmental factors such as temperature, salinity, oxygen, and depth (Carlisle and Starr, 2009; Froeschke, Stunz and Wildhaber, 2010; Speed *et al.*, 2010; Ward-Paige *et al.*, 2015; Bangley *et al.*, 2018; Hyatt and Anderson P. A. &. O'Donnell, 2018). Yet, it is still poorly understood how shark populations are going to respond to anticipated environmental change in these regions (Dye *et al.*, 2013; Mohamed Shaltout and Omstedt, 2014; Skliris *et al.*, 2018; Cheng *et al.*, 2019). For the same reasons shark populations are extremely vulnerable to overexploitation (Dulvy *et al.*, 2014), slow growth, late sexual maturity, and low fecundity also offers limited scope for adaptation in the next century. As such, it is important to understand the standing genetic variation in sharks and the key evolutionary drivers, in order to understand how they are likely to respond in future scenarios.

Most outlier loci were found to be significantly related to variation in temperature, with 26 of the 34 loci identified in at least two outlier analysis associated with at least one temperature variable. Sea temperature varies greatly over the small-spotted catsharks range, from 2 - 29 °C in NEA and 16 - 28 °C in Mediterranean (Assis *et al.*, 2017), and has shown to be a strong selective pressure in other marine species with similar distributions in the NEA and Mediterranean (Milano *et al.*, 2014; Diopere *et al.*, 2018; Vendrami *et al.*, 2019; Torrado *et al.*, 2020). In many regards, temperature is also thought to influence the biology and life-history of the small-spotted catshark, e.g. driving size variation (Henderson and Casey, 2001; Rodriguez-Cabello *et al.*, 2004; Ivory, Jeal and CP., 2005; Kousteni, Kontopoulou and Megalofonou, 2010; Finotto *et al.*, 2015), affecting spawning (Ellis and Shackley, 1997; Capape *et*

al., 2008, 2014) and embryotic development (Thomason *et al.*, 1996; Ellis and Shackley, 1997; Musa *et al.*, 2020). However, the degree to which traits are controlled by phenotypic plasticity or genetics is still largely unknown. Some studies show that growth performance in oviparous elasmobranchs could be a result of plasticity to temperature, particularly during the developmental stage, but they have failed to exclude the role of genetics (Rosa *et al.*, 2014; Hume, 2019; Izzo and BM., 2020; Musa *et al.*, 2020). This study suggests that some traits could be under genetic control. Allelic variation in a zinc finger protein linked with loci 44459 was significantly correlated with temperature. Although the ultimate function and purpose of most poly-zinc-finger proteins are unknown, they are believed to contribute to rapid morphological and behavioural evolution by modulating transcription of developmental genes (Emerson and Thomas, 2009). Thus, the expression of this protein may play an important role in physical adaptation of the small-spotted catshark to temperature, but further study is needed regarding the causal role of these polymorphism associations.

Both the NEA and Mediterranean show clines in salinity, increasing north to south in the NEA (~34.5 - 36 ppt) and increasing west to east in the Mediterranean (~36 – 39 ppt; Assis et al. 2017; Skliris et al. 2018). The most pronounced step comes at the Almeria-Oran Front (AOF), a common genetic break for many marine species (Pascual et al. 2017; Patarnello et al. 2007), which sees a 2 ppt increase in salinity over a distance of 2 km (Pascual *et al.*, 2017). Behavioural changes related to salinity in elasmobranch have been well described (Speed *et al.*, 2010; Bangley *et al.*, 2018; Hyatt and Anderson P. A. &. O'Donnell, 2018) indicating that salinity may be a strong selective pressure

for elasmobranchs, as suggested for other marine fish (Limborg *et al.*, 2012; Papakostas *et al.*, 2014; Berg *et al.*, 2015) and invertebrate species (Bernatchez, *et al.*, 2019; Carreras *et al.*, 2020). Interestingly, 41 outlier loci were related to salinity in this study, further suggesting its selective importance. Of these, loci 7213 showed high sequence homology to extracellular matrix (ECM) components such as glycosaminoglycans (GAGs). These are known to play an important role in homeostasis (Frantz, Stewart and Weaver, 2010) and could be highly important for regulation to salinity. Thus, adaptation to salinity could be maintaining genetic differentiation in the small-spotted catshark, particularly in areas that show stark differences in salinity, such as the transition zone between the NEA and the Mediterranean. Furthermore, salinity is predicted to shift in light of climate change and ocean warming (Mohamed Shaltout and Omstedt, 2014; Cheng *et al.*, 2019), particularly in the Mediterranean (Skliris *et al.*, 2018), posing a potential future selective pressure for coastal elasmobranch species.

Oxygen decreases west to east in the Mediterranean (Mavropoulou, Vervatis and Sofianos, 2020) and increases with latitude in the NEA (Assis *et al.*, 2017). It also decreases with depth (Miyake and Saruhashi, 1956). Here, 72 outlier loci were correlated with variation in oxygen, including three highlighted across two of the methods applied suggesting its potential importance as a selection pressure. Similar to temperature and salinity, oxygen has been previously reported to play a role in the distribution of several shark and ray species (Heithaus *et al.*, 2009; Espinoza, Farrugia and Lowe, 2011; Drymon *et al.*, 2013), alongside effecting swimming behaviour (Metcalf and Butler, 1984; Carlson and Parsons, 2001) and metabolic performance (Crear *et al.*, 2019).

Furthermore, hypoxia has been shown to increase embryotic mortality in the small-spotted catshark (Musa *et al.*, 2020), and could be a determinate as to why females often ascend to shallower depths to deposit their eggs (Compagno, Dando and Fowler, 2005; Powter and Gladstone, 2008). Interestingly, loci 55980, showed strong homology with Ferritin, a protein important for iron storage and release (Chasteen and Harrison, 1999), but which has also been linked to stresses such an anoxia in an marine invertebrate (Larade and Storey, 2004). Thus, it can be speculated that the expression of this protein may be important for adaptation to reduced oxygen levels, particularly for populations which inhabitant greater depth and/or warmer waters. Although these results can only be speculative at this stage, the selective importance of oxygen should not be underestimated in this species and for other elasmobranchs, particularly oviparous species which are unable to modulate their developmental environments (Compagno, Dando and Fowler, 2005; Powter and Gladstone, 2008).

Finally, 98 loci were significantly associated with depth, although predominately in LFMM. Depth is inversely related to many key environmental factors (temperature, food availability, oxygen) and it is difficult to assert the contribution it may be having in adaptive divergence. For example, five out of the seven loci associated with depth and identified in at least two outlier analysis methods, were also associated with variation in another environmental factor, notably temperature or oxygen (Table S3.5). Nonetheless, depth has often been ascribed to the complex genetic structuring of the small-spotted catshark in the Mediterranean (Gubili *et al.*, 2014; V. Kousteni *et al.*, 2015;

Ramírez-Amaro *et al.*, 2018) and its inter-connectivity with key environmental factors may be of a high selective importance in this species.

3.5.2. Elasmobranch genomics

Genetic and genomic research on elasmobranch has generally lagged behind studies involving other marine taxa, but increasing interest and concern over the status of elasmobranchs (Dulvy *et al.*, 2014, 2017) provides motivation to harness new genomic technologies and techniques (Li *et al.*, 2013; Bernatchez *et al.*, 2017; Ovenden *et al.*, 2018; Rodrigo Rodrigues Domingues, Hilsdorf and Gadig, 2018). Here, seascape genomics was applied to an elasmobranch species for the first time and showcases its usefulness in determining putative patterns of local adaptation and resolving fine-scale population structure for future management. However, it also highlights some pitfalls for future genomic studies of elasmobranchs. Unfortunately, only 65 of the 430 outlier loci yielding significant, unambiguous matches to the small-spotted catshark transcriptome (Mulley *et al.*, 2014), of which only 22 could then be annotated to functional proteins. This was partly a product of the small loci sequences generate from the ddRAD protocol, but also from the general lack of well-annotated genomic resources for elasmobranchs, although progress is being made with five elasmobranch genomes now sequenced (Venkatesh *et al.*, 2014; Read *et al.*, 2017; Yuichiro Hara *et al.*, 2018; Marra *et al.*, 2019). The lack of genomic resources is understandable given elasmobranchs have large genomes with large repeat content and sparse distribution of coding genes (Venkatesh *et al.*, 2014; Yuichiro Hara *et al.*, 2018), but nonetheless, makes it difficult to ascertain the selective importance of identified candidate SNPs.

The study also highlighted some limitations of the genomic approaches used. The ability of RAD-seq to identify adaptive loci has been questioned, with criticism largely reflective of the small percentage of the genome covered by RAD-seq methods and the sparseness of markers reducing the potential to identify functional loci (Fountain *et al.*, 2016; Lowry *et al.*, 2017; Benjelloun *et al.*, 2019). In a recent study, Benjelloun *et al.* (2019) found that although a panel of 5K to 10K random variants was enough for accurate estimations of genome diversity, much higher-density panels of at least 1M were required for the detection of signatures of selection, such as that produced by whole genome sequencing (WGS). For example, Naval-Sanchez *et al.* (2020) used 5,001,083 high quality SNPs to search for signatures of selection in Atlantic salmon (*Salmo salar*). Therefore, it has to be recognised that although the 9052 SNPs identified in this study offer greater resolution into population structure of the small-spotted catshark, the panel is only a small representation of the millions of SNPs, within the few tens of thousands functional loci, often found in vertebrates. Given the large genome size of elasmobranchs (3.8-6.7 Gb), it is highly probable that many functional loci would have been missed in the genome scans implemented in this study. That being said, this study does other an important first step in searching for adaptive variance in elasmobranchs and more studies are encouraged, particularly employing WGS.

Reassuringly, genomic studies of elasmobranchs have increased in the last five years, and as sequencing costs become ever cheaper, so does the prospect for more genomic resources and the potential for WGS studies. For example, the development of a well-annotated genome for *Scyliorhinus canicula* is underway as part of the Sanger Institute 25 Genomes for 25 Years project

(https://www.sanger.ac.uk/collaboration/25-genomes-25-years/) and offers exciting scope for elasmobranch evolutionary biology and conservation. Mapping the location of outlier SNPs from this study to a complete genome could help discern how many SNPs are located in a genomic island of differentiation and help link more loci to genes and function. Furthermore, alternative genomic approaches, such as hybridisation-based green capture (Li et al. 2013), show exciting promise and have been successfully used to determine the evolutionary history and demography of a black-tip reef shark *Carcharhinus melanopterus* by targeting known, highly divergent genes in fewer individuals (Delser *et al.*, 2016). As more elasmobranch genomes are sequenced, further comparison will continue to improve our understanding of the capacity of elasmobranchs to adapt to environmental stressors, and ultimately lead to their better management (Allendorf, Hohenlohe and Luikart, 2010; Hoban *et al.*, 2016; Domingues *et al.*, 2019; Waldvogel *et al.*, 2020). An important step to this is large comparative whole genome studies within and between species which, to date, have yet to be conducted on any elasmobranchs. Nevertheless, given that both the Mediterranean Sea and NEA have already seen a rise in sea temperatures (Dye *et al.*, 2013; M Shaltout and Omstedt, 2014; Cheng *et al.*, 2019), and elasmobranch populations continue to decline (Dulvy *et al.*, 2014, 2017), the importance expanding genomic resources and our understanding of elasmobranch biology, behaviour and conservation is paramount.

3.6 References

Aat, B., Mouffok, S. and Boutiba, Z. (2012) ‘Reproductive biology and growth of Lesser Spotted Dogfish Scyliorhinus canicula (Linnaeus, 1758) in Western Algerian coasts (Chondrichthyes, Scyliorhinidae)’, *Biodiversity Journal*, 3(1), pp. 41–48.

Ahrens, C. *et al.* (2018) ‘The search for loci under selection: Trends, biases and progress’, *Molecular Ecology*, 27(6), pp. 1342–1356. Available at: https://doi.

Allendorf, F. W., Hohenlohe, P. A. and Luikart, G. (2010) ‘Genomics and the future of conservation genetics’, *Nature Reviews Genetics*, 11, pp. 697–709.

Angilletta, M. J. and Dunham, A. E. (2003) ‘The temperature-size rule in ectotherms: simple evolutionary explanations may not be general’, *Am Nat*, 162(3), pp. 332–342. doi: 10.1086/377187.

Arnold, B. *et al.* (2013) ‘RADseq underestimates diversity and introduces genealogical biases due to nonrandom haplotype sampling’, *Molecular Ecology*, 22(11), pp. 3179–3190. doi: 10.1111/mec.12276.

Assis, J. *et al.* (2017) *Bio-ORACLE v2.0: Extending marine data layers for bioclimatic modelling*. Global Ecology and Biogeography.

Báez, J. C. *et al.* (2020) ‘Ensemble modeling of the potential distribution of the whale shark in the Atlantic Ocean’, *Ecology and Evolution*, 10(1), pp. 175–184. doi: 10.1002/ece3.5884.

Bangley, C. W. *et al.* (2018) ‘Delineation and mapping of coastal shark habitat within a shallow lagoonal estuary’, *PLOS ONE*, 13, p. 4. Available at: https://doi.org/10.1371/journal.pone.0195221.

Barausse, A. *et al.* (2014) ‘The role of fisheries and the environment in driving the decline of elasmobranchs in the northern Adriatic Sea’, *ICES Journal of Marine Science*, 71(7), pp. 1593–1603. doi: 10.1093/icesjms/fst222.

Barbieri, M. *et al.* (2014) ‘Molecular contribution to stock identification in the small-spotted catshark, Scyliorhinus canicula (Chondrichthyes, Scyliorhinidae)’, *Fisheries Research*, 154, pp. 11–16. doi: 10.1016/j.fishres.2014.01.021.

Barker, A. M. *et al.* (2019) ‘Hybridization between sympatric hammerhead sharks in the western North Atlantic Ocean’, *Biology Letters*, 15(4), p. 20190004. doi: 10.1098/rsbl.2019.0004.

Benestan, L. *et al.* (2016) ‘Seascape genomics provides evidence for thermal adaptation and current mediated population structure in {A}merican lobster (Homarus americanus)’, 25(20), pp. 5073–5092.

Benjamini, Y. and Hochberg, Y. (1995) ‘Controlling the false discovery rate: a practical and powerful approach to multiple testing’, *J. Royal Stat Soc. B*, 57(1), pp. 289–300.

Benjelloun, B. *et al.* (2019) ‘An evaluation of sequencing coverage and genotyping strategies to assess neutral and adaptive diversity’, *Molecular Ecology Resources*, 19(6), pp. 1497–1515. doi: 10.1111/1755-0998.13070.

Berg, P. R. *et al.* (2015) ‘Adaptation to Low Salinity Promotes Genomic Divergence in Atlantic Cod (Gadus morhua L.) ’, *Genome Biology and Evolution*, 7(6), pp. 1644–1663. doi: 10.1093/gbe/evv093.

Bernatchez, L. *et al.* (2017) ‘Harnessing the Power of Genomics to Secure the Future of Seafood’, *Trends in Ecology & Evolution*, 32(9), pp. 665–680. doi: 10.1016/j.tree.2017.06.010.

Bernatchez, S *et al.* (2019) ‘Seascape genomics of eastern oyster (Crassostrea virginica) along the Atlantic coast of Canada’, 12(3), pp. 587–609. Available at: https://doi.org/10.1111/eva.12741.

Bernatchez, Simon *et al.* (2019) ‘Seascape genomics of eastern oyster (Crassostrea virginica) along the Atlantic coast of Canada’, *Evolutionary Applications*, 12(3), pp. 587–609. doi: 10.1111/eva.12741.

Bierne, N., Gagnaire, P. A. and David, P. (2013) ‘The geography of introgression in a patchy environment and the thorn in the side of ecological speciation’, *Current Zoology*, 10(1093), pp. 72–86. Available at: https.

Birkmanis, C. A. *et al.* (2020) ‘Future Distribution of Suitable Habitat for Pelagic Sharks in Australia Under Climate Change Models’, *Frontiers in Marine Science*, 7(July), pp. 1–11. doi: 10.3389/fmars.2020.00570.

Bohonak, A. J. (1999) ‘Dispersal, gene flow, and population structure’, *Q Rev Biol*, 74, pp. 21–45.

Budd, A. M. *et al.* (2021) ‘First detection of critically endangered scalloped hammerhead sharks (Sphyrna lewini) in Guam, Micronesia, in five decades using environmental DNA’, *Ecological Indicators*, 127. doi: 10.1016/j.ecolind.2021.107649.

Capape, C. *et al.* (2008) ‘Biological observations on the smallspotted catshark Scyliorhinus canicula (Chondrichthyes: Scyliorhinidae) off the Languedocian coast (southern France, northern Mediterranean)’, *Pan-American Journal of Aquatic Sciences*, 3, pp. 282–289.

Capape, C. *et al.* (2014) ‘Production, maturity, reproductive cycle and fecundity of small-spotted catshark, Scyliorhinus canicula (Chondrichthyes: Scyliorhinidae) from the northern coast of Tunisia (Central Mediterranean)’, *J. Ichthyol*, 54, pp. 111–126. doi: 10.1134/S0032945214010020.

Cardeñosa, D. *et al.* (2017) ‘A multiplex PCR mini-barcode assay to identify processed shark products in the global trade’, *PLoS ONE*, 12(10), pp. 3–11. doi: 10.1371/journal.pone.0185368.

Carlisle, A. B. and Starr, R. M. (2009) ‘Habitat use, residency, and seasonal distribution of female leopard sharks Triakis semifasciata in Elkhorn Slough, California’, *Mar Ecol Prog Ser*, 380, pp. 213–228.

Carlson, J. K. and Parsons, G. R. (2001) ‘The Effects of Hypoxia on Three Sympatric Shark Species: Physiological and Behavioral Responses’, *Environmental Biology of Fishes*, 61, pp. 427–433. Available at: https://doi.org/10.1023/A:1011641302048.

Carreras, C. *et al.* (2017) ‘Population genomics of an endemic Mediterranean fish: Differentiation by fine scale dispersal and adaptation’, *Scientific Reports*, 7, p. 43417. Available at: https.

Carreras, C *et al.* (2020) ‘East is East and West is West: Population genomics and hierarchical analyses reveal genetic structure and adaptation footprints in the keystone species Paracentrotus lividus (Echinoidea)’, *Diversity and Distributions*, 26(3), pp. 382–398. Available at: https://doi.org/10.1111/ddi.13016.

Carreras, Carlos *et al.* (2020) ‘East is East and West is West: Population genomics and hierarchical analyses reveal genetic structure and adaptation footprints in the keystone species Paracentrotus lividus (Echinoidea)’, *Diversity and Distributions*, 26(3), pp. 382–398. doi: 10.1111/ddi.13016.

Castro, J., Picornell, A. and Ramon, M. (1999) ‘Mitochondrial DNA: A tool for populational genetics studies’, *International microbiology : the official journal of the Spanish Society for Microbiology*, 1, pp. 327–332.

Catchen, J. *et al.* (2011) ‘Stacks: building and genotyping loci de novo from short-read sequences’, *Genes, Genomes, Genetics,* 3(1), pp. 171–182.

Chapman, D. D. *et al.* (2015) ‘There and Back Again: A Review of Residency and Return Migrations in Sharks, with Implications for Population Structure and Management’, *Annual Review of Marine Science*, 7(1), pp. 547–570. doi: 10.1146/annurev-marine-010814-015730.

Chasteen, N. D. and Harrison, P. M. (1999) ‘Mineralization in Ferritin: An Efficient Means of Iron Storage’, *Journal of Structural Biology*, 126(3), pp. 182–194. Available at: https://doi.org/10.1006/jsbi.1999.4118.

Chen, H. and Boutros, P. C. V. (2011) ‘a package for the generation of highly-customizable Venn and Euler diagrams in R’, *BMC Bioinformatics*, 12, p. 35. Available at: https://doi.org/10.1186/1471-2105-12-35.

Cheng, L. *et al.* (2019) ‘How fast are the oceans warming’, *Science*, 363(6423), pp. 128–129.

Colloca, F., Scarcella, G. and Libralato, S. (2017) ‘Recent Trends and Impacts of Fisheries Exploitation on Mediterranean Stocks and Ecosystems’, *Frontiers in Marine Science*, 4, p. 244. doi: 10.3389/fmars.2017.00244.

Compagno, L., Dando, M. and Fowler, S. (2005) *A Field Guide to Sharks of the World*. Princeton: Princeton University Press.

Corrigan, S. *et al.* (2016) ‘A multilocus comparative study of dispersal in three codistributed demersal sharks from eastern Australia’, *Canadian Journal of Fisheries and Aquatic Sciences*, 73(3), pp. 406–415. doi: 10.1139/cjfas-2015-0085.

Crear, D. P. *et al.* (2019) ‘The impacts of warming and hypoxia on the performance of an obligate ram ventilator’, *Conservation physiology*, 7, p. 1. Available at: https://doi.org/10.1093/conphys/coz026.

Cure, Katherine *et al.* (2017) ‘Genomic signatures of local adaptation reveal source-sink dynamics in a high gene flow fish species’, *Scientific Reports*, 7(1), pp. 1–10. doi: 10.1038/s41598-017-09224-y.

Cure, K *et al.* (2017) ‘Genomic signatures of local adaptation reveal source-sink dynamics in a high gene flow fish species’, *Scientific Reports*, 7(1), pp. 1–10. Available at: https://doi.org/10.1038/s41598-017-09224-y.

Currie, D. J. *et al.* (2004) ‘Predictions and tests of climate-based hypotheses of broad-scale variation in taxonomic richness’, *Ecology Letters*, 7(12), pp. 1121–1134. doi: 10.1111/j.1461-0248.2004.00671.x.

Danecek, P. *et al.* (2011) ‘The variant call format and VCFtools.’, *Bioinformatics*, 27, pp. 2156–2158.

Daniels, R. R. *et al.* (2019) ‘Looking for local adaptation: Convergent microevolution in aleppo pine (pinus halepensis)’, *Genes*, 10(9). doi: 10.3390/genes10090673.

Davey, J. W. and Blaxter, M. L. (2010) ‘RADSeq: next-generation population genetics’, *Briefings in Functional Genomics.*, 9(5–6), pp. 416–423.

Davidson, L. N. K. (2012) ‘Shark Sanctuaries: Substance or Spin?’, *Science*, 338(6114), pp. 1538–1539. doi: 10.1126/science.338.6114.1538.

Delser, M. *et al.* (2016) ‘Population genomics of C. melanopterus using target gene capture data: demographic inferences and conservation perspectives’, *Sci Rep*, 6, p. 33753. doi: 10.1038/srep33753.

Díaz-Jaimes, P. *et al.* (2020) ‘Population genetic divergence of bonnethead sharks Sphyrna tiburo in the western North Atlantic: Implications for conservation’, *Aquatic Conservation: Marine and Freshwater Ecosystems*, (June), pp. 1–16. doi: 10.1002/aqc.3434.

Diopere, E. *et al.* (2018) ‘Seascape genetics of a flatfish reveals local selection under high levels of gene flow’, *ICES Journal of Marine Science*, 75(2), pp. 675–689. Available at: https://doi.org/10.1093/icesjms/fsx160.

Domingues, R. R. *et al.* (2019) ‘Genetic connectivity and phylogeography of the night shark (Carcharhinus signatus) in the western Atlantic Ocean: Implications for conservation management’, *Aquatic Conservation: Marine and Freshwater Ecosystems*, 29(1), pp. 102–114. doi: 10.1002/aqc.2961.

Domingues, Rodrigo Rodrigues, Hilsdorf, A. W. S. and Gadig, O. B. F. (2018) ‘The importance of considering genetic diversity in shark and ray conservation policies’, *Conservation Genetics*, 19(3), pp. 501–525. doi: 10.1007/s10592-017-1038-3.

Domingues, R R, Hilsdorf, A. W. S. and Gadig, O. B. F. (2018) ‘The importance of considering genetic diversity in shark and ray conservation policies’, *Conservation Genetics*, 19(3), pp. 501–525. Available at: https://doi.org/10.1007/s10592-017-1038-3.

Drymon, J. M. *et al.* (2013) ‘Multiscale analysis of factors that affect the distribution of sharks throughout the northern Gulf of Mexico’, *Fish Bull*, 111(4), pp. 370–380.

Dufresne, F. *et al.* (2013) ‘Recent progress and challenges in population genetics of polyploid organisms: An overview of current state-of-the-art molecular and statistical tools’, *Molecular ecology*, 23, pp. 40–69. doi: 10.1111/mec.12581.

Dulvy, N. *et al.* (2017) ‘Challenges and Priorities in Shark and Ray Conservation.’, *Current Biology*, 27, pp. 565–572. doi: 10.1016/j.cub.2017.04.038.

Dulvy, N. K. *et al.* (2014) ‘Extinction risk and conservation of the world’s sharks and rays’, *eLife*, 2014(3), pp. 1–34. doi: 10.7554/eLife.00590.

Dye, S. R. *et al.* (2013) ‘Impacts of climate change on temperature (air and sea)’, *MCCIP Sci Rev*, pp. 1–12.

Eales, N. (1949) ‘The Food of the Dogfish, Scyliorhinus Caniculus L’, *Journal of the Marine Biological Association of the United Kingdom*, 28(3), pp. 791–793. doi: 10.1017/S0025315400023584.

Ellis, J. R. and Shackley, S. E. (1997) ‘The reproductive biology of Scyliorhinus canicula in the Bristol Channel, UK’, *J. Fish. Biol.*, 51(19), pp. 361–372. doi: 10.1111/j.1095-8649.1997.tb01672.x).

Emerson, R. O. and Thomas, J. H. (2009) ‘Adaptive Evolution in Zinc Finger Transcription Factors’, *PLOS Genetics*, 5(1), p. e1000325. Available at: https://doi.org/10.1371/journal.pgen.1000325.

Espinoza, M., Farrugia, T. J. and Lowe, C. G. (2011) ‘Habitat use, movements and site fidelity of the gray smooth-hound shark (Mustelus californicus Gill 1863) in a newly restored southern California estuary’, *J Exp Mar Bio Ecol*, 401, pp. 63–67.

Evanno, G., Regnaut, S. and Goudet, J. (2005) ‘Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study’, *Molecular Ecology*, 14(2), pp. 2611–2620.

Excoffier, L., Hofer, T. and Foll, M. (2009) ‘Detecting loci under selection in a hierarchically structured population’, *Heredity*, 103(4), pp. 285–298. doi: 10.1038/hdy.2009.74.

Feitosa, L. M. *et al.* (2020) ‘Potential distribution and population trends of the smalltail shark Carcharhinus porosus inferred from species distribution models and historical catch data’, *Aquatic Conservation: Marine and Freshwater Ecosystems*, 30(5), pp. 882–891. doi: 10.1002/aqc.3293.

Ferrari, A. *et al.* (2018) ‘Natural history and molecular evolution of demersal Mediterranean sharks and skates inferred by comparative phylogeographic and demographic analyses’, *PeerJ*, 2018(9). doi: 10.7717/peerj.5560.

Field, I. C. *et al.* (no date) ‘Susceptibility of sharks, rays and chimaeras to global extinction’, *Adv Mar Biol*, 56, pp. 275–363.

Finotto, L. *et al.* (2015) ‘Contrasting life history and reproductive traits in two populations of Scyliorhinus canicula’, *Mar Biol*, 162, pp. 1175–1186. doi: 10.1007/s00227-015-2659-z.

Foll, M. and Gaggiotti, O. (2008) ‘A genome scan method to identify selected loci appropriate for both dominant and codominant markers: A {B}ayesian perspective’, *Genetics*, 180, pp. 977–993. doi: 10.1534/genet ics.108.092221.

Ford, E. (1921) ‘A contribution to our knowledge of the life histories of the dogfishes landed at Plymouth’, *Journ. Mar. Biol. Assoc.*, XII, pp. 468–505.

Fountain, E. D. *et al.* (2016) ‘Finding the right coverage: the impact of coverage and sequence quality on single nucleotide polymorphism genotyping error rates’, *Molecular ecology resources*, 16(4), pp. 966–978. doi: 10.1111/1755-0998.12519.

Francis, R. M. (2017) ‘pophelper: An r package and web app to analyse and visualize population structure’, *Molecular Ecology Resources*, 17, pp. 27–32. Available at: https://doi.org/10.1111/1755-0998.12509.

Frantz, C., Stewart, K. M. and Weaver, V. M. (2010) ‘The extracellular matrix at a glance’, *J. Cell Sci*, 123, pp. 4195–4200. doi: 10.1242/jcs.023820.

Frichot, E. and Francois, O. (2015) ‘LEA: An R package for landscape and ecological association studies’, *Methods Ecol Evol*, 6, pp. 925–929. doi: 10.1111/2041-210X.12382.

Froeschke, J., Stunz, G. W. and Wildhaber, M. L. (2010) ‘Environmental influences on the occurrence of coastal sharks in estuarine waters’, *Mar Ecol Prog Ser*, 407, pp. 279–292. Available at: https://doi.org/10.3354/meps08546.

Fuentes-Pardo, A. P. and Ruzzante, D. E. (2017) ‘Whole-genome sequencing approaches for conservation biology: Advantages, limitations and practical recommendations’, *Molecular Ecology*, 26(20), pp. 5369–5406. doi: 10.1111/mec.14264.

Gaggiotti, O. E. *et al.* (2009) ‘Disentangling the effects of evolutionary, demographic, and environmental factors influencing genetic structure of natural populations: Atlantic herring as a case study’, *Evolution*, 63, pp. 2939–2951. doi: 10.1111/j.1558-5646.2009.00779.x.

Gallego-García, N. *et al.* (2019) ‘Landscape genomic signatures indicate reduced gene flow and forest-associated adaptive divergence in an endangered neotropical turtle’, *Molecular Ecology*, 28(11), pp. 2757–2771. doi: 10.1111/mec.15112.

Gautier, M. *et al.* (2013) ‘Estimation of population allele frequencies from next-generation sequencing data: pool-versus individual-based genotyping’, *Molecular Ecology*, 22(14), pp. 3766–3779. doi: 10.1111/mec.12360.

Gautier, M. (2015) ‘Genome-wide scan for adaptive divergence and association with population-specific covariates’, *Genetics*, 201(4), pp. 1555–1579.

Griffiths, A. *et al.* (2013) ‘DNA barcoding unveils skate (Chondrichthyes: Rajidae) species diversity in “ray” products sold across Ireland and the UK’, *PeerJ*, 1, p. e129. doi: 10.7717/peerj.129.

Griffiths, A. M. *et al.* (2011) 'Levels of connectivity between longnose skate (Dipturus oxyrinchus) in the Mediterranean Sea and the north-eastern Atlantic Ocean', *Conservation Genetics*, 12(2), pp. 577–582. doi: 10.1007/s10592-010-0127-3.

Grummer, J. A. *et al.* (2019) 'Aquatic Landscape Genomics and Environmental Effects on Genetic Variation', *Trends in Ecology and Evolution*, 34(7), pp. 641–654. doi: 10.1016/j.tree.2019.02.013.

Gubili, C. *et al.* (2014) 'A tale of two seas: contrasting patterns of population structure in the small-spotted catshark across Europe', *Royal Society Open Science*, 1(3), pp. 140175–140175. doi: 10.1098/rsos.140175.

Gugger, P. F. *et al.* (2018) 'Applying landscape genomic tools to forest management and restoration of Hawaiian koa (Acacia koa) in a changing environment', *Evolutionary Applications*, 11(2), pp. 231–242. doi: 10.1111/eva.12534.

Hällfors, M. *et al.* (2016) 'Addressing potential local adaptation in species distribution models: Implications for conservation under climate change', *Ecological Applications*, 26. doi: 10.1890/15-0926.

Hara, Yuichiro *et al.* (2018) 'Shark genomes provide insights into elasmobranch evolution and the origin of vertebrates', *Nature Ecology and Evolution*, 2(11), pp. 1761–1771. doi: 10.1038/s41559-018-0673-5.

Hara, Y *et al.* (2018) 'Shark genomes provide insights into elasmobranch evolution and the origin of vertebrates', *Nat Ecol Evol*, 2, pp. 1761–1771. doi: 10.1038/s41559-018-0673-5.

Harvey, M. G. *et al.* (2016) 'Sequence Capture versus Restriction Site Associated DNA Sequencing for Shallow Systematics', *Systematic Biology*, 65(5), pp. 910–924. doi: 10.1093/sysbio/syw036.

Hedrick, P. W. (2005) ‘A standardized genetic differentiation measure’, *Evolution*, 59(8), pp. 1633–1638. doi: PMID: 1632923.

Heithaus, M. R. *et al.* (2009) ‘Physical factors influencing the distribution of a top predator in a subtropical oligotrophic estuary’, *Limnol Oceanogr*, 54(2), pp. 472–482.

Helyar, S. J. *et al.* (2011) ‘Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges’, *Molecular Ecology Resources*, 11, pp. 123–136. doi: 10.1111/j.1755-0998.2010.02943.x.

Henderson, A. C. and Casey, A. (2001) ‘Reproduction and growth in the lesser-spotted dogfish Scyliorhinus canicula (Elasmobranchii; Scyliorhinidae), from the west coast of Ireland’, *Cah. Biol. Mar.*, 42, pp. 397–405.

Heupel, M. R. *et al.* (2014) ‘Sizing up the ecological role of sharks as predators’, *Marine Ecology Progress Series*, 495, pp. 291–298. doi: 10.3354/meps10597.

Hoban, S. *et al.* (2016) ‘Finding the genomic basis of local adaptation: Pitfalls, practical solutions, and future directions’, *American Naturalist*, 188(4), pp. 379–397. doi: 10.1086/688018.

Hobbs, C. A. D. *et al.* (2019) ‘Using DNA Barcoding to Investigate Patterns of Species Utilisation in UK Shark Products Reveals Threatened Species on Sale’, *Scientific Reports*, 9(1), pp. 1–10. doi: 10.1038/s41598-018-38270-3.

Hohenlohe, P. A. *et al.* (2010) ‘Population Genomics of Parallel Adaptation in Threespine Stickleback using Sequenced RAD Tags’, *PLOS Genetics*, 6(2), p. e1000862. Available at: https://doi.org/10.1371/journal.pgen.1000862.

Hueter, R. *et al.* (2004) ‘Evidence of Philopatry in Sharks and Implications for the Management of Shark Fisheries’, *Journal of Northwest Atlantic Fishery Science*, 37, pp. 239–247. doi: 10.2960/J.v35.m493.

Hume, J. B. (2019) ‘Higher temperatures increase developmental rate & reduce body size at hatching in the small?eyed skate Raja microocellata: implications for exploitation of an elasmobranch in warming seas’, *Journal of Fish biology*, 95, pp. 655–658.

Hyatt, M. W. and Anderson P. A. &. O’Donnell, P. (2018) ‘Influence of Temperature, Salinity, and Dissolved Oxygen on the Stress Response of Bull (Carcharhinus leucas) and Bonnethead (Sphyrna tiburo) Sharks after Capture and Handling’, *Journal of Coastal Research*, 34(4), pp. 818–827. doi: 10.2112/JCOASTRES-D-17-00118.1.

ICES (2019) *Working Group on Elasmobranch Fishes (WGEF)*, *ICES Scientific Reports*. Available at: http://doi.org/10.17895/ices.pub.5594%0AEditors.

Ikeda, D. H. *et al.* (2017) ‘Genetically informed ecological niche models improve climate change predictions’, *Global Change Biology*, 23(1), pp. 164–176. doi: 10.1111/gcb.13470.

Ivory, P., Jeal, F. and CP., N. (2005) ‘Age determination, growth and reproduction in the lesser- spotted dogfish, Scyliorhinus canicula (L.)’, *J Northw Atl Fish Sci*, 35, pp. 89–106.

Izzo, C. and BM., G. (2020) ‘Port jackson shark growth is sensitive to temperature change’, *Frontiers in Marine Science*, 7, p. 240. doi: 10.3389/fmars.2020.00240.

Jenkins, T. L. *et al.* (2019) ‘Single nucleotide polymorphisms reveal a genetic cline across the north-east Atlantic and enable powerful population assignment in the European lobster’, *Evolutionary Applications*, 12(10), pp. 1881–1899. doi: 10.1111/eva.12849.

Jirik, K. E. and Lowe, C. G. (2012) ‘An elasmobranch maternity ward: female round stingrays Urobatis halleri use warm, restored estuarine habitat during gestation’, *Journal of Fish Biology*, 80(5), pp. 1227–1245. doi: https://doi.org/10.1111/j.1095-8649.2011.03208.x.

Johri, S., Solanki, J., *et al.* (2019) ‘“Genome skimming” with the MinION hand-held sequencer identifies CITES-listed shark species in India’s exports market’, *Scientific Reports*, 9(1), p. 4476. doi: 10.1038/s41598-019-40940-9.

Johri, S., Doane, M. P., *et al.* (2019) ‘Taking advantage of the genomics revolution for monitoring and conservation of chondrichthyan populations’, *Diversity*, 11(4). doi: 10.3390/d11040049.

Jombart, T. (2008) ‘adegenet: a R package for the multivariate analysis of genetic markers’, *Bioinformatics*, 24(11), pp. 1403–1405. doi: 10.1093/bioinformatics/btn129.

Jones, M. R. and Good, J. M. (2016) ‘Targeted capture in evolutionary and ecological genomics’, *Molecular ecology*. 2015/07/30, 25(1), pp. 185–202. doi: 10.1111/mec.13304.

Junge, C. *et al.* (2019) ‘Comparative population genomics confirms little population structure in two commercially targeted carcharhinid sharks’, *Marine Biology*, 166(2), pp. 1–15. doi: 10.1007/s00227-018-3454-4.

Kawecki, T. J. and Ebert, D. (2004) ‘Conceptual issues in local adaptation’, *Ecology Letters*, 7, pp. 1225–1241.

Keenan, K. *et al.* (2013) ‘diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors’, *Methods in Ecology and Evolution*, 4, pp. 782–788. doi: 10.1111/2041-210X.12067.

Kelley, J. L. *et al.* (2016) ‘The life aquatic: Advances in marine vertebrate genomics’, *Nature Reviews Genetics*, 17(9), pp. 523–534. doi: 10.1038/nrg.2016.66.

Kopelman, N. M. *et al.* (2015) ‘Clumpak: a program for identifying clustering modes and packaging population structure inferences across K’, *Mol Ecol Resour*, 15, pp. 1179–1191.

Kousteni, V. *et al.* (2015) ‘Strong population genetic structure and contrasting demographic histories for the small-spotted catshark (Scyliorhinus canicula) in the Mediterranean Sea’, *Heredity*, 114(3), pp. 333–343. doi: 10.1038/hdy.2014.107.

Kousteni, V *et al.* (2015) ‘Strong population genetic structure and contrasting demographic histories for the small-spotted catshark (Scyliorhinus canicula) in the Mediterranean Sea’, *Heredity*, 114(3), pp. 333–343. doi: 10.1038/hdy.2014.107.

Kousteni, V., Kontopoulou, M. and Megalofonou, P. (2010) ‘Sexual maturity and fecundity of Scyliorhinus canicula (Linnaeus, 1758) in the Aegean Sea’, *Mar. Biol. Res.*, 6, pp. 390–398.

Kraft, D. W. *et al.* (2020) ‘Genomics versus mtDNA for resolving stock structure in the silky shark (Carcharhinus falciformis)’, *PeerJ*. Edited by A. Amorim, 8, p. e10186. doi: 10.7717/peerj.10186.

Larade, K. and Storey, K. B. (2004) ‘Accumulation and translation of ferritin heavy chain transcripts following anoxia exposure in a marine invertebrate’, *The Journal of Experimental Biology*, 207, pp. 1353–1360. doi: 10.1242/jeb.00872.

Laver, T. *et al.* (2015) ‘Assessing the performance of the Oxford Nanopore Technologies MinION’, *Biomolecular Detection and Quantification*, 3, pp. 1–8. doi: https://doi.org/10.1016/j.bdq.2015.02.001.

Leone, A. *et al.* (2017) ‘Genetic differentiation and phylogeography of Mediterranean-North Eastern Atlantic blue shark (Prionace glauca, L. 1758) using mitochondrial DNA: Panmixia or complex stock structure?’, *PeerJ*, 2017(12). doi: 10.7717/peerj.4112.

Li, C. *et al.* (2013) ‘Capturing protein-coding genes across highly divergent species’, *Biotechniques*, 54, pp. 321–326.

Liggins, L., Treml, E. A. and Riginos, C. (2020) ‘Seascape Genomics: Contextualizing Adaptive and Neutral Genomic Variation in the Ocean Environment’, in Oleksiak, M. F. and Rajora, O. P. (eds) *Population Genomics: Marine Organisms*. Cham: Springer International Publishing, pp. 171–218. doi: 10.1007/13836_2019_68.

Limborg, M. T. *et al.* (2012) ‘Environmental selection on transcriptome derived SNPs in a high gene flow marine fish, the Atlantic herring (Clupea harengus). Molecular Ecology’, *Molecular Ecology*, 21, pp. 3686–3703.

Litvinov, F. (2003) ‘Sexual dimorphism as an index of the isolation of West African populations of the catshark S. canicula’, *J. Ichthyol.*, 43, pp. 81–85.

Lowry, D. B. *et al.* (2017) ‘Breaking RAD: an evaluation of the utility of restriction site-associated DNA sequencing for genome scans of adaptation’, *Molecular ecology resources*, 17(2), pp. 142–152. doi: 10.1111/1755-0998.12635.

Maggio, T. *et al.* (2019) ‘Historical separation and present-day structure of common dolphinfish (Coryphaena hippurus) populations in the Atlantic Ocean and Mediterranean Sea’, *ICES Journal of Marine Science*, 76(4), pp. 1028–1038. doi: 10.1093/icesjms/fsy174.

Maggs, C. *et al.* (2008) 'Evaluating signatures of glacial refugia for North Atlantic Benthic marine taxa', *Ecology*, 89, pp. S108–S122. doi: 10.1890/08-0257.1.

Mamanova, L. *et al.* (2010) 'Target-enrichment strategies for next-generation sequencing', *Nature Methods*, 7(2), pp. 111–118. doi: 10.1038/nmeth.1419.

Manuzzi, A. *et al.* (2019) 'Population genomics and phylogeography of a benthic coastal shark (Scyliorhinus canicula) using 2b-RAD single nucleotide polymorphisms', *Biological Journal of the Linnean Society*, 126(2), pp. 289–303. doi: 10.1093/biolinnean/bly185.

Marandel, F. *et al.* (2020) 'Estimating effective population size using RADseq: Effects of SNP selection and sample size', *Ecology and evolution*, 10(4), pp. 1929–1937. doi: 10.1002/ece3.6016.

Marra, N. J. *et al.* (2019) 'White shark genome reveals ancient elasmobranch adaptations associated with wound healing and the maintenance of genome stability', *Proceedings of the National Academy of Sciences*, 116(10), pp. 4446 LP – 4455. doi: 10.1073/pnas.1819778116.

Martin, C. S. *et al.* (2012) 'Modelled distributions of ten demersal elasmobranchs of the eastern English Channel in relation to the environment', *Journal of Experimental Marine Biology and Ecology*, 418–419, pp. 91–103. doi: https://doi.org/10.1016/j.jembe.2012.03.010.

Martinsohn, J. T. and Ogden, R. (2009) 'FishPopTrace—Developing SNP-based population genetic assignment methods to investigate illegal fishing', *Forensic Science International: Genetics Supplement Series*, 2(1), pp. 294–296. doi: https://doi.org/10.1016/j.fsigss.2009.08.108.

Mavropoulou, A., Vervatis, V. and Sofianos, S. (2020) ‘Dissolved oxygen variability in the Mediterranean Sea’, *Journal of Marine Systems*, 208, p. 103348. doi: 10.1016/j.jmarsys.2020.103348.

Metcalf, J. D. and Butler, P. J. (1984) ‘Changes in activity and ventilation response to hypoxia in unrestrained, unoperated dogfish, Scyliorhinus canicula’, *J. Exp. Biol.*, 108, pp. 411–418.

Milano, I. *et al.* (2014) ‘Outlier SNP markers reveal fine-scale genetic structuring across European hake populations (Merluccius merluccius).’, *Molecular Ecology*, 23, pp. 118–135.

Miyake, Y. and Saruhashi, K. (1956) ‘On the vertical distribution of the dissolved oxygen in the ocean’, *Deep Sea Research*, 3(4), pp. 242–247. doi: 10.1016/0146-6313(56)90012-0.

Momigliano, P. *et al.* (2017) ‘Genetic structure and signatures of selection in grey reef sharks (Carcharhinus amblyrhynchos)’, *Heredity*, 119(December), pp. 1–12. doi: 10.1038/hdy.2017.21.

Mucientes, G. R. *et al.* (2009) ‘Sexual segregation of pelagic sharks and the potential threat from fisheries’, *Biology letters*. 2009/02/25, 5(2), pp. 156–159. doi: 10.1098/rsbl.2008.0761.

Mulley, J. F. *et al.* (2014) ‘Transcriptomic analysis of the lesser spotted catshark (Scyliorhinus canicula) pancreas, liver and brain reveals molecular level conservation of vertebrate pancreas function’, *BMC Genomics*, 15(1), p. 1074. doi: 10.1186/1471-2164-15-1074.

Musa, S. M. *et al.* (2020) ‘Ocean warming and hypoxia affect embryonic growth, fitness and survival of small-spotted catsharks, Scyliorhinus canicula’, *J Fish Biol*, 97, pp. 257–264. doi: 10.1111/jfb.14370.

Narum, S. R. *et al.* (2013) 'Genotyping-by-sequencing in ecological and conservation genomics', *Molecular ecology*. 2013/05/25, 22(11), pp. 2841–2847. doi: 10.1111/mec.12350.

Naval-Sanchez, M. *et al.* (2020) 'Changed Patterns of Genomic Variation Following Recent Domestication: Selection Sweeps in Farmed Atlantic Salmon', *Frontiers in Genetics*, 11(April). doi: 10.3389/fgene.2020.00264.

Nielsen, E. E. *et al.* (2009) 'Population genomics of marine fishes: identifying adaptive variation in space and time', *Mol. Ecol*, 18, pp. 3128–3150.

Nielsen, E. E. *et al.* (2012) 'Gene-associated markers provide tools for tackling illegal fishing and false eco-certification', *Nature Communications*, 3, pp. 851–856. doi: 10.1038/ncomms1845.

Ovenden, J. R. *et al.* (2018) *Genetics and genomics for fundamental and applied research on elasmobranchs. In: Carrier JC, Heithaus MR, Simpfendorfer CA (eds) Shark research: emerging technologies and applications for the field and laboratory.* Boca Raton, FL: CRC Press.

Papakostas, S. *et al.* (2014) 'Proteome variance differences within populations of European whitefish (Coregonus lavaretus) originating from contrasting salinity environments', *J Proteomics.*, 105, pp. 144–150.

Paradis, E., Claude, J. and Strimmer, K. (2004) 'APE: Analyses of Phylogenetics and Evolution in R Language', *Bioinformatics*, 20, pp. 289–290. doi: 10.1093/bioinformatics/btg412.

Paris, J. R., Stevens, J. R. and Catchen, J. M. (2017) 'Lost in parameter space: a road map for stacks', *Methods in Ecology and Evolution*, 8(10), pp. 1360–1373. doi: 10.1111/2041-210X.12775.

Pascual, M. *et al.* (2017) ‘Impact of life history traits on gene flow: A multispecies systematic review across oceanographic barriers in the Mediterranean Sea’, *PLoS ONE*, 12(5), p. e0176419. doi: 10.1371/journal.pone.0176419.

Patarnello, T., Volckaert, F. A. M. J. and Castilho, R. (2007) ‘Pillars of Hercules: Is the Atlantic-Mediterranean transition a phylogeographical break?’, *Molecular Ecology*, 16(21), pp. 4426–4444. doi: 10.1111/j.1365-294X.2007.03477.x.

Pazartzi, T. *et al.* (2019) ‘High levels of mislabeling in shark meat – Investigating patterns of species utilization with DNA barcoding in Greek retailers’, *Food Control*, 98, pp. 179–186. doi: https://doi.org/10.1016/j.foodcont.2018.11.019.

Pazmino, D. A. *et al.* (2017) ‘Genome-wide SNPs reveal low effective population size within confined management units of the highly vagile Galapagos shark (Carcharhinus galapagensis)’, *Conserv Genet*, 18, pp. 1151–1163. Available at: https://doi.org/10.1007/s10592-017-0967-1.

Peterson, B. K. *et al.* (2012) ‘Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species.’, *PloS one*, 7(5), p. e37135. doi: 10.1371/journal.pone.0037135.

Poland, J. A. *et al.* (2012) ‘Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach’, *PLoS ONE*, 7(2), p. e32253. Available at: https://doi.org/10.1371/journal.pone.0032253.

Portnoy, D S *et al.* (2015) ‘Selection and sex-biased dispersal in a coastal shark: the influence of philopatry on adaptive variation’, *Mol Ecol*, 24, pp. 5877–5885. doi: 10.1111/mec.13441.

Portnoy, D. S. *et al.* (2015) ‘Selection and sex-biased dispersal in a coastal shark: The influence of philopatry on adaptive variation’, *Molecular Ecology*, 24(23), pp. 5877–5885. doi: 10.1111/mec.13441.

Postaire, B. D. *et al.* (2020) ‘Environmental DNA detection tracks established seasonal occurrence of blacktip sharks (Carcharhinus limbatus) in a semi-enclosed subtropical bay’, *Scientific Reports*, 10(1), pp. 1–8. doi: 10.1038/s41598-020-68843-0.

Powter, D. M. and Gladstone, W. (2008) ‘Embryonic mortality and predation on egg capsules of the port Jackson shark Heterodontus portusjacksoni (Meyer)’, *Journal of Fish Biology*, 72(3), pp. 573–584.

Pritchard, J. K., Stephens, M. and Donnelly, P. (2000) ‘Inference of population structure using multilocus genotype data’, *Genetics*, 155, pp. 945–959.

Prive, F. *et al.* (2020) ‘Performing highly efficient genome scans for local adaptation with R package pcadapt version 4’, *Molecular Biology and Evolution*, 37, pp. 2153–2154.

Purcell, S. *et al.* (2007) ‘PLINK: a toolset for whole-genome association and population-based linkage analysis’, *American Journal of Human Genetics*, 81(3), pp. 559–575.

Ramirez-Amaro, S. *et al.* (2018) ‘Contrasting evolutionary patterns in populations of demersal sharks throughout the western Mediterranean’, *Marine Biology*, 165(1), p. 3. doi: 10.1007/s00227-017-3254-2.

Ramírez-Amaro, S. *et al.* (2018) ‘Contrasting evolutionary patterns in populations of demersal sharks throughout the western Mediterranean’, *Marine Biology*, 165(1). doi: 10.1007/s00227-017-3254-2.

Razgour, O. *et al.* (2019) ‘Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections’, *Proceedings of the National Academy of Sciences*, 116(21), pp. 10418 LP – 10423. doi: 10.1073/pnas.1820663116.

Read, T. D. *et al.* (2017) ‘Draft sequencing and assembly of the genome of the world’s largest fish, the whale shark: Rhincodon typus Smith 1828’, *BMC Genomics*, 18(1), p. 532. doi: 10.1186/s12864-017-3926-9.

Rellstab, C. *et al.* (2015) ‘A practical guide to environmental association analysis in landscape genomics’, *Molecular Ecology*, 24(17), pp. 4348–4370. doi: 10.1111/mec.13322.

Rochette, N. C. and Catchen, J. M. (2017) ‘Deriving genotypes from RAD-seq short-read data using Stacks’, *Nature Protocols*, 12(12), pp. 2640–2659. doi: 10.1038/nprot.2017.123.

Rochette, N., River-Colon, A. and Catchen, J. (2019) ‘Stacks 2: Analytical methods for paired?end sequencing improve RADseq-based population genomics’, *Molecular Ecology*, 28(21), pp. 4737–4754.

Rodriguez-Cabello, C. *et al.* (2004) ‘Is the lesser spotted dogfish (Scyliorhinus canicula) population from the Cantabrian Sea a unique stock?’, *Fisheries Research*, 69, pp. 57–71. doi: 10.1016/j.fishres.2004.04.002.

Rodriguez-Cabello, C., Sanchez, F. and Olaso, I. (2007) ‘Distribution patterns and sexual segregations of Scyliorhinus canicula (L.) in the Cantabrian Sea’, *Journal of Fish Biology*, 70, pp. 1568–1586. doi: 10.1111/j.1095-8649.2007.01444.x.

Rohde, K. (1992) ‘Latitudinal gradients in species diversity: the search for the primary cause’, *Oikos*, 65, pp. 514–527.

Rosa, R. *et al.* (2014) ‘Early-life exposure to climate change impairs tropical shark survival’, *Proceedings of the Royal Society B*, 281(1793), p. 20141738.

Rubinoff, D., Cameron, S. and Will, K. (2006) ‘A genomic perspective on the shortcomings of mitochondrial DNA for “barcoding” identification’, *Journal of Heredity*, 97(6), pp. 581–594. doi: 10.1093/jhered/esl036.

Ruegg, K. *et al.* (2018) ‘Ecological genomics predicts climate vulnerability in an endangered southwestern songbird’, *Ecology Letters*, 21(7), pp. 1085–1096. doi: 10.1111/ele.12977.

Selkoe, K. A. *et al.* (2016) ‘A decade of seascape genetics: Contributions to basic and applied marine connectivity’, *Marine Ecology Progress Series*, 554, pp. 1–19. Available at: https://doi.org/10.3354/meps11792.

Shaltout, Mohamed and Omstedt, A. (2014) ‘Recent sea surface temperature trends and future scenarios for the Mediterranean Sea’, *Oceanologia*, 56(3), pp. 411–443. doi: 10.5697/oc.56-3.411.

Shaltout, M and Omstedt, A. (2014) ‘Recent sea surface temperature trends and future scenarios for the Mediterranean Sea’, *Oceanologia*, 56(3), pp. 411–443. doi: 10.5697/oc.56-3.411.

Sharma, K. *et al.* (2019) ‘The Chemosensory Receptor Repertoire of a True Shark Is Dominated by a Single Olfactory Receptor Family’, *Genome Biology and Evolution*, 11(2), pp. 398–405. doi: 10.1093/gbe/evz002.

Simpfendorfer, C. A. *et al.* (2016) ‘Environmental DNA detects Critically Endangered largetooth sawfish in the wild’, *Endangered Species Research*, 30(1), pp. 109–116. doi: 10.3354/esr00731.

Sims, D., Nash, J. and Morritt, D. (2001) ‘Movements and activity of male and female dogfish in a tidal sea lough: Alternative behavioural strategies and apparent sexual segregation’, *Marine Biology*, 139, pp. 1165–1175. doi:

10.1007/s002270100666.

Skliris, N. *et al.* (2018) ‘Mediterranean sea water budget long-term trend inferred from salinity observations’, *Clim Dyn*, 51, pp. 2857–2876. doi: 10.1007/s00382-017-4053-7.

Speed, C. W. *et al.* (2010) ‘Complexities of coastal shark movements and their implications for management’, *Mar Ecol Prog Ser*, 408, pp. 275–293. doi: 10.3354/meps08581.

Suchan, T. *et al.* (2016) ‘Hybridization Capture Using RAD Probes (hyRAD), a New Tool for Performing Genomic Analyses on Collection Specimens’, *PLOS ONE*, 11(3), p. e0151651. Available at: https://doi.org/10.1371/journal.pone.0151651.

Sunday, J. M., Bates, A. E. and Dulvy, N. K. (2012) ‘Thermal tolerance and the global redistribution of animals’, *Nature Climate Change*, 2(9), pp. 686–690. doi: 10.1038/nclimate1539.

Supek, F. *et al.* (2011) ‘Summarizes and Visualizes Long Lists of Gene Ontology Terms’, *PLoS ONE*, 6, p. e21800.

Tan, M. *et al.* (2019) ‘The whale shark genome reveals patterns of vertebrate gene family evolution’, *bioRxiv*. doi: 10.1101/685743.

Tewhey, R. *et al.* (2009) ‘Microdroplet-based PCR enrichment for large-scale targeted sequencing’, *Nature Biotechnology*, 27(11), pp. 1025–1031. doi: 10.1038/nbt.1583.

Thomason, J. C. *et al.* (1996) ‘Effect of temperature and photoperiod on the growth of the embryonic dogfish, Scyliorhinus canicula’, *Journal of Fish Biology*, 49(4), pp. 739–742. doi: 10.1111/j.1095-8649.1996.tb00071.x.

Tiffin, P. and Ross-Ibarra, J. (2014) ‘Advances and limits of using population genetics to understand local adaptation’, *Trends in Ecology and Evolution*, 29(12), pp. 673–680. doi: 10.1016/j.tree.2014.10.004.

Torrado, H. *et al.* (2020) ‘Individual-based population genomics reveal different drivers of adaptation in sympatric fish’, *Scientific Reports*, 10(1), pp. 1–14. doi: 10.1038/s41598-020-69160-2.

Trenkel, V. M. *et al.* (2020) ‘Methods for identifying and interpreting sex-linked SNP markers and carrying out sex assignment: application to thornback ray (Raja clavata)’, *Molecular Ecology Resources*, 20(6), pp. 1610–1619. doi: 10.1111/1755-0998.13225.

Vaha, J.-P. *et al.* (2007) ‘Life-history and habitat features influence the within-river genetic structure of Atlantic salmon’, *Molecular Ecology*, 16(13), pp. 2638–2654. doi: 10.1111/j.1365-294X.2007.03329.x.

Vendrami, D. L. J. *et al.* (2019) ‘RAD sequencing sheds new light on the genetic structure and local adaptation of European scallops and resolves their demographic histories’, *Scientific Reports*, 9(1), pp. 1–13. doi: 10.1038/s41598-019-43939-4.

Venkatesh, B. *et al.* (2014) ‘Elephant shark genome provides unique insights into gnathostome evolution’, *Nature*, 505, pp. 174–179. doi: 10.1038/nature12826.

Veríssimo, A. *et al.* (2018) ‘ Population genomics and phylogeography of a benthic coastal shark (Scyliorhinus canicula) using 2b-RAD single nucleotide polymorphisms ’, *Biological Journal of the Linnean Society*, 126(2), pp. 289–303. doi: 10.1093/biolinnean/bly185.

Waldvogel, A. M. *et al.* (2020) ‘Evolutionary genomics improves prediction of species responses to climate change’, *Evolution Letters*, 4(1), pp. 4–18. doi: 10.1002/evl3.154.

Wang, Q. *et al.* (2012) ‘Community annotation and bioinformatics workforce development in concert—Little Skate Genome Annotation Workshops and Jamborees’, *Database : the journal of biological databases and curation*, 2012, p. bar064. doi: 10.1093/database/bar064.

Wannell, G. *et al.* (2020) ‘A new minibarcode assay to facilitate species identification from processed, degraded or historic ray (batoidea) samples’, *Conservation Genetics Resources*. doi: 10.1007/s12686-020-01158-4.

Ward-Paige, C. A. *et al.* (2015) ‘Characterizing and predicting essential habitat features for juvenile coastal sharks’, *Mar Ecol*, 36, pp. 419–431. doi: 10.1111/maec.12151.

Weber, J. A. *et al.* (2020) ‘The whale shark genome reveals how genomic and physiological properties scale with body size’, *Proceedings of the National Academy of Sciences*, 117(34), pp. 20662–20671. doi: 10.1073/pnas.1922576117.

Weir, B. S. and Cockerham, C. C. (1984) ‘Estimating F-Statistics for the Analysis of Population Structure’, *Evolution*, 38(6), pp. 1358–1370. Available at: http://www.jstor.org/stable/2408641.

Wheeler, C. R. *et al.* (2020) ‘Anthropogenic stressors influence reproduction and development in elasmobranch fishes’, *Reviews in Fish Biology and Fisheries*, 30(2), pp. 373–386. doi: 10.1007/s11160-020-09604-0.

White, T. A., Stamford, J. and Hoelzel, A. R. (2010) ‘Local selection and population structure in a deep-sea fish, the roundnose grenadier (Coryphaenoides rupestris)’, *Mol Ecol*, 19, pp. 216–226. doi: https://doi.org/10.1111/j.1365-294X.2009.04446.x.

Zimmerman, S. J. *et al.* (2019) ‘Signatures of adaptive divergence among populations of an avian species of conservation concern’, *Evolutionary Applications*, 12(8), pp. 1661–1677. doi: 10.1111/eva.12825.

3.7 Supplementary Information

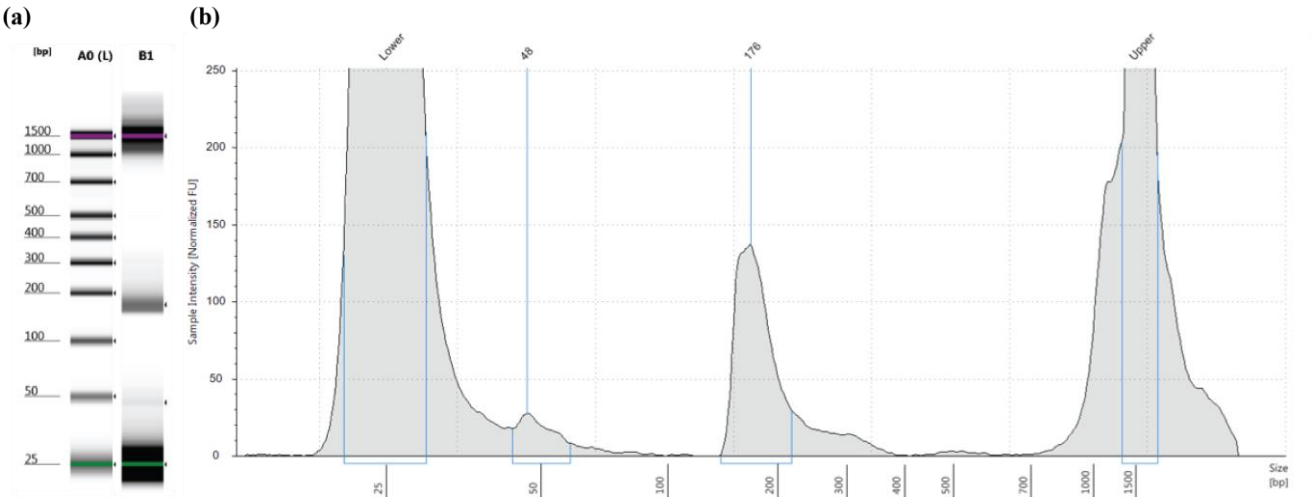


Figure S3.1. Agilent® 4200 TapeStation genomic DNA analysis. (a) Virtual electrophoretic gel of genomic DNA extracted from *Scyliorhinus canicula* individuals. Green lines at 25 bp and 1500 bp of the gel image are internal standards added to permit quantitation. (b) Representative electropherogram of ddRAD library. The combination of restriction enzymes *PstI-HF* (NEB) and *MspI* (NEB) resulted in a peak at 176 bp for the library.

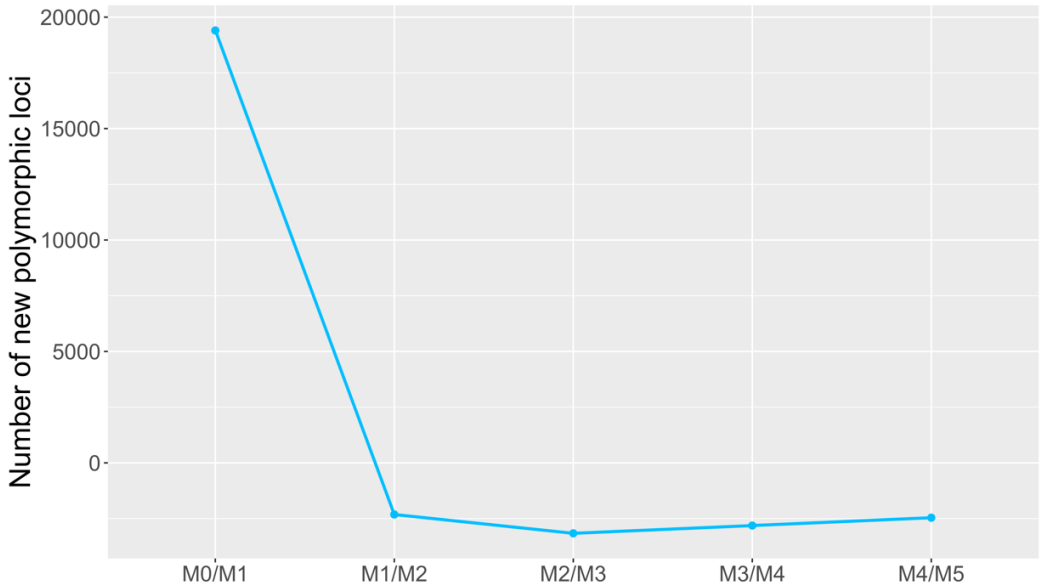


Figure S3.2. Number of new polymorphic loci (r80 loci) added for each iteration of *M* (mismatches allowed between stacks to form loci) and *n* (mismatches allowed between stacks during catalog construction) using the wrapper script *denovo_map.pl* in STACKS v 2.0.

Table S3.1. ddRAD *de novo* assembly of loci in STACKS v 2.0 for each *Scyliorhinus canicula* sample. Individuals highlighted in bold were dropped due to poor read recovery in process_radtags and/or poor coverage following loci building using the *denovo_map.pl wrapper* script.

		***process_radtags* filtering**		***denovo_map* assembly (m3 M1 n2)**		
Sample	**Barcode**	**Total (R1 & R2)**	**Retained Reads**	**Coverage**	**Polymorphic Loci**	**% of total loci**
Norway1	TGCTT	28222490	14411601	24.03	6215	9.86
Norway2	GCAAGCCAT	17158178	8738560	16.74	11946	10.61
Norway3	CGCACCAATT	9584104	6071830	13.44	12413	10.35
Norway4	CTCGCGG	13935274	8046232	16.51	12275	10.75
Nsea24	AACTGG	22015044	11391276	20.39	14013	10.91
NSea25	ATGAGCAA	16348260	8307814	16.85	14370	10.91
Nsea26	CTTGA	7404452	4064518	11.56	17019	10.71
Nsea27	GCGTCCT	28858762	15666484	24.31	10154	10.83
NSea30	ACCAGGA	15756078	8284303	17.04	3745	9.77
NSea31	CCACTCA	10401436	6151409	14.29	7107	10.13
Port8	TCACGGAAG	17119392	8465080	16.98	12368	10.68
Port9	TATCA	33376616	15311885	24.12	17032	10.76
Port10	TAGCCAA	32682422	16080522	25.38	8930	10.78
Port12	ATATCGCCA	12450060	6099252	14.41	15561	11.05
Port13	**CTCTA**	**3197786**	**1480328**	**8.16**		
Port14	GCCAACAAGA	7798934	3826984	11.39	13383	11.27
Africa1	CTCTCGCAT	4598066	2663615	10.21	12658	11.58
Africa3	TGCCGCAT	16557312	8408082	17.23	13666	10.82
Africa4	GCGTACAAT	21229640	10777724	19.58	12061	10.46
Africa5	ACGCGCG	18532194	9851276	18.43	9644	10.07
Africa10	GTCGCCT	13174660	7179425	15.58	8879	9.98
Africa11	CGTGTCA	10531508	7053444	15.2	5209	9.42
Mallorca30	AATGAACGA	14008698	7055200	14.68	8844	9.70
Mallorca31	CGTCGCCACT	8501234	5386096	12.95	14091	10.48
Mallorca32	ATGGCAA	20029590	10451660	19.71	10919	10.25
Mallorca33	CAACCACACA	11041050	6115206	13.92	10098	10.07
Mallorca34	GCTCCGA	19738214	10205450	18.71	14209	10.76
Mallorca38	**AACGTGCCT**	**76628**	**36339**	**6.04**		
Crete2	CTCAT	7915588	4200214	11.74	9873	10.18
Crete3	ACGGTACT	24918552	12812020	21.63	14392	10.73
Crete4	GCGCCG	26801768	13130081	19.75	15763	10.93
Crete5	CAAGT	15473648	8099262	16.33	12824	10.89
Crete6	TCCGAG	16298270	7597029	16.4	8612	10.28
Crete7	TAGATGA	20406826	9870386	18.32	18099	11.05
Cyprus114	TGGCCAG	13906004	6984846	13.26	13035	10.89
Cyprus115	GCACGAT	12213308	5623777	13.19	11760	10.80
Cyprus116	TTGCTG	10702688	4626021	11.73	17330	11.20
Cyprus117	CGCAACCAGT	4557536	2497634	11.34	13178	10.74
Cyprus123	TCACTG	10042980	4468218	10.7	11300	10.48
Cyprus127	ACAGT	25799986	11362158	19.73	14598	10.79

Table S3.2. Summary of filtering steps applied to the original single nucleotide polymorphism dataset obtained from running the *denovo_map.sh* in STACKS v. 2.0.

Filtering step	Loci filtered	Loci retained	% retained
STACKS: denovo_map			
Initial loci from *denovo_map*	0	158063	100
STACKS: populations			
R 0.95	143,134	14929	9.45
p 7	0	14929	9.45
r 0.5	0	14929	9.45
min-mac3	4419	10521	6.65
max-obs-het 0.5	454	10056	6.36
VCFTools			
min_depth 5, max depth 87.5	494	9562	6.05
PLINK			
Linkage disequilibrium	510	9052	5.73

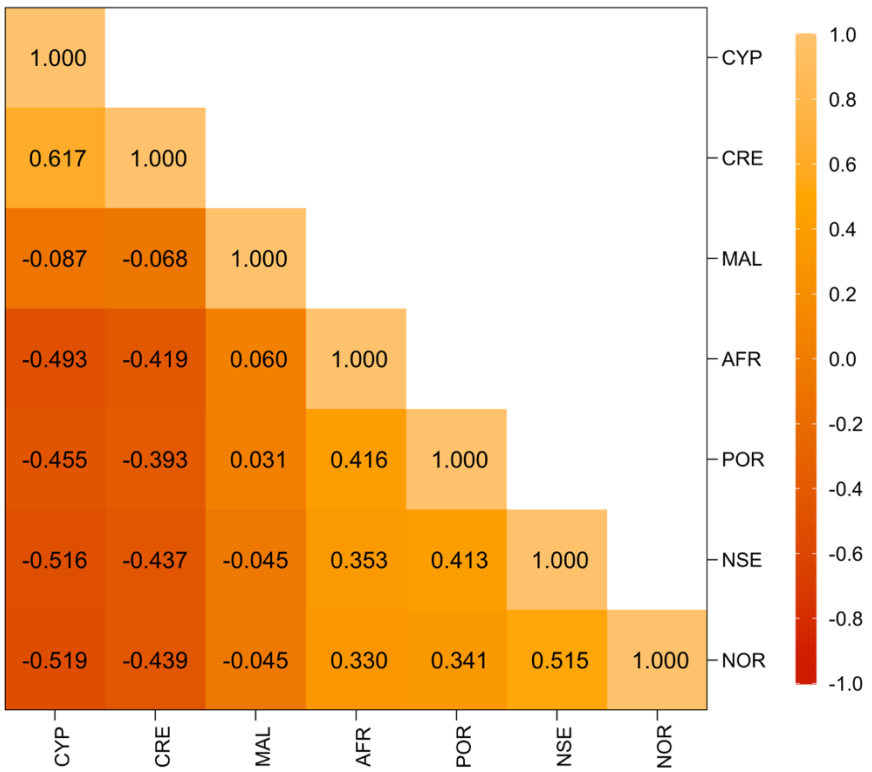


Figure S3.3. Correlation heatmap derived from the allele frequency covariance matrix used in BayPass to account for the demographic history of populations. Correlation should resemble population differentiation derived from FST statistics. See Table 3.2 for location codes.

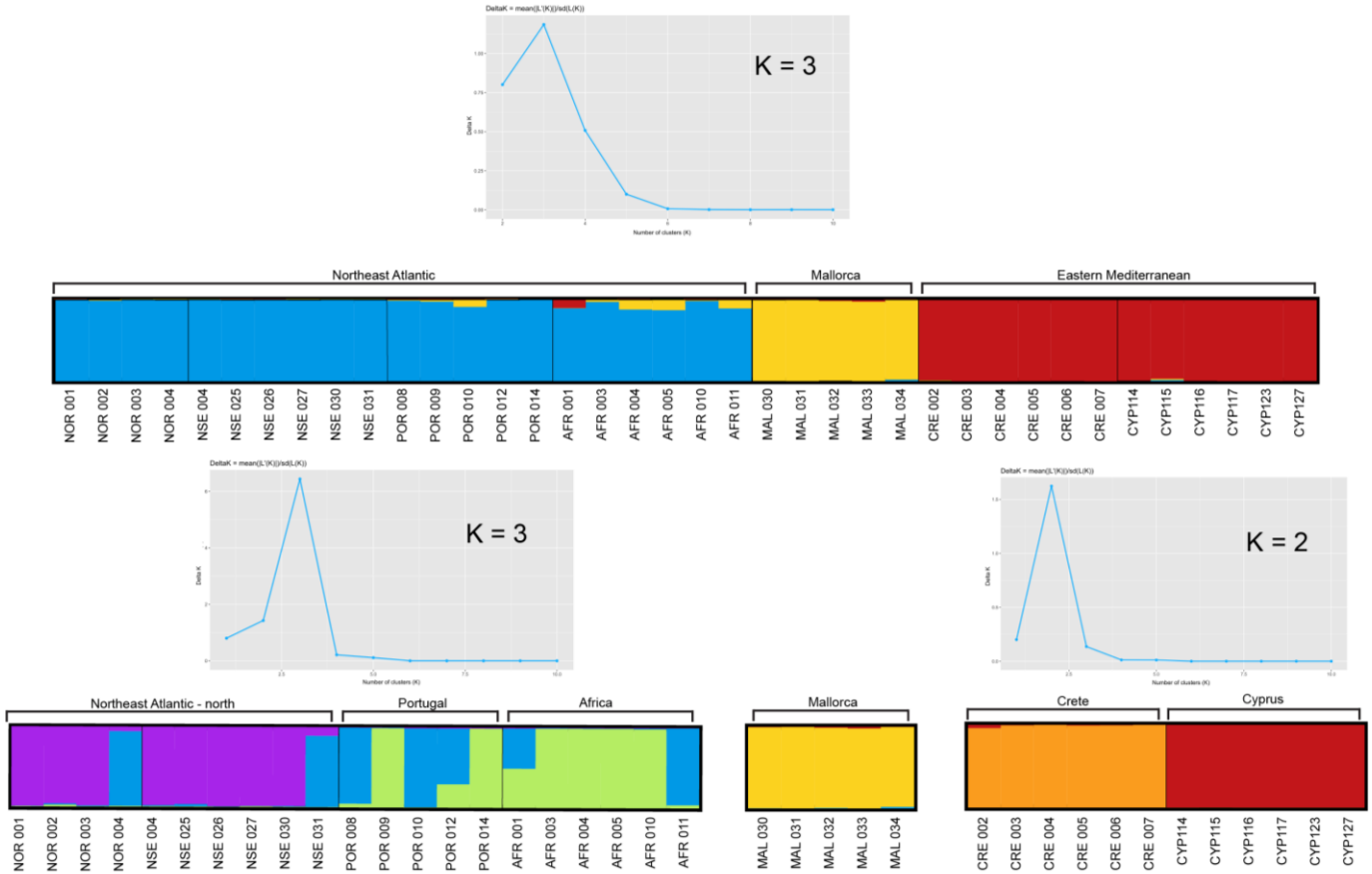


Figure S3.4. Structure analysis demonstrating the clustering of samples into regional groupings. Each horizontal line represents an individual which are partitioned into *K* coloured segments representing an individual's estimated membership in K clusters. A 'hierarchical' approach (Vaha *et al*., 2007), with two rounds of analysis was employed to capture major structure within the RAD data. To judge the correct *K*, the *ΔK* method of Evanno *et al.* (2005) was applied.

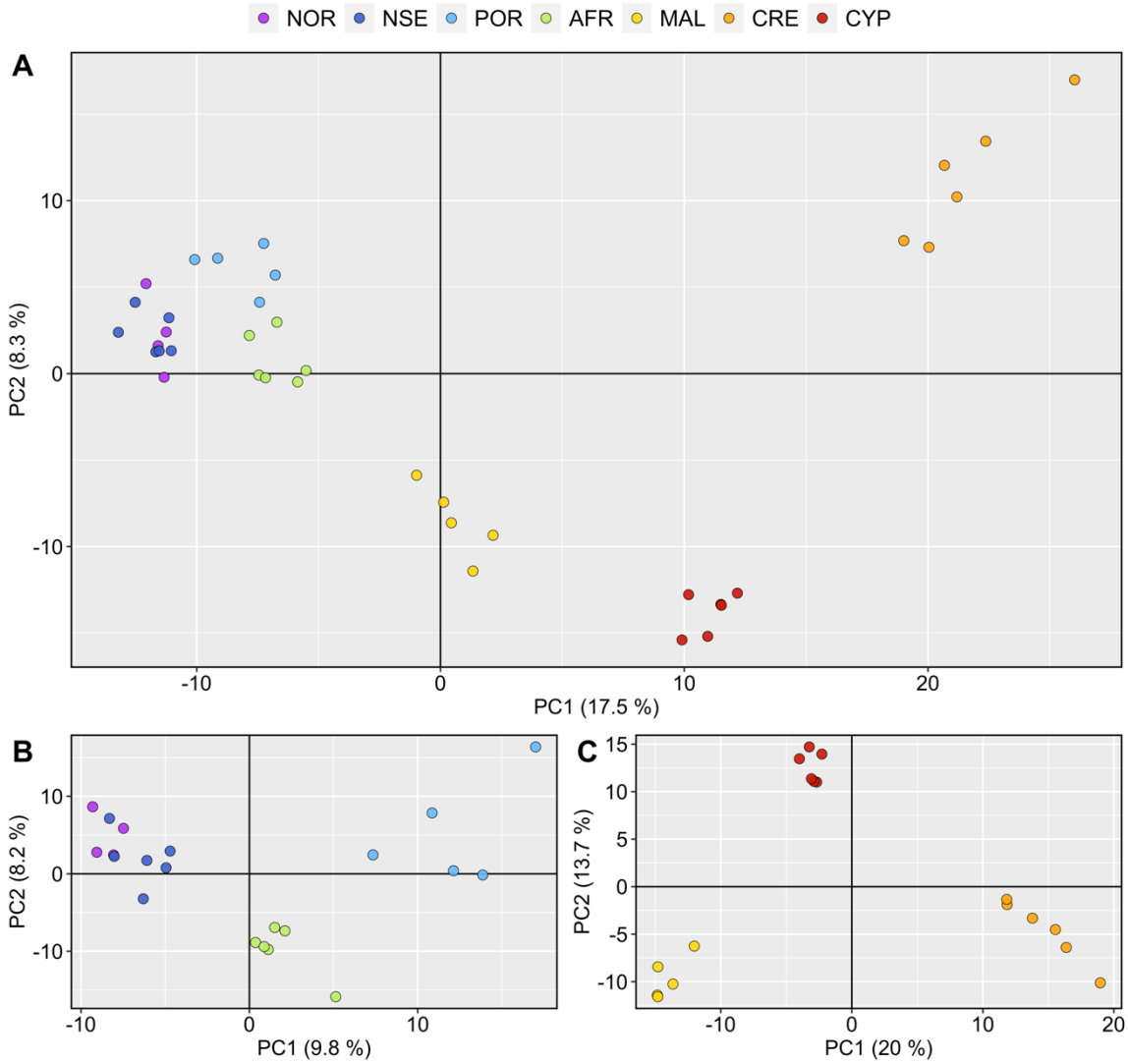


Figure S3.5. Principal component analysis (PCoA) of genetic distance between sample localities using 430 identified outlier SNPs: (a) all populations within both the Atlantic and Mediterranean; (b) only Atlantic localities; (c) only Mediterranean localities. The first principal component (PC1) is graphed along the x-axis, and the second component (PC2) along the y-axis and explain percentage of genetic variation. See Table 3.2 for location codes.

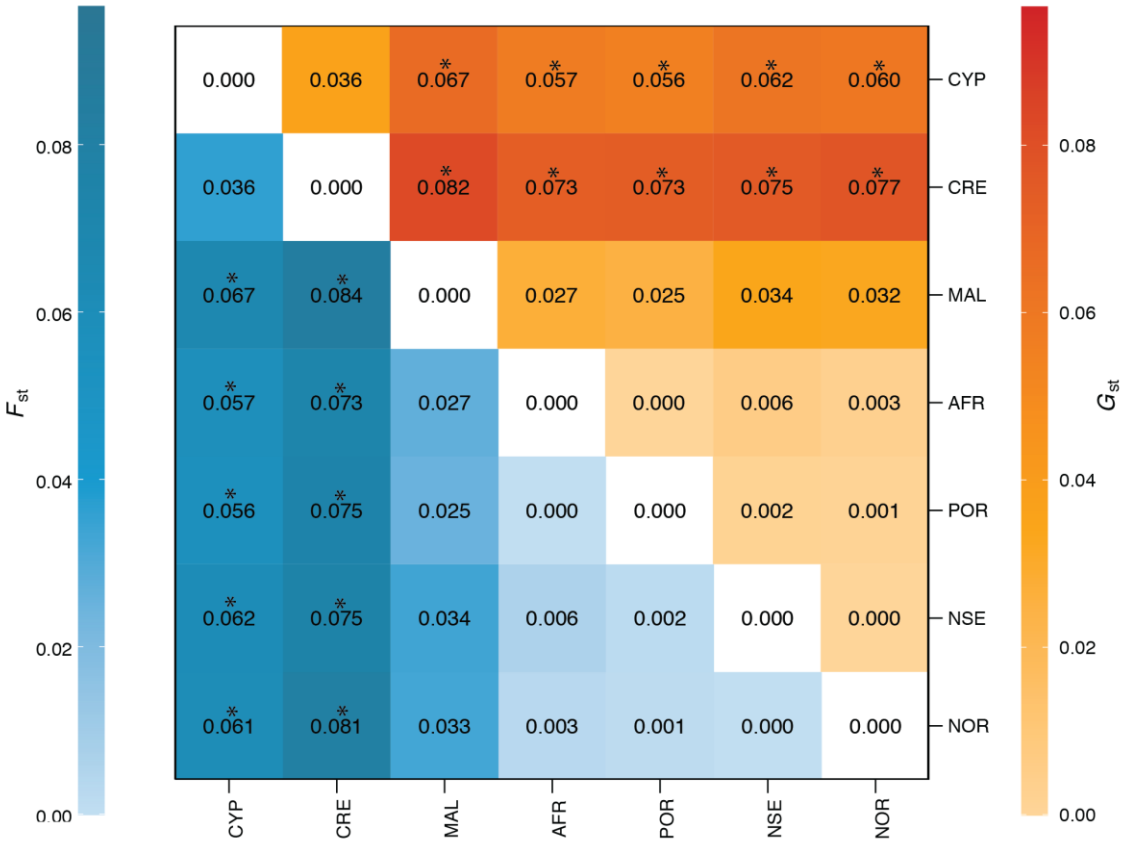


Figure S3.6. Pairwise differentiation among all seven sampling localities of *Scyliorhinus canicula* using a panel of 8,622 neutral SNPs. Below diagonal, pairwise F_{ST}. Above diagonal, pairwise G'_{ST}. Values marked with asterisks (*) were significant at 95 % confidence interval. See Table 3.2 for location codes.

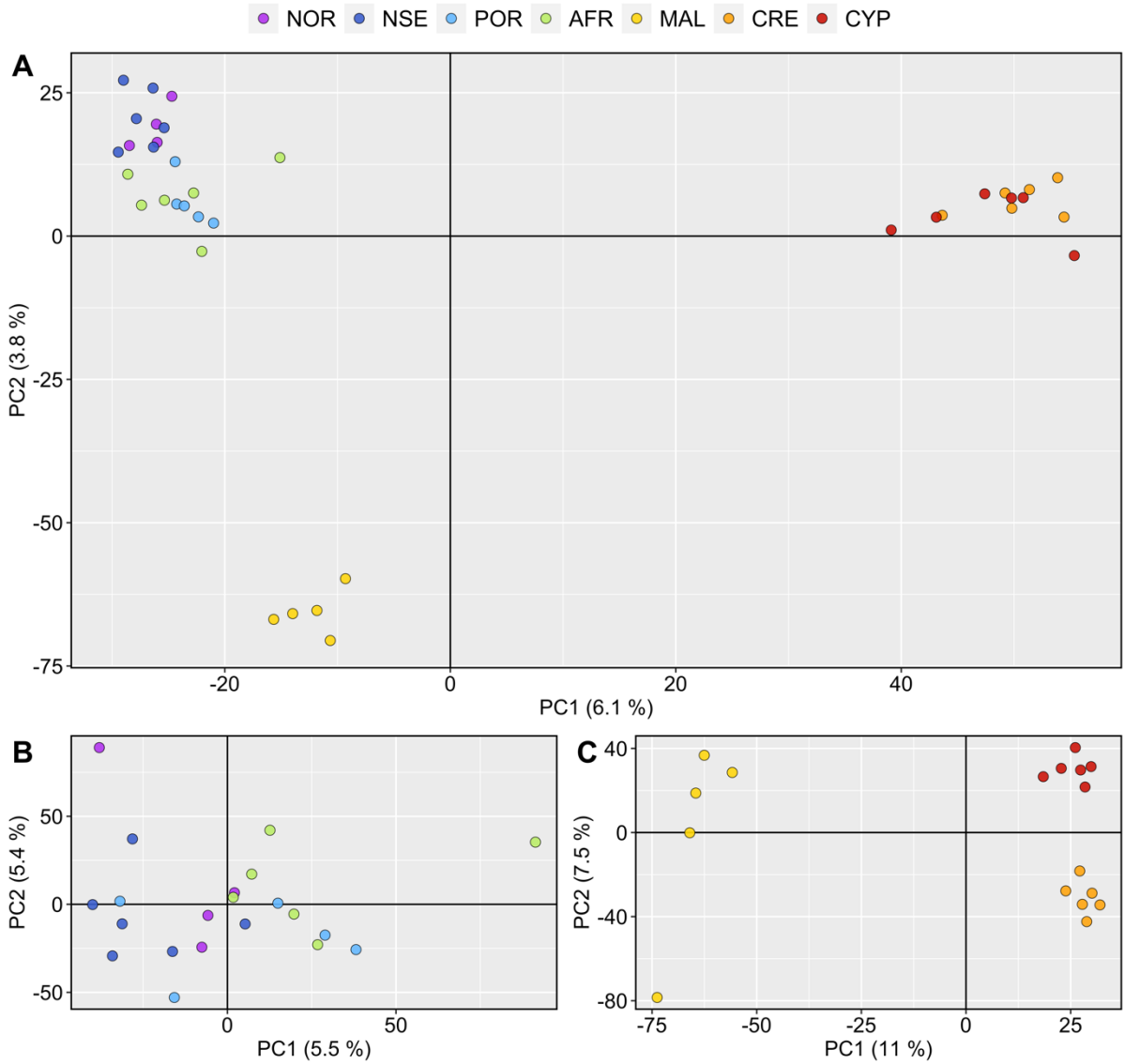


Figure S3.7. Principal component analysis (PCoA) of genetic distance between sample localities using 8,622 neutral SNPs: (a) all populations within both the Atlantic and Mediterranean; (b) only Atlantic localities; (c) only Mediterranean localities. The first principal component (PC1) is graphed along the x-axis, and the second component (PC2) along the y-axis and explain percentage of genetic variation. See Table 3.2 for location codes.

Table S3.3. Pearson correlations between environmental variables obtained from the Bio-ORACLE repository (Assis *et al*., 2017). Those with a correlation ≥ 0.70 are highlighted in bold.

	Min SST	Max SST	Mean SST	SST range	Min SBT	Max SBT	Mean SBT	SBT range	SB Salinity	Mean Depth	SB dissox
Min SST	1										
Max SST	**0.957**	1									
Mean SST	**0.991**	**0.986**	1								
SST range	-0.236	0.053	-0.111	1							
Min SBT	0.238	0.28	0.265	0.1	1						
Max SBT	0.162	0.232	0.201	0.199	**0.961**	1					
Mean SBT	0.205	0.261	0.238	0.148	**0.991**	**0.989**	1				
SBT range	-0.135	-0.021	-0.082	0.378	0.475	0.695	0.586	1			
SB Salinity	0.153	0.099	0.133	-0.204	0.085	-0.038	0.031	-0.364	1		
Mean Depth	-0.199	-0.153	-0.18	0.155	0.534	0.6	0.567	0.58	-0.175	1	
SB dissox	-0.487	-0.497	-0.498	0.006	-0.129	-0.03	-0.085	0.273	-0.194	0.121	1

Table S3.4. Raw table of all outlier loci identified in each of three outlier methods used and their corresponding environmental association. Loci are ranked in descending order depending from: identified in all methods, two of the methods, either BayPass or LFMM and only PCAdapt. P, PCAdapt; B, BayPass; C, LFMM. Those SNPs identified in two or more methods were considered strong candidates of selection.

Loci	Length	PCAdapt	SST mean	SST range	SBT mean	SBT range	SB salinity	SB dissox	Depth	# of methods	Common variable	Environmental variable
7213	69	P		B			LB			3	Yes	SB salinity
59521	79	P		LB						3	Yes	SST range
64932	66	P		L			B			3	No	
69065	50	P	B					L		3	No	
110233	53	P						LB		3	Yes	SB dissox
4282	52			B	L					2	No	
18937	53		L		LB					2	Yes	SBT mean
26549	76			B					L	2	No	
28854	54		L		L		LB			2	Yes	SB salinity
31218	51		B		LB					2	Yes	SBT mean
34042	71							LB	L	2	Yes	SB dissox
40098	70					B		L	L	2		
42921	50		L			B		L		2	No	
55980	54		L	LB	L		L	B		2	Yes	SST range
68648	68			L			B			2		
75256	53		B		LB					2	Yes	SBT mean
152056	52		LB		LB			B		2	Yes	SST mean/ SBT mean
168688	55			B	L					2		
8810	81	P			B					2		
14560	50	P					B			2		
23571	72	P		B			B			2		
33951	63	P		B						2		
41797	56	P		B			B			2		
44459	66	P		B						2		
52226	64	P			L					2		
53010	67	P							L	2		
55416	61	P						L	L	2		
57439	63	P							L	2		
66839	62	P		B						2		
92037	59	P						L	L	2		
142793	52	P				B		B		2		
147374	53	P		B						2		
116086	54	P					B			2		
148799	78	P	B							2		
43028	73		L		L			L		1		
45226	62		L		L			L		1		
51911	71		L		L					1		
53181	53					B		B		1		
62507	54		L		L			L		1		
66041	67		L		L			L		1		
98684	53		L		L			L		1		
101556	61		L		L			L		1		
118980	54		L		L			L		1		
152924	71		B		B			B		1		
168712	68		L		L			L		1		
14829	50		L		L					1		
16836	57		B				B			1		
19917	52							L	L	1		
21101	51		L		L					1		
28324	58		L		L					1		
30848	88		L		L			L		1		
33090	89							L	L	1		
36803	91							L	L	1		
38377	50			B				B		1		
42109	68					B			B	1		
54420	53		L		L					1		
54457	53		L		L					1		
59659	57		L		L					1		
62982	57		L		L					1		
63172	53							L	L	1		
63218	73			L					L	1		
63323	60							L	L	1		
65623	50		L		L					1		
67787	64		L		L					1		
68297	52		L					L		1		
70795	62			B				B		1		
74978	67		L		L					1		
75554	60		L					L		1		
77169	62		L		L					1		
78901	57		L		L					1		
80766	51							L	L	1		
84575	75			L					L	1		
84581	51		B	B						1		
86427	50		L		L					1		
86981	53		L		L			L		1		
88146	52		L		L					1		
88199	61		L		L					1		
89763	66		L		L					1		
92709	53		L		L					1		
104092	53							L	L	1		
105872	58		L		L							
110356	59		L		L					1		
115654	53					B			B	1		
128050	51		L		L					1		
131266	55					B			B	1		
138279	73					B		B		1		

144722	75		L			L							1
148651	63		B				B						1
169803	68		L			L							1
334801	88		L			L							1
1198	70					L							1
3034	61											L	1
3357	52											L	1
4822	54											L	1
5703	51											L	1
8126	55									B			1
8801	54									B			1
8915	59									B			1
10874	65											L	1
16969	50											L	1
17190	72											L	1
17253	79					L							1
17281	54					L							1
18027	53									L			1
18388	80				B								1
18777	51											L	1
20716	59											L	1
20897	51										B		1
22407	74											L	1
23499	55											L	1
23631	62					L							1
25292	66								B				1
25431	81											L	1
25586	59							B					1
27340	51											L	1
28521	51											L	1
28563	92			L									1
28641	50				B								1
29169	66									L			1
29562	60											L	1
29829	54										B		1
30822	68		B										1
31263	69				B								1
32852	56											B	1
33027	69					L							1
33145	52											L	1
33596	66					L							1
33659	59					L							1
33967	84											B	1
34590	64											L	1
35188	63											L	1
36740	72			L									1
37292	78								B				1
38302	75		B										1
38342	82				B								1
38513	83											L	1
39552	82										B		1
41126	62										B		1
41538	59									L			1
41766	51										B		1
41886	75							B					1
43147	56										B		1
43440	67		B										1
44431	75											L	1
44603	56										B		1
45577	62											B	
46612	55											L	1
47295	65											L	1
47762	63										B		1
48969	58											L	1
49296	51				B								1
49543	71											L	1
49818	65											L	1
51075	55										B		1
51468	54							B					1
52243	56					L							1
52387	61				B								1
53730	79				B								1
53864	58											L	1
55354	51						B						1
56126	57											L	1
57519	62											L	1
58421	68											L	1
59880	72				B								1
60820	65					L							1
61411	57											L	1
61586	51				B								1
62390	65											B	1
62644	70											L	1
63109	50				B								1
63258	59											L	1
63563	53				B								1
63781	66							B					1
63853	57											L	1
64900	75				B								1
65041	52									L			1
66390	52											L	1
66761	111					L							1
68045	110											L	1
68504	53	B											1
68652	54											B	1
68714	52		L										1
69385	66				B								1
69672	64											L	1
70175	51				B								1
70320	54										B		1
71748	67				B								1
71959	79				B								1
72298	52											L	1
73084	64										B		1
73448	90											L	1
73666	51				B								1
74360	70										B		1
74366	65					L							1

75152	59							L																	1
75175	53					B																			1
75889	56																B								1
76268	57																			L					1
76572	69																			L					1
76983	50																B								1
78637	60					B																			1
80216	58					B																			1
81202	59					B																			1
81472	62					B																			1
81623	72					B																			1
82473	50																			L					1
82793	61							B																	1
82834	54				B																				1
82879	110					B																			1
83035	50							L																	1
83517	56										B														1
83618	55																			L					1
84207	88																B								1
84415	53									B															1
85015	75							L																	1
86145	124																			L					1
86258	71							L																	1
86555	64							L																	1
86800	64																			L					1
86844	55																			L					1
87027	60																			L					1
87206	51																B								1
91041	76					B																			1
91127	81			B																					1
91191	60							L																	1
91294	53																B								1
91443	58							B																	1
91669	57																B								1
91935	67			B																					1
91965	50																			L					1
92008	55							B																	1
92073	58							L																	1
93425	57																			L					1
93843	65																			L					1
94749	65					B																			1
95541	56																			L					1
95652	63																B								1
96224	55																			L					1
98331	75																B								1
98970	77					B																			1
101176	64					B																			1
101402	82																			L					1
102024	54							B																	1
102096	60																B								1
102427	52																			L					1
102624	56																			L					1
102785	52			B																					1
105290	83															L									1
105639	66																			L					1
106047	55															L				L					1
106245	59			B																					1
107065	55			B																					1
107090	50										B														1
107174	59																			L					1
110833	55					B																			1
111501	68																			L					1
113187	63																			L					1
113422	58			B																					1
113526	63																			L					1
114102	74																			L					1
114223	61					B																			1
114402	68																B								1
114411	86																			L					1
114636	81																			L					1
114972	66																			L					1
116010	71																			L					1
116426	53					B																			1
117688	53			L																					1
123514	80																			L					1
125711	50																B								1
126414	70					B																			1
128861	76																B								1
129206	87																B								1
130076	61					B																			1
130299	64			L																					1
131092	51									B															1
133363	50					B																			1
134093	76							L																	1
134605	52					B																			1
135987	82																B								1
136254	51					B																			1
137312	74										B														1
137766	53															L									1
138491	81			B																					1
139625	55				B																				1
142698	55																B								1
150976	78																			L					1
151791	84																			B					1
151950	82																			L					1
152638	82										B														1
152691	53																			L					1
162364	59			L																					1
296622	80																			L					1
336105	56							L																	1
359203	74															L									1
115395	74	P																							1
7285	52	P																							1
8324	57	P																							1
10066	54	P																							1
12536	58	P																							1
12701	64	P																							1
18811	63	P																							1

24867	51	P	1
25959	66	P	1
36289	61	P	1
36427	110	P	1
39411	82	P	1
39709	65	P	1
40444	62	P	1
41254	58	P	1
41621	66	P	1
46670	58	P	1
47933	50	P	1
48799	66	P	1
49962	57	P	1
50047	52	P	1
50589	55	P	1
51325	53	P	1
54149	69	P	1
54293	91	P	1
55564	53	P	1
55625	72	P	1
56007	54	P	1
56700	84	P	1
57897	79	P	1
58280	75	P	1
59318	50	P	1
60025	61	P	1
60443	63	P	1
61795	64	P	1
63312	50	P	1
63968	73	P	1
65543	57	P	1
65903	62	P	1
66049	52	P	1
66327	66	P	1
67747	50	P	1
67825	68	P	1
68030	80	P	1
68705	57	P	1
69300	78	P	1
71605	95	P	1
72364	110	P	1
72666	57	P	1
72768	54	P	1
73131	50	P	1
73414	55	P	1
74371	63	P	1
75022	66	P	1
75963	55	P	1
76103	51	P	1
77825	53	P	1
78258	64	P	1
79000	51	P	1
79115	53	P	1
80253	61	P	1
80408	50	P	1
80755	70	P	1
81278	79	P	1
81571	52	P	1
82467	86	P	1
82715	50	P	1
85303	60	P	1
85325	57	P	1
86518	64	P	1
87806	74	P	1
87989	59	P	1
89748	75	P	1
89890	71	P	1
91961	62	P	1
92935	57	P	1
93603	59	P	1
94638	62	P	1
98027	52	P	1
100005	52	P	1
100911	86	P	1
101494	51	P	1
103494	67	P	1
103569	61	P	1
105604	51	P	1
106693	53	P	1
106960	51	P	1
108310	54	P	1
109979	65	P	1
110053	50	P	1
110221	64	P	1
112397	62	P	1
112768	76	P	1
113382	60	P	1
113426	52	P	1
113809	58	P	1
113981	55	P	1
114685	60	P	1
114877	74	P	1
115680	63	P	1
115749	51	P	1
115991	53	P	1
116817	66	P	1
117123	81	P	1
117167	69	P	1
118215	57	P	1
119105	64	P	1
123215	56	P	1
123243	61	P	1
124274	55	P	1
124718	50	P	1
125276	66	P	1
126046	54	P	1
126082	57	P	1
127101	82	P	1

127371	77	P	1
127822	60	P	1
129291	58	P	1
131803	55	P	1
134360	54	P	1
135783	56	P	1
138695	84	P	1
139326	59	P	1
140952	57	P	1
141099	66	P	1
142435	52	P	1
144481	79	P	1
146204	53	P	1
146574	57	P	1
152922	67	P	1
252246	71	P	1
313354	53	P	1
369196	50	P	1

Chapter 4: General Discussion

4.1 Summary of findings

This thesis has considered two main objectives aiming to increase the understanding of neutral and evolutionary mechanisms underlying population structure and local adaptation in elasmobranchs. These were successfully addressed using both population genetic and genomic approaches focusing on the small-spotted catshark, *Scyliorhinus canicula*. Chapter two utilised the mtDNA control region (CR) to reveal significant population structure within the catsharks distribution, covering the widest geographical range to date and included the first genetic assessment of many edge populations. Chapter three employed double-digest restriction site associated DNA (ddRAD) SNP markers to reveal further population structuring of catsharks across their range. This coupled with environmental inference and outlier detection, demonstrated strong signatures of divergent selection suggestive of adaptation to local conditions.

4.2 Interplay of neutral and adaptive processes

The inferences gained through chapter two and three have undoubtedly improved the understanding of the interacting effects of gene-flow, genetic drift and natural selection in the varying environments across the small-spotted catsharks range. One of the key findings of this thesis was the role that both neutral and adaptive processes appear to have in driving genetic differentiation in this catshark. In the Mediterranean, evidence of neutral divergence was found using both the mtDNA CR in chapter two and putatively neutral SNPs in chapter three, but failed to reveal clear structuring in the northeast Atlantic

(NEA). A reasonable conclusion is that the more complex geomorphology of the Mediterranean basin, alongside the limited dispersal capacity of the catshark (Sims, Nash and Morritt, 2001; Rodriguez-Cabello *et al.*, 2004), could be significantly restricting gene flow, even on a small spatial scale. The lack of barriers in the NEA instead promote homogenising gene flow potentially buffering any edge effects that were hypothesised in this region. In fact, edge effects seemed to only influence eastern Mediterranean populations which showed distinct clustering. Evidently, this is a species likely to be highly sensitive to dispersal barriers (Barbieri *et al.*, 2014; Gubili *et al.*, 2014; Ramirez-Amaro *et al.*, 2018) and highlights the strong influence life-history traits can have on the genetic structuring of elasmobranch, particularly in smaller, demersal species with restricted gene flow (Chapman *et al.*, 2015; Corrigan *et al.*, 2016). It is also interesting to speculate if the CR marker, being found in the mitochondrial genome, is more sensitive/powerful in detecting population structure in the Mediterranean. It has a smaller effective population size compared to nuclear DNA (Castro, Picornell and Ramon, 1999) and a different, maternal, mode of inheritance (Rubinoff, Cameron and Will, 2006), potentially reflecting patterns of female philopatry well known in sharks (Hueter *et al.*, 2004; Mucientes *et al.*, 2009; Martin *et al.*, 2012) not male-mediated gene flow. Similar patterns were documented in other species of shark (Chapman *et al.*, 2015; D S Portnoy *et al.*, 2015) and suggests sex-biased dispersal may be a key driver of population structure in this region.

Utilising ddRAD sequencing (ddRAD-seq) combined with outlier analysis, Chapter three identified a suite of markers putatively under selection, and for the first time in the small-spotted catshark, suggested the influence of local

adaptation on population structure. The large panel of SNP markers were able to reveal fine-scale population structure in both the NEA and Mediterranean that was previously undetected with mitochondrial and microsatellite markers (Barbieri *et al.*, 2014; Gubili *et al.*, 2014; V Kousteni *et al.*, 2015; Ferrari *et al.*, 2018); further evidence of the greater resolution often attained with large SNP datasets (Milano *et al.*, 2014; Carreras *et al.*, 2017; Jenkins *et al.*, 2019). This was perhaps best demonstrated in the NEA where SNPs revealed genetic structure that contrasts with the homogenous population proposed in both Chapter two and previous studies (Gubili *et al.*, 2014; V Kousteni *et al.*, 2015). The signal was even clearer when analysing SNPs putatively under selection generated with outlier and seascape analysis. This suggested that divergence is likely being driven by environmental differences e.g. in temperature, salinity, oxygen, and depth. Indeed, the versatility and effectiveness of seascape/landscape genomic approaches have already been recognised in a range of organisms including birds (Zimmerman *et al.*, 2019), trees (Gugger *et al.*, 2018; Daniels *et al.*, 2019), reptiles (Gallego-García *et al.*, 2019), marine invertebrates (Benestan *et al.*, 2016; S Bernatchez *et al.*, 2019; Vendrami *et al.*, 2019) and fish (K Cure *et al.*, 2017; Diopere *et al.*, 2018; Torrado *et al.*, 2020). Although the majority of studies are still based on terrestrial organisms with only 4 % of all landscape genetics/genomics papers published since 1991 being on marine systems (Grummer *et al.*, 2019), which partly reflects the lack of genomic resources for many aquatic species (Kelley *et al.*, 2016). Overall, being able to distinguish neutral and adaptive divergence and their drivers holds promise for the better detection of demographically independent stocks, which should serve as important conservation units, and as such, more SNP based seascape genomic studies are highly recommended for elasmobranchs.

4.3 RAD-seq: applications in elasmobranch research

In this project, the ddRAD library preparation costs were low, reflecting the use of the reduced genome representation protocol (Poland *et al.*, 2012) and the fact consumables (restriction enzymes, adapters, reagents) existed within Dr Fraser's lab group at the University of Exeter. The costs saved allowed more individuals to be included in the library and more money spent on the sequencing run. Given that chondrichthyan genomes are very large (1-7 gigabses (Venkatesh *et al.*, 2014; Read *et al.*, 2017; Y Hara *et al.*, 2018; Marra *et al.*, 2019) this was a very cost-effective approach to take. It was further shown that this method can be accurate and allow SNPS to be confidently genotyped *de novo* (i.e. without a reference genome). This is highly advantageous given the lack of genomic resources currently available for elasmobranchs (R R Domingues, Hilsdorf and Gadig, 2018; Johri, Doane, *et al.*, 2019). As such, ddRAD-seq holds enormous potential for the affordable and prompt acquisition of genomic data from previously unevaluated elasmobranchs, helping to reduce current rates of data deficiency in many elasmobranchs (Dulvy *et al.*, 2014, 2017; R R Domingues, Hilsdorf and Gadig, 2018).

Although the ddRAD-seq approach utilised in this work identified thousands of genome-wide SNPs at low cost and with good coverage, there were a couple key limitations that needs to be addressed in the context of this discussion. First, using the protocol of (Poland *et al.*, 2012) did result in relatively short fragment lengths (average 62 bp) which caused some issues mapping SNPs to the few genomic resources available (chapter three). This was initially identified as a concern after *in silico* estimates of fragment size, but was determined the

best approach with limited resources to allow for individual-level information. For example, a much larger number of individuals could have been sequenced with a pooled RAD-seq approach (Gautier *et al.*, 2013) but this would only allow for population-level information. Alternative enzymes were trialled (e.g. SbfI) but were limited by the reverse adapters' available and only resulted in even shorter fragments. Plus, this protocol was already proven to work reliably in other projects within the lab group. Nevertheless, when not restriction by available adapters or resources, future studies should consider using a less frequent second cutting enzyme during library preparation, which should increase fragment length.

Secondly, mapping candidate loci to determine function proved difficult given the lack of genomic resources available for elasmobranchs (and the small RAD-tag sequence lengths discussed above). Some inferences of gene function were gained from mapping to transcribed regions of the small-spotted catshark transcriptome (Mulley *et al.*, 2014), however, it was impossible to determine whether these were indeed under selection or in linkage with a near-by region of the genome. Fortunately, more elasmobranch genome projects are ongoing that will add to the four shark and one Chimaera genomes currently available (Venkatesh *et al.*, 2014; Read *et al.*, 2017; Y Hara *et al.*, 2018; Marra *et al.*, 2019). These include the little skate *Leucoraja erinacae* genome (Wang *et al.*, 2012, https://skatebase.org) and two separate attempts at the *S. canicula* genome; one from a Mediterranean shark (unpublished but see Sharma *et al.*, 2019) and one from a Atlantic shark (https://www.sanger.ac.uk/collaboration/25-genomes-25-years/). These projects have already provided considerable insight into elasmobranch evolution (Y Hara *et al.*, 2018; Tan *et al.*, 2019) physiology

(Weber *et al.*, 2020) and gene function (Marra *et al.*, 2019). However, key to the future work of this thesis is the release of the *S. canicula* genome from the Sanger Institute, which is in its final stages and will seemingly be well annotated. Upon the release of the genome, candidate loci identified in chapter three should be remapped to help discern how many SNPs are indeed located in regions under selection and to aid in the discovery of more loci linked to functional genes.

With higher resolution of population structure, reduced costs and *de novo* mapping in non-model organism shown within this thesis, it is perhaps not surprising RAD-seq has become one of the most widespread genomic methods for population genetic, phylogeography and evolutionary studies (Davey and Blaxter, 2010; Narum *et al.*, 2013). Although it is potential for elasmobranchs remains largely unexplored with few RAD-seq studies on elasmobranchs (Johri, Doane, *et al.*, 2019). Some work has begun to emerge in recent years utilising various RAD-seq methodologies and has provided novel insight into population divergence in the bonnethead shark (ddRAD; Díaz-Jaimes *et al.*, 2020), small-spotted catshark (2bRAD, Manuzzi *et al.*, 2019) and silky shark (ezRAD; Kraft *et al.*, 2020) hybridisation in hammerhead sharks (ddARD, (Barker *et al.*, 2019) and estimates of effective population size in the thornback ray (RADseq; (Marandel *et al.*, 2020). Continuing to use such approaches will unlock further information on elasmobranch biology and help to address key issues in elasmobranch conservation, such as the high rates of data deficiency (Dulvy *et al.*, 2014; R R Domingues, Hilsdorf and Gadig, 2018) and the impacts of environmental change (Dulvy *et al.*, 2014).

4.4 Alternative genomic approaches and resources

Although advantageous for the study on natural populations, RAD-seq is not the only genomic approach to show exciting promise for future studies of elasmobranchs. Others include hybridisation-based gene capture (Li *et al.*, 2013) and genome skimming (Johri, Solanki, *et al.*, 2019) alongside the latest advent of high-throughput sequencing technologies (e.g. MinION; Laver *et al.*, 2015). It is perhaps hybridisation gene capture that has been most widely used in elasmobranchs and shows particular promise (for a full review of methods see (Johri, Doane, *et al.*, 2019). It uses predesigned probes to hybridise and capture genetic regions of the genomes that have been determined *a priori* to be informative (i.e. protein coding genes/exomes, (Mamanova *et al.*, 2010). Normally, the main challenge in non-model species of this approach is designing a capture probe set, which by definition requires *a priori* knowledge of target sequences (Harvey *et al.*, 2016). Fortunately, using the elephant shark genome (Venkatesh *et al.*, 2014), a multi-species capture probe has been developed for elasmobranch and able to target more than 1000 different genes across species (Li *et al.*, 2013). This approach has been used to determine the evolutionary history and demography of a black-tip reef shark *Carcharhinus melanopterus* (Delser *et al.*, 2016), and for the excellent ongoing phylogenetic Chondrichthyan Tree of Life project, which has genotype over 800 species (https://sharksray.org/).

Unlike RAD-seq data, which are essentially one-off data sets, gene capture data represents a lasting, amplifiable resources for comparative studies in which new samples can continuously be added (Harvey *et al.*, 2016). This could be highly beneficial for threatened elasmobranch species where samples are

hard to collect. Furthermore, as targeted genes are often conserved across taxa, gene capture loci often have far better mapping scores to divergent genomes to determine function (Li *et al.*, 2013; Harvey *et al.*, 2016). For approaches requiring more information per locus this might be the preferable approach, particularly when genomic resources are lacking (Jones and Good, 2016). However, conserved regions targeted by gene capture may be insufficiently dispersed across the genome for use in genome-wide scans to search for signatures of selection (Hohenlohe *et al.*, 2010; Harvey *et al.*, 2016; Jones and Good, 2016). These studies rely on the identification of heterogeneous genomic regions impacted by neutral and adaptive processes (Diopere *et al.*, 2018; Bernatchez, *et al.*, 2019; Daniels *et al.*, 2019), which in essence is why outlier loci sequences can be difficult to map to currently available genomes. For the same reasons, RAD-seq is still preferable for identifying fine-scale population differentiation, at least in recently diverged samples, because the greater number of polymorphisms increases the chances of detecting rare or shared alleles (Davey and Blaxter, 2010; Narum *et al.*, 2013). However, this doesn't mean that both approaches could not be used in conjunction, through a method known as hyRAD (Jones and Good, 2016; Suchan *et al.*, 2016). In this approach, capture probes can be developed to target a panel of informative RAD markers identified form an initial RADseq experiment. This two-step approach combines the ability of *de novo* SNP discovery with the higher repeatability and lower variability of gene capture (Jones and Good, 2016; Suchan *et al.*, 2016). This could enable the cost-effective generation of large population datasets currently lacking for elasmobranchs (Dulvy *et al.*, 2014; R R Domingues, Hilsdorf and Gadig, 2018). Similar approaches have also been used for high-throughput genotyping of pre-

defined SNPs, such microfluidic approaches (Tewhey *et al.*, 2009), e.g. Fluidigm® Access Array™, which was recently employed to reveal population structure in the European lobster (Jenkins *et al.*, 2019). Implementing such an approach with the SNPs generated in this thesis would allow new individuals and samples sites to be added to form a larger spatial and temporal SNP dataset, further enabling the ability to search for signatures of local adaptation. Nevertheless, as sequencing technologies rapid improve and cost fall, new methods are sure to appear, alongside existing methods such as whole genome sequencing becoming more affordable.

4.5 Predicting the impacts of environmental change

The higher resolution of ddRAD SNP markers identified in this thesis provided the ability to interrogate evolutionary forces driving differentiation at a much more detailed level (chapter three). Such inferences will hopefully enable more accurate predictions surrounding the response of elasmobranch populations to climatic change (Waldvogel *et al.*, 2020). For example, the inclusions of local adaptation data in ecological niche models (ENM) should enable more accurate forecasts of species vulnerability and extinction risk as already shown in bats (Razgour *et al.*, 2019), birds (Ruegg *et al.*, 2018) and trees (Ikeda *et al.*, 2017). However, there is little evidence of such an approach being applied in marine environments, despite the 'trailing-edge, leading-edge' patterns of range shifts often occurring more frequently in marine species (Sunday, Bates and Dulvy, 2012). Indeed, there is a distinct lack of genetic data in studies utilising ENMs to predict future distribution of sharks (Báez *et al.*, 2020; Birkmanis *et al.*, 2020; Feitosa *et al.*, 2020). The exclusion of adaptation data/parameters can also lead to incorrect range prediction and therefore misplaced conservation effort

(Hällfors *et al.*, 2016). This highlights the need for increased genomic data in elasmobranchs to aid successful conservation and management (R R Domingues, Hilsdorf and Gadig, 2018; Waldvogel *et al.*, 2020).

4.6 Conservation and management

Elasmobranchs are a group of great interest to conservationist given their ecological importance (Heupel *et al.*, 2014) and their current high levels of overexploitation (Dulvy *et al.*, 2014). Yet, the assessment and management of populations/stock is not well established and the inclusion of genetic data is severely lacking (R R Domingues, Hilsdorf and Gadig, 2018). The results from this thesis clearly show the potential of small-spotted catshark to form multiple populations/stocks and has important applications for sustainable stock management. From a fisheries perspective, the genetic structure uncovered in chapter 3 are consistent with two of the existing management stocks currently proposed in this region: the North Sea and Atlantic Iberian waters (ICES, 2019). Comments cannot be made on the other two categorised stocks (Celtic Sea and west of Scotland and Northern Bay of Biscay) given samples weren't included from these localities in chapter 3. Inclusion of Norwegian catsharks within the North Sea stock should be considered given the putative genetic similarity, however, much more sampling within the Norwegian Sea is needed for confirmation. Furthermore, results from chapter 3 suggest the putative west African population should be managed as its own discrete stock, but again, further sampling is needed and capture coordinates recorded.

In the Mediterranean, results were consistent with the multiple genetic stocks currently proposed in this region (Barbieri *et al.*, 2014; Gubili *et al.*, 2014).

However, populations exhibiting low genetic diversity and a high degree of genetic differentiation, such as populations within the eastern Mediterranean (Crete, Cyprus) should be further considered as discrete stocks and managed accordingly. The vulnerability of *S. canicula* to the effects of overexploitation could also be exaggerated at the range edge which makes these population a particular concern. This is somewhat worrying given the inadequate management systems in place in regions of the Mediterranean (Colloca, Scarcella and Libralato, 2017) that have previously resulted in drastic stock declines in this species (Barausse *et al.*, 2014). Overall, these results have wider implications for fisheries management of other exploited elasmobranchs, particularly species exhibiting similar life-history traits and distribution, which could display similar patterns of spatial genetic structure. Nevertheless, more species-specific studies are needed on elasmobranchs to accurately assess population dynamics and determine fishery stocks.

The SNP panel developed in this thesis offers exciting scope for future studies of the small-spotted catshark that will hopefully provide further insight into elasmobranch biology and enable better management. The use of SNP panels composed of highly-ranking loci (i.e. those that best define population structure) have proven to be an extremely informative tool for fisheries management (Bernatchez *et al.*, 2017), helping to determine the origin of fished individuals and for tackling illegal fishing (Martinsohn and Ogden, 2009; Nielsen *et al.*, 2012). Somewhat similar mtDNA barcoding methods have proven highly effective for determining species of origin in elasmobranch trade (Griffiths *et al.*, 2013; Cardeñosa *et al.*, 2017; Hobbs *et al.*, 2019; Wannell *et al.*, 2020) but to the best of our knowledge, no studies have employed informative SNPs to

determine location of origin in elasmobranchs. As such, it would be interesting to determine the ability of SNPs developed in this thesis to assign individuals back to their sampling location. Although the small-spotted catsharks is listed as Least Concern on the IUCN Red List (Serena et al. 2016), the development of such methods would enable its application for tackling illegal activity in marine protected areas (Davidson, 2012) and mislabelling in threatened species (Pazartzi *et al.*, 2019) - two pressing issues in elasmobranch conservation.

In a recent study of the thornback ray *raja clavata* authors were able to identify 19 putative sex-linked markers from 4604 SNP generated using RADseq (Trenkel *et al.*, 2020). Although considerably more individuals were used in their study, the sex of samples analysed in chapter three are known. By adopting a similar method to Trenkel *et al.* (2020), it would be interesting to see if sex-linked SNPs could be identified in the small-spotted catshark. Developing such methods for sex assignment could have important applications in the management and conservation of elasmobranchs. For example, elasmobranchs species are known to segregate by sex and exhibit sex based dispersal (Jirik and Lowe, 2012; Martin *et al.*, 2012; Chapman *et al.*, 2015), although how this effects sex-biased catch in fisheries is still unknown (Mucientes et al. 2009). Sex-linked markers could help determine the sex from fisheries derived meat products and allow a better understanding of catch sex-ratios or fishing grounds dominate by a single sex.

4.7 Concluding remarks

In conclusion, both mtDNA and SNP markers have proven to be effective tools for resolving population structure within this thesis offering important insights

into small-spotted catshark population dynamics. Although, it is the greater resolution and versatility of genome-wide SNP data that holds the greatest potential for future studies of elasmobranchs. ddRAD generated a panel of highly informative and robust SNPs that have exciting future applications in the continued research of this small shark. Seascape genomic approaches proved to be an excellent tool for searching for signatures of adaptation and key environmental drivers in the small-spotted catsharks range. By applying these methods, alongside the integration of newly emerging techniques, we can better understand spatial patterns of neutral and adaptive genetic variation, key drivers of selection and how elasmobranch populations are likely to respond to environmental change. Furthermore, continued dedication to genomic studies will help to drastically reduce data deficiency, improve conservation practices and sustainable management of populations and help improve product traceability in our markets and fisheries. In tandem, significant effort is needed to increase the number of genomic resources available for elasmobranchs in order for future genomic resources to be most beneficial.

4.8 References

Aat, B., Mouffok, S. and Boutiba, Z. (2012) ‘Reproductive biology and growth of Lesser Spotted Dogfish Scyliorhinus canicula (Linnaeus, 1758) in Western Algerian coasts (Chondrichthyes, Scyliorhinidae)’, *Biodiversity Journal*, 3(1), pp. 41–48.

Ahrens, C. *et al.* (2018) ‘The search for loci under selection: Trends, biases and progress’, *Molecular Ecology*, 27(6), pp. 1342–1356. Available at: https://doi.

Allendorf, F. W., Hohenlohe, P. A. and Luikart, G. (2010) ‘Genomics and the future of conservation genetics’, *Nature Reviews Genetics*, 11, pp. 697–709.

Angilletta, M. J. and Dunham, A. E. (2003) ‘The temperature-size rule in ectotherms: simple evolutionary explanations may not be general’, *Am Nat*, 162(3), pp. 332–342. doi: 10.1086/377187.

Arnold, B. *et al.* (2013) ‘RADseq underestimates diversity and introduces genealogical biases due to nonrandom haplotype sampling’, *Molecular Ecology*, 22(11), pp. 3179–3190. doi: 10.1111/mec.12276.

Assis, J. *et al.* (2017) *Bio-ORACLE v2.0: Extending marine data layers for bioclimatic modelling*. Global Ecology and Biogeography.

Báez, J. C. *et al.* (2020) ‘Ensemble modeling of the potential distribution of the whale shark in the Atlantic Ocean’, *Ecology and Evolution*, 10(1), pp. 175–184. doi: 10.1002/ece3.5884.

Bangley, C. W. *et al.* (2018) ‘Delineation and mapping of coastal shark habitat within a shallow lagoonal estuary’, *PLOS ONE*, 13, p. 4. Available at: https://doi.org/10.1371/journal.pone.0195221.

Barausse, A. *et al.* (2014) ‘The role of fisheries and the environment in driving the decline of elasmobranchs in the northern Adriatic Sea’, *ICES Journal of Marine Science*, 71(7), pp. 1593–1603. doi: 10.1093/icesjms/fst222.

Barbieri, M. *et al.* (2014) ‘Molecular contribution to stock identification in the small-spotted catshark, Scyliorhinus canicula (Chondrichthyes, Scyliorhinidae)’, *Fisheries Research*, 154, pp. 11–16. doi: 10.1016/j.fishres.2014.01.021.

Barker, A. M. *et al.* (2019) ‘Hybridization between sympatric hammerhead sharks in the western North Atlantic Ocean’, *Biology Letters*, 15(4), p. 20190004. doi: 10.1098/rsbl.2019.0004.

Benestan, L. *et al.* (2016) ‘Seascape genomics provides evidence for thermal adaptation and current mediated population structure in {A}merican lobster (Homarus americanus)’, 25(20), pp. 5073–5092.

Benjamini, Y. and Hochberg, Y. (1995) ‘Controlling the false discovery rate: a practical and powerful approach to multiple testing’, *J. Royal Stat Soc. B*, 57(1), pp. 289–300.

Benjelloun, B. *et al.* (2019) ‘An evaluation of sequencing coverage and genotyping strategies to assess neutral and adaptive diversity’, *Molecular Ecology Resources*, 19(6), pp. 1497–1515. doi: 10.1111/1755-0998.13070.

Berg, P. R. *et al.* (2015) ‘Adaptation to Low Salinity Promotes Genomic Divergence in Atlantic Cod (Gadus morhua L.) ’, *Genome Biology and Evolution*, 7(6), pp. 1644–1663. doi: 10.1093/gbe/evv093.

Bernatchez, L. *et al.* (2017) ‘Harnessing the Power of Genomics to Secure the Future of Seafood’, *Trends in Ecology & Evolution*, 32(9), pp. 665–680. doi: 10.1016/j.tree.2017.06.010.

Bernatchez, S *et al.* (2019) ‘Seascape genomics of eastern oyster (Crassostrea virginica) along the Atlantic coast of Canada’, 12(3), pp. 587–609. Available at: https://doi.org/10.1111/eva.12741.

Bernatchez, Simon *et al.* (2019) ‘Seascape genomics of eastern oyster (Crassostrea virginica) along the Atlantic coast of Canada’, *Evolutionary Applications*, 12(3), pp. 587–609. doi: 10.1111/eva.12741.

Bierne, N., Gagnaire, P. A. and David, P. (2013) ‘The geography of introgression in a patchy environment and the thorn in the side of ecological speciation’, *Current Zoology*, 10(1093), pp. 72–86. Available at: https.

Birkmanis, C. A. *et al.* (2020) ‘Future Distribution of Suitable Habitat for Pelagic Sharks in Australia Under Climate Change Models’, *Frontiers in Marine Science*, 7(July), pp. 1–11. doi: 10.3389/fmars.2020.00570.

Bohonak, A. J. (1999) ‘Dispersal, gene flow, and population structure’, *Q Rev Biol*, 74, pp. 21–45.

Budd, A. M. *et al.* (2021) ‘First detection of critically endangered scalloped hammerhead sharks (Sphyrna lewini) in Guam, Micronesia, in five decades using environmental DNA’, *Ecological Indicators*, 127. doi: 10.1016/j.ecolind.2021.107649.

Capape, C. *et al.* (2008) ‘Biological observations on the smallspotted catshark Scyliorhinus canicula (Chondrichthyes: Scyliorhinidae) off the Languedocian coast (southern France, northern Mediterranean)’, *Pan-American Journal of Aquatic Sciences*, 3, pp. 282–289.

Capape, C. *et al.* (2014) ‘Production, maturity, reproductive cycle and fecundity of small-spotted catshark, Scyliorhinus canicula (Chondrichthyes: Scyliorhinidae) from the northern coast of Tunisia (Central Mediterranean)’, *J. Ichthyol*, 54, pp. 111–126. doi: 10.1134/S0032945214010020.

Cardeñosa, D. *et al.* (2017) ‘A multiplex PCR mini-barcode assay to identify processed shark products in the global trade’, *PLoS ONE*, 12(10), pp. 3–11. doi: 10.1371/journal.pone.0185368.

Carlisle, A. B. and Starr, R. M. (2009) ‘Habitat use, residency, and seasonal distribution of female leopard sharks Triakis semifasciata in Elkhorn Slough, California’, *Mar Ecol Prog Ser*, 380, pp. 213–228.

Carlson, J. K. and Parsons, G. R. (2001) ‘The Effects of Hypoxia on Three Sympatric Shark Species: Physiological and Behavioral Responses’, *Environmental Biology of Fishes*, 61, pp. 427–433. Available at: https://doi.org/10.1023/A:1011641302048.

Carreras, C. *et al.* (2017) ‘Population genomics of an endemic Mediterranean fish: Differentiation by fine scale dispersal and adaptation’, *Scientific Reports*, 7, p. 43417. Available at: https.

Carreras, C *et al.* (2020) ‘East is East and West is West: Population genomics and hierarchical analyses reveal genetic structure and adaptation footprints in the keystone species Paracentrotus lividus (Echinoidea)’, *Diversity and Distributions*, 26(3), pp. 382–398. Available at: https://doi.org/10.1111/ddi.13016.

Carreras, Carlos *et al.* (2020) ‘East is East and West is West: Population genomics and hierarchical analyses reveal genetic structure and adaptation footprints in the keystone species Paracentrotus lividus (Echinoidea)’, *Diversity and Distributions*, 26(3), pp. 382–398. doi: 10.1111/ddi.13016.

Castro, J., Picornell, A. and Ramon, M. (1999) ‘Mitochondrial DNA: A tool for populational genetics studies’, *International microbiology : the official journal of the Spanish Society for Microbiology*, 1, pp. 327–332.

Catchen, J. *et al.* (2011) ‘Stacks: building and genotyping loci de novo from short-read sequences’, *Genes, Genomes, Genetics,* 3(1), pp. 171–182.

Chapman, D. D. *et al.* (2015) ‘There and Back Again: A Review of Residency and Return Migrations in Sharks, with Implications for Population Structure and Management’, *Annual Review of Marine Science*, 7(1), pp. 547–570. doi: 10.1146/annurev-marine-010814-015730.

Chasteen, N. D. and Harrison, P. M. (1999) ‘Mineralization in Ferritin: An Efficient Means of Iron Storage’, *Journal of Structural Biology*, 126(3), pp. 182–194. Available at: https://doi.org/10.1006/jsbi.1999.4118.

Chen, H. and Boutros, P. C. V. (2011) ‘a package for the generation of highly-customizable Venn and Euler diagrams in R’, *BMC Bioinformatics*, 12, p. 35. Available at: https://doi.org/10.1186/1471-2105-12-35.

Cheng, L. *et al.* (2019) ‘How fast are the oceans warming’, *Science*, 363(6423), pp. 128–129.

Colloca, F., Scarcella, G. and Libralato, S. (2017) ‘Recent Trends and Impacts of Fisheries Exploitation on Mediterranean Stocks and Ecosystems’, *Frontiers in Marine Science*, 4, p. 244. doi: 10.3389/fmars.2017.00244.

Compagno, L., Dando, M. and Fowler, S. (2005) *A Field Guide to Sharks of the World*. Princeton: Princeton University Press.

Corrigan, S. *et al.* (2016) ‘A multilocus comparative study of dispersal in three codistributed demersal sharks from eastern Australia’, *Canadian Journal of Fisheries and Aquatic Sciences*, 73(3), pp. 406–415. doi: 10.1139/cjfas-2015-0085.

Crear, D. P. *et al.* (2019) ‘The impacts of warming and hypoxia on the performance of an obligate ram ventilator’, *Conservation physiology*, 7, p. 1. Available at: https://doi.org/10.1093/conphys/coz026.

Cure, Katherine *et al.* (2017) ‘Genomic signatures of local adaptation reveal source-sink dynamics in a high gene flow fish species’, *Scientific Reports*, 7(1), pp. 1–10. doi: 10.1038/s41598-017-09224-y.

Cure, K *et al.* (2017) ‘Genomic signatures of local adaptation reveal source-sink dynamics in a high gene flow fish species’, *Scientific Reports*, 7(1), pp. 1–10. Available at: https://doi.org/10.1038/s41598-017-09224-y.

Currie, D. J. *et al.* (2004) ‘Predictions and tests of climate-based hypotheses of broad-scale variation in taxonomic richness’, *Ecology Letters*, 7(12), pp. 1121–1134. doi: 10.1111/j.1461-0248.2004.00671.x.

Danecek, P. *et al.* (2011) ‘The variant call format and VCFtools.’, *Bioinformatics*, 27, pp. 2156–2158.

Daniels, R. R. *et al.* (2019) ‘Looking for local adaptation: Convergent microevolution in aleppo pine (pinus halepensis)’, *Genes*, 10(9). doi: 10.3390/genes10090673.

Davey, J. W. and Blaxter, M. L. (2010) ‘RADSeq: next-generation population genetics’, *Briefings in Functional Genomics.*, 9(5–6), pp. 416–423.

Davidson, L. N. K. (2012) ‘Shark Sanctuaries: Substance or Spin?’, *Science*, 338(6114), pp. 1538–1539. doi: 10.1126/science.338.6114.1538.

Delser, M. *et al.* (2016) ‘Population genomics of C. melanopterus using target gene capture data: demographic inferences and conservation perspectives’, *Sci Rep*, 6, p. 33753. doi: 10.1038/srep33753.

Díaz-Jaimes, P. *et al.* (2020) ‘Population genetic divergence of bonnethead sharks Sphyrna tiburo in the western North Atlantic: Implications for conservation’, *Aquatic Conservation: Marine and Freshwater Ecosystems*, (June), pp. 1–16. doi: 10.1002/aqc.3434.

Diopere, E. *et al.* (2018) ‘Seascape genetics of a flatfish reveals local selection under high levels of gene flow’, *ICES Journal of Marine Science*, 75(2), pp. 675–689. Available at: https://doi.org/10.1093/icesjms/fsx160.

Domingues, R. R. *et al.* (2019) ‘Genetic connectivity and phylogeography of the night shark (Carcharhinus signatus) in the western Atlantic Ocean: Implications for conservation management’, *Aquatic Conservation: Marine and Freshwater Ecosystems*, 29(1), pp. 102–114. doi: 10.1002/aqc.2961.

Domingues, Rodrigo Rodrigues, Hilsdorf, A. W. S. and Gadig, O. B. F. (2018) ‘The importance of considering genetic diversity in shark and ray conservation policies’, *Conservation Genetics*, 19(3), pp. 501–525. doi: 10.1007/s10592-017-1038-3.

Domingues, R R, Hilsdorf, A. W. S. and Gadig, O. B. F. (2018) ‘The importance of considering genetic diversity in shark and ray conservation policies’, *Conservation Genetics*, 19(3), pp. 501–525. Available at: https://doi.org/10.1007/s10592-017-1038-3.

Drymon, J. M. *et al.* (2013) ‘Multiscale analysis of factors that affect the distribution of sharks throughout the northern Gulf of Mexico’, *Fish Bull*, 111(4), pp. 370–380.

Dufresne, F. *et al.* (2013) ‘Recent progress and challenges in population genetics of polyploid organisms: An overview of current state-of-the-art molecular and statistical tools’, *Molecular ecology*, 23, pp. 40–69. doi: 10.1111/mec.12581.

Dulvy, N. *et al.* (2017) ‘Challenges and Priorities in Shark and Ray Conservation.’, *Current Biology*, 27, pp. 565–572. doi: 10.1016/j.cub.2017.04.038.

Dulvy, N. K. *et al.* (2014) ‘Extinction risk and conservation of the world’s sharks and rays’, *eLife*, 2014(3), pp. 1–34. doi: 10.7554/eLife.00590.

Dye, S. R. *et al.* (2013) ‘Impacts of climate change on temperature (air and sea)’, *MCCIP Sci Rev*, pp. 1–12.

Eales, N. (1949) ‘The Food of the Dogfish, Scyliorhinus Caniculus L’, *Journal of the Marine Biological Association of the United Kingdom*, 28(3), pp. 791–793. doi: 10.1017/S0025315400023584.

Ellis, J. R. and Shackley, S. E. (1997) ‘The reproductive biology of Scyliorhinus canicula in the Bristol Channel, UK’, *J. Fish. Biol.*, 51(19), pp. 361–372. doi: 10.1111/j.1095-8649.1997.tb01672.x).

Emerson, R. O. and Thomas, J. H. (2009) ‘Adaptive Evolution in Zinc Finger Transcription Factors’, *PLOS Genetics*, 5(1), p. e1000325. Available at: https://doi.org/10.1371/journal.pgen.1000325.

Espinoza, M., Farrugia, T. J. and Lowe, C. G. (2011) ‘Habitat use, movements and site fidelity of the gray smooth-hound shark (Mustelus californicus Gill 1863) in a newly restored southern California estuary’, *J Exp Mar Bio Ecol*, 401, pp. 63–67.

Evanno, G., Regnaut, S. and Goudet, J. (2005) ‘Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study’, *Molecular Ecology*, 14(2), pp. 2611–2620.

Excoffier, L., Hofer, T. and Foll, M. (2009) ‘Detecting loci under selection in a hierarchically structured population’, *Heredity*, 103(4), pp. 285–298. doi: 10.1038/hdy.2009.74.

Feitosa, L. M. *et al.* (2020) ‘Potential distribution and population trends of the smalltail shark Carcharhinus porosus inferred from species distribution models and historical catch data’, *Aquatic Conservation: Marine and Freshwater*

Ecosystems, 30(5), pp. 882–891. doi: 10.1002/aqc.3293.

Ferrari, A. *et al.* (2018) 'Natural history and molecular evolution of demersal Mediterranean sharks and skates inferred by comparative phylogeographic and demographic analyses', *PeerJ*, 2018(9). doi: 10.7717/peerj.5560.

Field, I. C. *et al.* (no date) 'Susceptibility of sharks, rays and chimaeras to global extinction', *Adv Mar Biol*, 56, pp. 275–363.

Finotto, L. *et al.* (2015) 'Contrasting life history and reproductive traits in two populations of Scyliorhinus canicula', *Mar Biol*, 162, pp. 1175–1186. doi: 10.1007/s00227-015-2659-z.

Foll, M. and Gaggiotti, O. (2008) 'A genome scan method to identify selected loci appropriate for both dominant and codominant markers: A {B}ayesian perspective', *Genetics*, 180, pp. 977–993. doi: 10.1534/genet ics.108.092221.

Ford, E. (1921) 'A contribution to our knowledge of the life histories of the dogfishes landed at Plymouth', *Journ. Mar. Biol. Assoc.*, XII, pp. 468–505.

Fountain, E. D. *et al.* (2016) 'Finding the right coverage: the impact of coverage and sequence quality on single nucleotide polymorphism genotyping error rates', *Molecular ecology resources*, 16(4), pp. 966–978. doi: 10.1111/1755-0998.12519.

Francis, R. M. (2017) 'pophelper: An r package and web app to analyse and visualize population structure', *Molecular Ecology Resources*, 17, pp. 27–32. Available at: https://doi.org/10.1111/1755-0998.12509.

Frantz, C., Stewart, K. M. and Weaver, V. M. (2010) 'The extracellular matrix at a glance', *J. Cell Sci*, 123, pp. 4195–4200. doi: 10.1242/jcs.023820.

Frichot, E. and Francois, O. (2015) 'LEA: An R package for landscape and ecological association studies', *Methods Ecol Evol*, 6, pp. 925–929. doi: 10.1111/2041-210X.12382.

Froeschke, J., Stunz, G. W. and Wildhaber, M. L. (2010) ‘Environmental influences on the occurrence of coastal sharks in estuarine waters’, *Mar Ecol Prog Ser*, 407, pp. 279–292. Available at: https://doi.org/10.3354/meps08546.

Fuentes-Pardo, A. P. and Ruzzante, D. E. (2017) ‘Whole-genome sequencing approaches for conservation biology: Advantages, limitations and practical recommendations’, *Molecular Ecology*, 26(20), pp. 5369–5406. doi: 10.1111/mec.14264.

Gaggiotti, O. E. *et al.* (2009) ‘Disentangling the effects of evolutionary, demographic, and environmental factors influencing genetic structure of natural populations: Atlantic herring as a case study’, *Evolution*, 63, pp. 2939–2951. doi: 10.1111/j.1558-5646.2009.00779.x.

Gallego-García, N. *et al.* (2019) ‘Landscape genomic signatures indicate reduced gene flow and forest-associated adaptive divergence in an endangered neotropical turtle’, *Molecular Ecology*, 28(11), pp. 2757–2771. doi: 10.1111/mec.15112.

Gautier, M. *et al.* (2013) ‘Estimation of population allele frequencies from next-generation sequencing data: pool-versus individual-based genotyping’, *Molecular Ecology*, 22(14), pp. 3766–3779. doi: 10.1111/mec.12360.

Gautier, M. (2015) ‘Genome-wide scan for adaptive divergence and association with population-specific covariates’, *Genetics*, 201(4), pp. 1555–1579.

Griffiths, A. *et al.* (2013) ‘DNA barcoding unveils skate (Chondrichthyes: Rajidae) species diversity in “ray” products sold across Ireland and the UK’, *PeerJ*, 1, p. e129. doi: 10.7717/peerj.129.

Griffiths, A. M. *et al.* (2011) ‘Levels of connectivity between longnose skate (Dipturus oxyrinchus) in the Mediterranean Sea and the north-eastern Atlantic Ocean’, *Conservation Genetics*, 12(2), pp. 577–582. doi: 10.1007/s10592-010-

0127-3.

Grummer, J. A. *et al.* (2019) ‘Aquatic Landscape Genomics and Environmental Effects on Genetic Variation’, *Trends in Ecology and Evolution*, 34(7), pp. 641–654. doi: 10.1016/j.tree.2019.02.013.

Gubili, C. *et al.* (2014) ‘A tale of two seas: contrasting patterns of population structure in the small-spotted catshark across Europe’, *Royal Society Open Science*, 1(3), pp. 140175–140175. doi: 10.1098/rsos.140175.

Gugger, P. F. *et al.* (2018) ‘Applying landscape genomic tools to forest management and restoration of Hawaiian koa (Acacia koa) in a changing environment’, *Evolutionary Applications*, 11(2), pp. 231–242. doi: 10.1111/eva.12534.

Hällfors, M. *et al.* (2016) ‘Addressing potential local adaptation in species distribution models: Implications for conservation under climate change’, *Ecological Applications*, 26. doi: 10.1890/15-0926.

Hara, Yuichiro *et al.* (2018) ‘Shark genomes provide insights into elasmobranch evolution and the origin of vertebrates’, *Nature Ecology and Evolution*, 2(11), pp. 1761–1771. doi: 10.1038/s41559-018-0673-5.

Hara, Y *et al.* (2018) ‘Shark genomes provide insights into elasmobranch evolution and the origin of vertebrates’, *Nat Ecol Evol*, 2, pp. 1761–1771. doi: 10.1038/s41559-018-0673-5.

Harvey, M. G. *et al.* (2016) ‘Sequence Capture versus Restriction Site Associated DNA Sequencing for Shallow Systematics’, *Systematic Biology*, 65(5), pp. 910–924. doi: 10.1093/sysbio/syw036.

Hedrick, P. W. (2005) ‘A standardized genetic differentiation measure’, *Evolution*, 59(8), pp. 1633–1638. doi: PMID: 1632923.

Heithaus, M. R. *et al.* (2009) ‘Physical factors influencing the distribution of a top predator in a subtropical oligotrophic estuary’, *Limnol Oceanogr*, 54(2), pp. 472–482.

Helyar, S. J. *et al.* (2011) ‘Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges’, *Molecular Ecology Resources*, 11, pp. 123–136. doi: 10.1111/j.1755-0998.2010.02943.x.

Henderson, A. C. and Casey, A. (2001) ‘Reproduction and growth in the lesser-spotted dogfish Scyliorhinus canicula (Elasmobranchii; Scyliorhinidae), from the west coast of Ireland’, *Cah. Biol. Mar.*, 42, pp. 397–405.

Heupel, M. R. *et al.* (2014) ‘Sizing up the ecological role of sharks as predators’, *Marine Ecology Progress Series*, 495, pp. 291–298. doi: 10.3354/meps10597.

Hoban, S. *et al.* (2016) ‘Finding the genomic basis of local adaptation: Pitfalls, practical solutions, and future directions’, *American Naturalist*, 188(4), pp. 379–397. doi: 10.1086/688018.

Hobbs, C. A. D. *et al.* (2019) ‘Using DNA Barcoding to Investigate Patterns of Species Utilisation in UK Shark Products Reveals Threatened Species on Sale’, *Scientific Reports*, 9(1), pp. 1–10. doi: 10.1038/s41598-018-38270-3.

Hohenlohe, P. A. *et al.* (2010) ‘Population Genomics of Parallel Adaptation in Threespine Stickleback using Sequenced RAD Tags’, *PLOS Genetics*, 6(2), p. e1000862. Available at: https://doi.org/10.1371/journal.pgen.1000862.

Hueter, R. *et al.* (2004) ‘Evidence of Philopatry in Sharks and Implications for the Management of Shark Fisheries’, *Journal of Northwest Atlantic Fishery Science*, 37, pp. 239–247. doi: 10.2960/J.v35.m493.

Hume, J. B. (2019) ‘Higher temperatures increase developmental rate & reduce body size at hatching in the small?eyed skate Raja microocellata: implications for exploitation of an elasmobranch in warming seas’, *Journal of Fish biology*,

95, pp. 655–658.

Hyatt, M. W. and Anderson P. A. &. O'Donnell, P. (2018) 'Influence of Temperature, Salinity, and Dissolved Oxygen on the Stress Response of Bull (Carcharhinus leucas) and Bonnethead (Sphyrna tiburo) Sharks after Capture and Handling', *Journal of Coastal Research*, 34(4), pp. 818–827. doi: 10.2112/JCOASTRES-D-17-00118.1.

ICES (2019) *Working Group on Elasmobranch Fishes (WGEF)*, *ICES Scientific Reports*. Available at: http://doi.org/10.17895/ices.pub.5594%0AEditors.

Ikeda, D. H. *et al.* (2017) 'Genetically informed ecological niche models improve climate change predictions', *Global Change Biology*, 23(1), pp. 164–176. doi: 10.1111/gcb.13470.

Ivory, P., Jeal, F. and CP., N. (2005) 'Age determination, growth and reproduction in the lesser- spotted dogfish, Scyliorhinus canicula (L.)', *J Northw Atl Fish Sci*, 35, pp. 89–106.

Izzo, C. and BM., G. (2020) 'Port jackson shark growth is sensitive to temperature change', *Frontiers in Marine Science*, 7, p. 240. doi: 10.3389/fmars.2020.00240.

Jenkins, T. L. *et al.* (2019) 'Single nucleotide polymorphisms reveal a genetic cline across the north-east Atlantic and enable powerful population assignment in the European lobster', *Evolutionary Applications*, 12(10), pp. 1881–1899. doi: 10.1111/eva.12849.

Jirik, K. E. and Lowe, C. G. (2012) 'An elasmobranch maternity ward: female round stingrays Urobatis halleri use warm, restored estuarine habitat during gestation', *Journal of Fish Biology*, 80(5), pp. 1227–1245. doi: https://doi.org/10.1111/j.1095-8649.2011.03208.x.

Johri, S., Solanki, J., *et al.* (2019) '"Genome skimming" with the MinION hand-held sequencer identifies CITES-listed shark species in India's exports market', *Scientific Reports*, 9(1), p. 4476. doi: 10.1038/s41598-019-40940-9.

Johri, S., Doane, M. P., *et al.* (2019) 'Taking advantage of the genomics revolution for monitoring and conservation of chondrichthyan populations', *Diversity*, 11(4). doi: 10.3390/d11040049.

Jombart, T. (2008) 'adegenet: a R package for the multivariate analysis of genetic markers', *Bioinformatics*, 24(11), pp. 1403–1405. doi: 10.1093/bioinformatics/btn129.

Jones, M. R. and Good, J. M. (2016) 'Targeted capture in evolutionary and ecological genomics', *Molecular ecology*. 2015/07/30, 25(1), pp. 185–202. doi: 10.1111/mec.13304.

Junge, C. *et al.* (2019) 'Comparative population genomics confirms little population structure in two commercially targeted carcharhinid sharks', *Marine Biology*, 166(2), pp. 1–15. doi: 10.1007/s00227-018-3454-4.

Kawecki, T. J. and Ebert, D. (2004) 'Conceptual issues in local adaptation', *Ecology Letters*, 7, pp. 1225–1241.

Keenan, K. *et al.* (2013) 'diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors', *Methods in Ecology and Evolution*, 4, pp. 782–788. doi: 10.1111/2041-210X.12067.

Kelley, J. L. *et al.* (2016) 'The life aquatic: Advances in marine vertebrate genomics', *Nature Reviews Genetics*, 17(9), pp. 523–534. doi: 10.1038/nrg.2016.66.

Kopelman, N. M. *et al.* (2015) 'Clumpak: a program for identifying clustering modes and packaging population structure inferences across K', *Mol Ecol*

Resour, 15, pp. 1179–1191.

Kousteni, V. *et al.* (2015) ‘Strong population genetic structure and contrasting demographic histories for the small-spotted catshark (Scyliorhinus canicula) in the Mediterranean Sea’, *Heredity*, 114(3), pp. 333–343. doi: 10.1038/hdy.2014.107.

Kousteni, V *et al.* (2015) ‘Strong population genetic structure and contrasting demographic histories for the small-spotted catshark (Scyliorhinus canicula) in the Mediterranean Sea’, *Heredity*, 114(3), pp. 333–343. doi: 10.1038/hdy.2014.107.

Kousteni, V., Kontopoulou, M. and Megalofonou, P. (2010) ‘Sexual maturity and fecundity of Scyliorhinus canicula (Linnaeus, 1758) in the Aegean Sea’, *Mar. Biol. Res.*, 6, pp. 390–398.

Kraft, D. W. *et al.* (2020) ‘Genomics versus mtDNA for resolving stock structure in the silky shark (Carcharhinus falciformis)’, *PeerJ*. Edited by A. Amorim, 8, p. e10186. doi: 10.7717/peerj.10186.

Larade, K. and Storey, K. B. (2004) ‘Accumulation and translation of ferritin heavy chain transcripts following anoxia exposure in a marine invertebrate’, *The Journal of Experimental Biology*, 207, pp. 1353–1360. doi: 10.1242/jeb.00872.

Laver, T. *et al.* (2015) ‘Assessing the performance of the Oxford Nanopore Technologies MinION’, *Biomolecular Detection and Quantification*, 3, pp. 1–8. doi: https://doi.org/10.1016/j.bdq.2015.02.001.

Leone, A. *et al.* (2017) ‘Genetic differentiation and phylogeography of Mediterranean-North Eastern Atlantic blue shark (Prionace glauca, L. 1758) using mitochondrial DNA: Panmixia or complex stock structure?’, *PeerJ*, 2017(12). doi: 10.7717/peerj.4112.

Li, C. *et al.* (2013) ‘Capturing protein-coding genes across highly divergent species’, *Biotechniques*, 54, pp. 321–326.

Liggins, L., Treml, E. A. and Riginos, C. (2020) ‘Seascape Genomics: Contextualizing Adaptive and Neutral Genomic Variation in the Ocean Environment’, in Oleksiak, M. F. and Rajora, O. P. (eds) *Population Genomics: Marine Organisms*. Cham: Springer International Publishing, pp. 171–218. doi: 10.1007/13836_2019_68.

Limborg, M. T. *et al.* (2012) ‘Environmental selection on transcriptome derived SNPs in a high gene flow marine fish, the Atlantic herring (Clupea harengus). Molecular Ecology’, *Molecular Ecology*, 21, pp. 3686–3703.

Litvinov, F. (2003) ‘Sexual dimorphism as an index of the isolation of West African populations of the catshark S. canicula’, *J. Ichthyol.*, 43, pp. 81–85.

Lowry, D. B. *et al.* (2017) ‘Breaking RAD: an evaluation of the utility of restriction site-associated DNA sequencing for genome scans of adaptation’, *Molecular ecology resources*, 17(2), pp. 142–152. doi: 10.1111/1755-0998.12635.

Maggio, T. *et al.* (2019) ‘Historical separation and present-day structure of common dolphinfish (Coryphaena hippurus) populations in the Atlantic Ocean and Mediterranean Sea’, *ICES Journal of Marine Science*, 76(4), pp. 1028–1038. doi: 10.1093/icesjms/fsy174.

Maggs, C. *et al.* (2008) ‘Evaluating signatures of glacial refugia for North Atlantic Benthic marine taxa’, *Ecology*, 89, pp. S108–S122. doi: 10.1890/08-0257.1.

Mamanova, L. *et al.* (2010) ‘Target-enrichment strategies for next-generation sequencing’, *Nature Methods*, 7(2), pp. 111–118. doi: 10.1038/nmeth.1419.

Manuzzi, A. *et al.* (2019) ‘Population genomics and phylogeography of a benthic coastal shark (Scyliorhinus canicula) using 2b-RAD single nucleotide polymorphisms’, *Biological Journal of the Linnean Society*, 126(2), pp. 289–303. doi: 10.1093/biolinnean/bly185.

Marandel, F. *et al.* (2020) ‘Estimating effective population size using RADseq: Effects of SNP selection and sample size’, *Ecology and evolution*, 10(4), pp. 1929–1937. doi: 10.1002/ece3.6016.

Marra, N. J. *et al.* (2019) ‘White shark genome reveals ancient elasmobranch adaptations associated with wound healing and the maintenance of genome stability’, *Proceedings of the National Academy of Sciences*, 116(10), pp. 4446 LP – 4455. doi: 10.1073/pnas.1819778116.

Martin, C. S. *et al.* (2012) ‘Modelled distributions of ten demersal elasmobranchs of the eastern English Channel in relation to the environment’, *Journal of Experimental Marine Biology and Ecology*, 418–419, pp. 91–103. doi: https://doi.org/10.1016/j.jembe.2012.03.010.

Martinsohn, J. T. and Ogden, R. (2009) ‘FishPopTrace—Developing SNP-based population genetic assignment methods to investigate illegal fishing’, *Forensic Science International: Genetics Supplement Series*, 2(1), pp. 294–296. doi: https://doi.org/10.1016/j.fsigss.2009.08.108.

Mavropoulou, A., Vervatis, V. and Sofianos, S. (2020) ‘Dissolved oxygen variability in the Mediterranean Sea’, *Journal of Marine Systems*, 208, p. 103348. doi: 10.1016/j.jmarsys.2020.103348.

Metcalf, J. D. and Butler, P. J. (1984) ‘Changes in activity and ventilation response to hypoxia in unrestrained, unoperated dogfish, Scyliorhinus canicula’, *J. Exp. Biol.*, 108, pp. 411–418.

Milano, I. *et al.* (2014) ‘Outlier SNP markers reveal fine-scale genetic structuring across European hake populations (Merluccius merluccius).’, *Molecular Ecology*, 23, pp. 118–135.

Miyake, Y. and Saruhashi, K. (1956) ‘On the vertical distribution of the dissolved oxygen in the ocean’, *Deep Sea Research*, 3(4), pp. 242–247. doi: 10.1016/0146-6313(56)90012-0.

Momigliano, P. *et al.* (2017) ‘Genetic structure and signatures of selection in grey reef sharks (Carcharhinus amblyrhynchos)’, *Heredity*, 119(December), pp. 1–12. doi: 10.1038/hdy.2017.21.

Mucientes, G. R. *et al.* (2009) ‘Sexual segregation of pelagic sharks and the potential threat from fisheries’, *Biology letters*. 2009/02/25, 5(2), pp. 156–159. doi: 10.1098/rsbl.2008.0761.

Mulley, J. F. *et al.* (2014) ‘Transcriptomic analysis of the lesser spotted catshark (Scyliorhinus canicula) pancreas, liver and brain reveals molecular level conservation of vertebrate pancreas function’, *BMC Genomics*, 15(1), p. 1074. doi: 10.1186/1471-2164-15-1074.

Musa, S. M. *et al.* (2020) ‘Ocean warming and hypoxia affect embryonic growth, fitness and survival of small-spotted catsharks, Scyliorhinus canicula’, *J Fish Biol*, 97, pp. 257–264. doi: 10.1111/jfb.14370.

Narum, S. R. *et al.* (2013) ‘Genotyping-by-sequencing in ecological and conservation genomics’, *Molecular ecology*. 2013/05/25, 22(11), pp. 2841–2847. doi: 10.1111/mec.12350.

Naval-Sanchez, M. *et al.* (2020) ‘Changed Patterns of Genomic Variation Following Recent Domestication: Selection Sweeps in Farmed Atlantic Salmon’, *Frontiers in Genetics*, 11(April). doi: 10.3389/fgene.2020.00264.

Nielsen, E. E. *et al.* (2009) 'Population genomics of marine fishes: identifying adaptive variation in space and time', *Mol. Ecol*, 18, pp. 3128–3150.

Nielsen, E. E. *et al.* (2012) 'Gene-associated markers provide tools for tackling illegal fishing and false eco-certification', *Nature Communications*, 3, pp. 851–856. doi: 10.1038/ncomms1845.

Ovenden, J. R. *et al.* (2018) *Genetics and genomics for fundamental and applied research on elasmobranchs. In: Carrier JC, Heithaus MR, Simpfendorfer CA (eds) Shark research: emerging technologies and applications for the field and laboratory.* Boca Raton, FL: CRC Press.

Papakostas, S. *et al.* (2014) 'Proteome variance differences within populations of European whitefish (Coregonus lavaretus) originating from contrasting salinity environments', *J Proteomics.*, 105, pp. 144–150.

Paradis, E., Claude, J. and Strimmer, K. (2004) 'APE: Analyses of Phylogenetics and Evolution in R Language', *Bioinformatics*, 20, pp. 289–290. doi: 10.1093/bioinformatics/btg412.

Paris, J. R., Stevens, J. R. and Catchen, J. M. (2017) 'Lost in parameter space: a road map for stacks', *Methods in Ecology and Evolution*, 8(10), pp. 1360–1373. doi: 10.1111/2041-210X.12775.

Pascual, M. *et al.* (2017) 'Impact of life history traits on gene flow: A multispecies systematic review across oceanographic barriers in the Mediterranean Sea', *PLoS ONE*, 12(5), p. e0176419. doi: 10.1371/journal.pone.0176419.

Patarnello, T., Volckaert, F. A. M. J. and Castilho, R. (2007) 'Pillars of Hercules: Is the Atlantic-Mediterranean transition a phylogeographical break?', *Molecular Ecology*, 16(21), pp. 4426–4444. doi: 10.1111/j.1365-294X.2007.03477.x.

Pazartzi, T. *et al.* (2019) ‘High levels of mislabeling in shark meat – Investigating patterns of species utilization with DNA barcoding in Greek retailers’, *Food Control*, 98, pp. 179–186. doi: https://doi.org/10.1016/j.foodcont.2018.11.019.

Pazmino, D. A. *et al.* (2017) ‘Genome-wide SNPs reveal low effective population size within confined management units of the highly vagile Galapagos shark (Carcharhinus galapagensis)’, *Conserv Genet*, 18, pp. 1151–1163. Available at: https://doi.org/10.1007/s10592-017-0967-1.

Peterson, B. K. *et al.* (2012) ‘Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species.’, *PloS one*, 7(5), p. e37135. doi: 10.1371/journal.pone.0037135.

Poland, J. A. *et al.* (2012) ‘Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach’, *PLoS ONE*, 7(2), p. e32253. Available at: https://doi.org/10.1371/journal.pone.0032253.

Portnoy, D S *et al.* (2015) ‘Selection and sex-biased dispersal in a coastal shark: the influence of philopatry on adaptive variation’, *Mol Ecol*, 24, pp. 5877–5885. doi: 10.1111/mec.13441.

Portnoy, D. S. *et al.* (2015) ‘Selection and sex-biased dispersal in a coastal shark: The influence of philopatry on adaptive variation’, *Molecular Ecology*, 24(23), pp. 5877–5885. doi: 10.1111/mec.13441.

Postaire, B. D. *et al.* (2020) ‘Environmental DNA detection tracks established seasonal occurrence of blacktip sharks (Carcharhinus limbatus) in a semi-enclosed subtropical bay’, *Scientific Reports*, 10(1), pp. 1–8. doi: 10.1038/s41598-020-68843-0.

Powter, D. M. and Gladstone, W. (2008) ‘Embryonic mortality and predation on egg capsules of the port Jackson shark Heterodontus portusjacksoni (Meyer)’, *Journal of Fish Biology*, 72(3), pp. 573–584.

Pritchard, J. K., Stephens, M. and Donnelly, P. (2000) ‘Inference of population structure using multilocus genotype data’, *Genetics*, 155, pp. 945–959.

Prive, F. *et al.* (2020) ‘Performing highly efficient genome scans for local adaptation with R package pcadapt version 4’, *Molecular Biology and Evolution*, 37, pp. 2153–2154.

Purcell, S. *et al.* (2007) ‘PLINK: a toolset for whole-genome association and population-based linkage analysis’, *American Journal of Human Genetics*, 81(3), pp. 559–575.

Ramirez-Amaro, S. *et al.* (2018) ‘Contrasting evolutionary patterns in populations of demersal sharks throughout the western Mediterranean’, *Marine Biology*, 165(1), p. 3. doi: 10.1007/s00227-017-3254-2.

Ramírez-Amaro, S. *et al.* (2018) ‘Contrasting evolutionary patterns in populations of demersal sharks throughout the western Mediterranean’, *Marine Biology*, 165(1). doi: 10.1007/s00227-017-3254-2.

Razgour, O. *et al.* (2019) ‘Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections’, *Proceedings of the National Academy of Sciences*, 116(21), pp. 10418 LP – 10423. doi: 10.1073/pnas.1820663116.

Read, T. D. *et al.* (2017) ‘Draft sequencing and assembly of the genome of the world’s largest fish, the whale shark: Rhincodon typus Smith 1828’, *BMC Genomics*, 18(1), p. 532. doi: 10.1186/s12864-017-3926-9.

Rellstab, C. *et al.* (2015) ‘A practical guide to environmental association analysis in landscape genomics’, *Molecular Ecology*, 24(17), pp. 4348–4370. doi: 10.1111/mec.13322.

Rochette, N. C. and Catchen, J. M. (2017) ‘Deriving genotypes from RAD-seq short-read data using Stacks’, *Nature Protocols*, 12(12), pp. 2640–2659. doi: 10.1038/nprot.2017.123.

Rochette, N., River-Colon, A. and Catchen, J. (2019) ‘Stacks 2: Analytical methods for paired?end sequencing improve RADseq-based population genomics’, *Molecular Ecology*, 28(21), pp. 4737–4754.

Rodriguez-Cabello, C. *et al.* (2004) ‘Is the lesser spotted dogfish (Scyliorhinus canicula) population from the Cantabrian Sea a unique stock?’, *Fisheries Research*, 69, pp. 57–71. doi: 10.1016/j.fishres.2004.04.002.

Rodriguez-Cabello, C., Sanchez, F. and Olaso, I. (2007) ‘Distribution patterns and sexual segregations of Scyliorhinus canicula (L.) in the Cantabrian Sea’, *Journal of Fish Biology*, 70, pp. 1568–1586. doi: 10.1111/j.1095-8649.2007.01444.x.

Rohde, K. (1992) ‘Latitudinal gradients in species diversity: the search for the primary cause’, *Oikos*, 65, pp. 514–527.

Rosa, R. *et al.* (2014) ‘Early-life exposure to climate change impairs tropical shark survival’, *Proceedings of the Royal Society B*, 281(1793), p. 20141738.

Rubinoff, D., Cameron, S. and Will, K. (2006) ‘A genomic perspective on the shortcomings of mitochondrial DNA for “barcoding” identification’, *Journal of Heredity*, 97(6), pp. 581–594. doi: 10.1093/jhered/esl036.

Ruegg, K. *et al.* (2018) ‘Ecological genomics predicts climate vulnerability in an endangered southwestern songbird’, *Ecology Letters*, 21(7), pp. 1085–1096. doi: 10.1111/ele.12977.

Selkoe, K. A. *et al.* (2016) ‘A decade of seascape genetics: Contributions to basic and applied marine connectivity’, *Marine Ecology Progress Series*, 554, pp. 1–19. Available at: https://doi.org/10.3354/meps11792.

Shaltout, Mohamed and Omstedt, A. (2014) ‘Recent sea surface temperature trends and future scenarios for the Mediterranean Sea’, *Oceanologia*, 56(3), pp. 411–443. doi: 10.5697/oc.56-3.411.

Shaltout, M and Omstedt, A. (2014) ‘Recent sea surface temperature trends and future scenarios for the Mediterranean Sea’, *Oceanologia*, 56(3), pp. 411–443. doi: 10.5697/oc.56-3.411.

Sharma, K. *et al.* (2019) ‘The Chemosensory Receptor Repertoire of a True Shark Is Dominated by a Single Olfactory Receptor Family’, *Genome Biology and Evolution*, 11(2), pp. 398–405. doi: 10.1093/gbe/evz002.

Simpfendorfer, C. A. *et al.* (2016) ‘Environmental DNA detects Critically Endangered largetooth sawfish in the wild’, *Endangered Species Research*, 30(1), pp. 109–116. doi: 10.3354/esr00731.

Sims, D., Nash, J. and Morritt, D. (2001) ‘Movements and activity of male and female dogfish in a tidal sea lough: Alternative behavioural strategies and apparent sexual segregation’, *Marine Biology*, 139, pp. 1165–1175. doi: 10.1007/s002270100666.

Skliris, N. *et al.* (2018) ‘Mediterranean sea water budget long-term trend inferred from salinity observations’, *Clim Dyn*, 51, pp. 2857–2876. doi: 10.1007/s00382-017-4053-7.

Speed, C. W. *et al.* (2010) ‘Complexities of coastal shark movements and their implications for management’, *Mar Ecol Prog Ser*, 408, pp. 275–293. doi: 10.3354/meps08581.

Suchan, T. *et al.* (2016) ‘Hybridization Capture Using RAD Probes (hyRAD), a New Tool for Performing Genomic Analyses on Collection Specimens’, *PLOS ONE*, 11(3), p. e0151651. Available at: https://doi.org/10.1371/journal.pone.0151651.

Sunday, J. M., Bates, A. E. and Dulvy, N. K. (2012) ‘Thermal tolerance and the global redistribution of animals’, *Nature Climate Change*, 2(9), pp. 686–690. doi: 10.1038/nclimate1539.

Supek, F. *et al.* (2011) ‘Summarizes and Visualizes Long Lists of Gene Ontology Terms’, *PLoS ONE*, 6, p. e21800.

Tan, M. *et al.* (2019) ‘The whale shark genome reveals patterns of vertebrate gene family evolution’, *bioRxiv*. doi: 10.1101/685743.

Tewhey, R. *et al.* (2009) ‘Microdroplet-based PCR enrichment for large-scale targeted sequencing’, *Nature Biotechnology*, 27(11), pp. 1025–1031. doi: 10.1038/nbt.1583.

Thomason, J. C. *et al.* (1996) ‘Effect of temperature and photoperiod on the growth of the embryonic dogfish, Scyliorhinus canicula’, *Journal of Fish Biology*, 49(4), pp. 739–742. doi: 10.1111/j.1095-8649.1996.tb00071.x.

Tiffin, P. and Ross-Ibarra, J. (2014) ‘Advances and limits of using population genetics to understand local adaptation’, *Trends in Ecology and Evolution*, 29(12), pp. 673–680. doi: 10.1016/j.tree.2014.10.004.

Torrado, H. *et al.* (2020) ‘Individual-based population genomics reveal different drivers of adaptation in sympatric fish’, *Scientific Reports*, 10(1), pp. 1–14. doi: 10.1038/s41598-020-69160-2.

Trenkel, V. M. *et al.* (2020) ‘Methods for identifying and interpreting sex-linked SNP markers and carrying out sex assignment: application to thornback ray (Raja clavata)’, *Molecular Ecology Resources*, 20(6), pp. 1610–1619. doi:

10.1111/1755-0998.13225.

Vaha, J.-P. *et al.* (2007) ‘Life-history and habitat features influence the within-river genetic structure of Atlantic salmon’, *Molecular Ecology*, 16(13), pp. 2638–2654. doi: 10.1111/j.1365-294X.2007.03329.x.

Vendrami, D. L. J. *et al.* (2019) ‘RAD sequencing sheds new light on the genetic structure and local adaptation of European scallops and resolves their demographic histories’, *Scientific Reports*, 9(1), pp. 1–13. doi: 10.1038/s41598-019-43939-4.

Venkatesh, B. *et al.* (2014) ‘Elephant shark genome provides unique insights into gnathostome evolution’, *Nature*, 505, pp. 174–179. doi: 10.1038/nature12826.

Veríssimo, A. *et al.* (2018) ‘ Population genomics and phylogeography of a benthic coastal shark (Scyliorhinus canicula) using 2b-RAD single nucleotide polymorphisms ’, *Biological Journal of the Linnean Society*, 126(2), pp. 289–303. doi: 10.1093/biolinnean/bly185.

Waldvogel, A. M. *et al.* (2020) ‘Evolutionary genomics improves prediction of species responses to climate change’, *Evolution Letters*, 4(1), pp. 4–18. doi: 10.1002/evl3.154.

Wang, Q. *et al.* (2012) ‘Community annotation and bioinformatics workforce development in concert—Little Skate Genome Annotation Workshops and Jamborees’, *Database : the journal of biological databases and curation*, 2012, p. bar064. doi: 10.1093/database/bar064.

Wannell, G. *et al.* (2020) ‘A new minibarcode assay to facilitate species identification from processed, degraded or historic ray (batoidea) samples’, *Conservation Genetics Resources*. doi: 10.1007/s12686-020-01158-4.

Ward-Paige, C. A. *et al.* (2015) ‘Characterizing and predicting essential habitat features for juvenile coastal sharks’, *Mar Ecol*, 36, pp. 419–431. doi: 10.1111/maec.12151.

Weber, J. A. *et al.* (2020) ‘The whale shark genome reveals how genomic and physiological properties scale with body size’, *Proceedings of the National Academy of Sciences*, 117(34), pp. 20662–20671. doi: 10.1073/pnas.1922576117.

Weir, B. S. and Cockerham, C. C. (1984) ‘Estimating F-Statistics for the Analysis of Population Structure’, *Evolution*, 38(6), pp. 1358–1370. Available at: http://www.jstor.org/stable/2408641.

Wheeler, C. R. *et al.* (2020) ‘Anthropogenic stressors influence reproduction and development in elasmobranch fishes’, *Reviews in Fish Biology and Fisheries*, 30(2), pp. 373–386. doi: 10.1007/s11160-020-09604-0.

White, T. A., Stamford, J. and Hoelzel, A. R. (2010) ‘Local selection and population structure in a deep-sea fish, the roundnose grenadier (Coryphaenoides rupestris)’, *Mol Ecol*, 19, pp. 216–226. doi: https://doi.org/10.1111/j.1365-294X.2009.04446.x.

Zimmerman, S. J. *et al.* (2019) ‘Signatures of adaptive divergence among populations of an avian species of conservation concern’, *Evolutionary Applications*, 12(8), pp. 1661–1677. doi: 10.1111/eva.12825.

Additional Published Work

Bound in this additional section is the further work I undertook during the course of my Masters by Research. It included the development of a new DNA minibarcode to facilitate species identification in processed batoid samples in the hope to improve trade monitoring. This work was published in Conservation Genetics Resources:

Wannell, G., Griffiths, A. M., Spinou, A., Batista, R., Mendonça, M., Wosiacki, W., Fraser, B., Wintner, S., Papadopoulos, A., Krey, G. & Gubili, C. (2020). A new minibarcode assay to facilitate species identification from processed, degraded or historic ray (batoidea) samples. *Conserv Genet Resour*. 12, 659-668.

Whilst it lies outside the tight focus of my dissertation title on the population genetics of *S. canicula*, it still focuses on molecular tools in elasmobranchs. Therefore, I hope that you will considered it as part of submission of work towards my Masters by Research.

Link to paper: https://link.springer.com/article/10.1007/s12686-020-01158-4