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Genetic diversity and differentiation of velvet belly lanternshark (*Etmopterus spinax*) in the Northeast Atlantic.

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Master's thesis in biology BIO-3950, June 2022

Acknowledgments

I would like to thank my supervisor Arve Lynghammar and my co-supervisor Claudia Junge for collaborating with me and making my dream of starting to work on sharks come true. And thanks for all the supervision and help you two have given me throughout the entire process.

Thanks to the Institute of Marine Research (IMR) for the samples and data I needed to execute this project. A special thanks to all the people on the research survey Reketokt 2021, who taught me the process of sampling fish, including sharks, and recording the data.

Thanks to Tanja Hanebrekke who helped me with the last steps on the genetic samples, to make them ready for sanger-sequencing.

Thank you to all members of the RGG genetics group at UIT for the social network, friendly and professional environment you created. And a special thanks to my fellow students that helped read my thesis early in the process.

Kristel Berg, Mathilde Horaud and Simon Nordblad Scmidt for helping me out and teaching me how DNA Extraction, Gel making, PCR-machine and other lab equipment and protocols worked in the laboratory.

Thanks to Gavin Naylor for sequences of *E. spinax* outside of Norway.

Forward

The purpose of this study was to investigate population structure of the velvet belly lanternshark (*Etmopterus spinax*). This project has been supervised by Associated Professor Arve Lynghammar, and co-supervised by Dr. Claudia Junge. The newly sequenced mitochondrial DNA NADH2 sequences will be added to NCBI GenBank.

This thesis is written in APA 7 format.

Abstract

Deep-sea sharks are little resilient to targeted harvesting and bycatch fisheries due to their life history strategy characterized by slow growth, late sexual maturity, low fecundity, and few offspring. The Northeast Atlantic component of *Etmopterus spinax* is an example of a species where substantial population changes in terms of abundances have occurred due to intensive fisheries induced mortality. This has led to the IUCN categorization of *E. spinax* as Vulnerable with a negative population trend. To reverse the trend, management and conservation criteria are needed. To give management advice, knowledge about the connectivity between potential populations need to be improved, together with an understanding of their habitat and life history. In this study, the potential populations and differences between the locations sampled in the NE Atlantic, and particularly Norway, are studied by analyzing the mitochondrial NADH2 gene. Overall, at all locations the haplotype diversity is moderate to high, and the nucleotide diversity low. This can indicate relatively recent population expansion. The results also show significant population structure between the Norwegian locations and the rest of NE Atlantic. On a smaller scale, there was weak population structure within Norway and none for the rest. Against expectations, no genetic difference was found between a fjord and coastal population off Trondheim, NO. The lack of identifying genetic differences and population structure can be due to migration, or that NADH2 is not a suitable marker to identify this on a relatively small scale.

Keywords

NADH2, Population structure, Connectivity, Genetic diversity, Vulnerable, Northeast Atlantic

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1 INTRODUCTION

1.1 Sharks, ecosystem, and vulnerability

Chondrichthyans, i.e., sharks, rays, skates, and chimaeras, have survived and evolved the past 400 million years, which make them one of the most successful and oldest group of vertebrates in terms of historical durability (Camhi et al., 1998; Coelho, 2007). There are approximately 1,114 described chondrichthyan species around the world (Kyne & Simpfendorfer, 2010). Chondrichthyans are morphologically diverse with body forms reflecting habitat preference (Lynghammar et al., 2013). Sharks make out about half (428 species) of the chondrichthyans (Kyne & Simpfendorfer, 2010), and occupying a wide range of habitats, from the epipelagic (0 – 200 m) all the way to the deep-sea (200 – 2000 m) (Kyne & Simpfendorfer, 2010).

Sharks have an important ecosystem role, many being on the top of the food chain and keeping balance of the marine life, with a top-down influence (Myers et al., 2007). By being a predator, they can affect their pray species composition of the lower trophic level. How strong the trophic effects of sharks of smaller size is, are largely unknown (Cailliet et al., 2005). In order to understand how the ecosystem will respond to natural or human-induces changes, knowledge about the deep-sea communities is urgently needed (Howell et al., 2021). Removal of sharks in an ecosystem will have effects on the structure and functions of the marine ecosystem (Stevens et al., 2000). Examples of direct effects are density-dependent changes, and indirect effects involve trophic interactions, e.g., through selectively removing a level in the food chain, disturbances are made (Stevens et al., 2000).

Sharks have a K-selected life history strategy, characterized by slow growth, late sexual maturity, reduced fecundity, and few offspring (Camhi et al., 1998). How long they live, age of maturity and litter size varies highly for different taxa and species. One example on the one extreme end is the Greenland shark (*Somniousus microcephalus*) with a lifespan of at least 272 years. And with the females not reaching maturity before they are > 400 cm long, the corresponding age would be 156 ± 22 years(Nielsen et al., 2016). Other deep-sea species, for example *E. spinax* and *E. pusillus* mature at 75 % and 87 % of their maximum body length,

respectively, which varies a bit from species and sex (Coelho & Erzini, 2005). Sexual dimorphism in terms of size at maturity is common in sharks and specifically for the *Etmopterus* genus, with females usually maturing later and at larger sizes than males (Jakobsdóttir, 2001).

Given these biological characteristics, sharks and other chondrichthyans are believed to have a low resilience to targeted harvesting and bycatch fisheries (McMillan et al., 2017; Neiva et al., 2006). With fishery pressure increasing on commercial fish, it indirectly and directly affects other species (ICES, 2020). With the technological improvement of fishing gear and vessels, they can expand to new areas, and move from the continental shelf to the slope to target other species, or increase catch on species with high commercial value (Ramírez-Amaro et al., 2020). For the latter being species such as Norway lobster, *Nephrops norvegicus* (Linnaeus 1758), deep water rose shrimp, *Parapenaeus longirostris* (Lucas 1846), and blue and red shrimp, *Aristeus antennatus* (Risso 1816) (Monteiro et al., 2001). These commercial species occur in the same depth range as the deep-sea shakes (Coelho & Erzini, 2010), which can increase the decline of the deep-sea shark species. For example, in the Mediterranean the movement of fisheries from shelf to slope has led to recovery of some species in the shelf area, but decreasing trends for the deep-water species *E. spinax* and *Dipturus oxyrinchus* (Ramírez-Amaro et al., 2020). Such alterations can further affect other parameters, such as maturation and fecundity (Coelho et al., 2010) and which habitats they occupy, a density-dependent change (Stevens et al., 2000).

1.2 Etmopterus spinax

Etmopterus spinax distribution span extending from northern Norwegian Sea and Iceland to the Gulf of Guinea and Gabon, including the Mediterranean Sea, the Azores, the Canary Islands, and Cape Verde (Ebert & Stehmann, 2013). There is a higher density of *E. spinax* in the Norwegian Sea than in the North Sea, where they are limited to the Norwegian Trench (Jac et al., 2021). *Etmopterus spinax* mainly inhabits the continental shelf and slope, at depths of 70-2000 m, with individuals recorded in shallower waters up to 20 m, but with the highest abundance between 200-500 m depth (Ebert et al., 2021) in soft-bottom habitats (Sion et al., 2004).

The preferred habitat of *E. spinax* is deep cold water (temperature around 2-8 °C). It is shown that the presence of the species depends on temperature in combination with other factors, mainly depth, while the abundance of the species seems dependent on salinity (Jac et al., 2021). The temperature and salinity of the water is affected by the currents and upwellings (Rodhe, 1989), which means that topography affects where we find the most *E. spinax*. These factors are most likely the reason for *E spinax* being in higher abundances in the Norwegian trench. But with relatively even temperature and salinity along the whole coast of Norway, the distribution of *E. spinax* follows the whole coast (Jac et al., 2021).

Etmopterus spinax have live-bearing strategy, aplacental viviparous (Capape et al., 2001; Jac et al., 2021), with the young feeding on yolk until birth (Capape et al., 2001) of 6-20 live offspring (Coelho & Erzini, 2008). At the time of hatching they are readily feed on larger prey and do not depend on plankton blooms like larvae of many bony fishes (Beaugrand et al., 2003).

They are sexually dimorphic (Aranha et al., 2009; Sion et al., 2004), with females growing to maximum 60 cm total length (TL), and the males to maximum 50 cm, but individuals over 40 cm are rare (Coelho & Erzini, 2005; Jac, 2020). Females are found to be distributed over a wider depth range than males (Coelho & Erzini, 2010). Size increases with depth regardless of sex (McMillan et al., 2017), with the larger older specimens occurring in deeper water, while the smaller and younger specimens occur mainly in shallow waters (Coelho & Erzini, 2010). Female age-at-maturity is 4.7 years and maximum age is 11 years (Coelho & Erzini, 2008) , resulting in a generation length of 7.8 years (Finucci et al., 2021). Generation length can be estimated in several ways, which also can alter the definition some. IUCN have defined generation based of analyzed trend data over three generation lengths (23 years), which yield an annual rate of change, giving a median change over three generation lengths (Finucci et al., 2021). Generation length is greater than the age at first breeding and less than the age of the oldest breeding individual. Under threat the generation time can vary, such as under exploitation of fish, the more natural, i.e., pre-disturbance, generation length should be used (IUCN, 2019).

1.3 Vulnerability of *E. spinax*

In the North east Atlantic (NE Atlantic) component of *E. spinax* substantial population changes in terms of abundances have occurred due to intensive fisheries induced mortality (Coelho et al., 2010). *Etmopterus spinax* is one of the most impacted shark species in the NE Atlantic fisheries, as a result of accidental bycatch (no commercial value) (Besnard et al., 2022). There are no exact number on how high the bycatch of *E. spinax* is (ICES, 2020). Estimated landing data provided to the ICES working group of elasmobranch fishes (WGEF) from 2005 onwards indicates that landings assigned to *E. spinax* should be considered as *Etmopterus* spp., due to only a small proportion of the catch is known *Etmopterus* species (ICES, 2020).There are very few countries where they identify *Etmopterus* spp., on species level, but in Portugal they mainly refer their landings to *E. spinax* and *E. pusillus* (ICES, 2020). Landings reported by Norway was 163 tons in 2019, being the highest value reported of *E. spinax* of six countries (Denmark, Norway, UK, France, Spain and Portugal) (ICES, 2020). Regardless of this high bycatch there is still a need of more knowledge on the species. Other factors making *E. spinax* vulnerable is climate change, pollution and habitat loss (Dulvy et al., 2021). Climate change is a threat by leading to change of temperatures, which potentially makes species move from one area to another (Sguotti et al., 2016).

Etmopterus spinax is an abundant deep-sea, demersal shark species, categorized as Least Concern (LC) by the International Union for Conservation of Nature (IUCN) in 2009 (Coelho et al., 2009). After the new evaluation by IUCN in 2021 the status has changed from LC to Vulnerable (VU) A2bd and having a declining trend, globally (Finucci et al., 2021). *Etmopterus spinax* has gotten the assessment criterion A2bd because: there has been a reduction in the population size of ≥ 30% over the last three generations (23 years) based on abundance indices and actual levels of exploitation (Coelho et al., 2009; Finucci et al., 2021; IUCN, 2012). These data were estimated to represent 80% of the species known spatial distribution. According to Hesthagen et al. (2021) *E. spinax* is of LC in Norwegian waters, evaluated as part of the Norwegian Red List assessment through Artsdatabanken in 2021. The evaluation is set based on it being too little data / time series to evaluate the effect of fisheries. Artsdatabanken follow the same criteria as IUCN (2012). The Norwegian component of the species comprise < 5% of the European and < 5% of the global population (Wienerroither et al., 2021). The shift on a global basis from LC to VU is a motivation for this study to obtain

more knowledge on the population structure of *E. spinax*, so that the correct population unit can be managed, and to reduce further decline.

1.4 Population genetics and Connectivity

What is a population and how can they be connected?

A population can have various definitions, and there is not one specific correct answer. The population concept is central for topics on ecology, evolutionary biology, and conservation biology (Waples & Gaggiotti, 2006). There are two major biological definitions of a population. First being the ecological paradigm, where the compatible forces are largely demographic, and focus on co-occurrence in space and time so that individuals can interact. Second definition being on the evolutionary paradigm, where the compatible forces are primarily genetic, and the focus is on reproductive interactions between the individuals (Waples & Gaggiotti, 2006).

It can be difficult to know when groups of individuals of a species are different enough to define them as separate populations. Andrewartha and Birch (1986) and IUCN (2012) categories and criteria, define a population (also known as natural – population) as all individuals of one species, which is supported by a locality (e.g. a pond, or a fjord). The localities can be separated by barriers, which to some degree can keep the local populations that building up the natural population separate (Andrewartha & Birch, 1986).
Population connectivity consists of both a genetic and a demographic component (Lowe & Allendorf, 2010; Marandel et al., 2018). Genetic and demographic connectivity are fundamentally different and they require different, but potentially complementary methods of assessment (Lowe & Allendorf, 2010). Genetic connectivity can be defined as; to which extend gene flow affects evolutionary processes within and between populations (Lowe & Allendorf, 2010). This can be studied by the use of genetic tests, such as F_{ST} to assess gene flow (Wright, 1943). Many questions can be answered by genetic methods, but alone they provide little information to the whole picture of basic population biology, therefore demographic connectivity is of central importance (Runge et al., 2007). Demographic connectivity can be defined as the degree to which population growth and vital rates are

affected by dispersal (Lowe & Allendorf, 2010). For populations to be connected at least one individual must immigrate per generation over long periods of evolutionary time. But this one immigrant is not enough to maintain nearly identical haplotype or allelic frequencies between populations (Lowe & Allendorf, 2010).

Barriers and dispersal

Ronce (2007) defines dispersal as any movement of individuals with potential consequences for gene flow across space. Dispersal movement can be divided into three stages: departure (or emigration), a roaming stage, and settling (or immigration). Biodiversity and distribution relies on dispersal of animals across the landscape (Hirschfeld et al., 2021). If there had been unrestricted dispersal and gene flow, there would be a lack of genetic population structure throughout a species geographical range (Slatkin, 1987). But dispersal is restricted by bio-physical problems (Cowen et al., 2002), such as geographical features or unfavorable environmental conditions, creating genetic divergence among populations (Hellberg et al., 2002). In the ocean these barriers can be land masses, bathymetry, gradients of temperature and salinity, currents, tides, and other movements of water masses (Cowen et al., 2002; Riginos & Liggins, 2013). Connectivity is not only defined by physical processes, but also behavior, such as preferred habitat for reproduction which can reduce the genetic connectivity, and larval development and behavioral issues including vertical migration, play an important role (Cowen et al., 2002).

Many marine organisms such as teleost fish and marine invertebrates have a juvenile planktonic stage that uses ocean currents as a dispersal agent, while the adults are more stationary at sites (Hellberg, 2009). While those marine animals lacking a planktonic larval stage depend on the active dispersal of individuals (Hirschfeld et al., 2021; Marandel et al., 2018). For sharks, the dispersal is conducted by the adults while the juveniles are more stationary (Ebert et al., 2013; Grubbs, 2010). There can be produced distinct geographical patterns of genetic variation due to these fundamental differences in life history and dispersal (Hirschfeld et al., 2021).

While the egg and larva can use the currents for dispersal, the currents can function as a barrier for species with active dispersal. Global connectivity might only be possible for species with large-scale horizontal dispersal, that also tolerate a wide spectrum of

environmental conditions and/or capable of extensive vertical migration (Hirschfeld et al., 2021). Connectivity of smaller deep-sea sharks across a wide geographical range can be explained by habitat preferences and maximum depth. Species with a large depth range are less likely to show genetic differentiation across depths and mid ocean barriers, e.g., a bathymetric barrier such as the strait of Gibraltar between the Atlantic and Mediterranean (Gubili et al., 2016; Hirschfeld et al., 2021)

How and why do we study genetic connectivity?

To study genetic connectivity requires a toolbox of techniques and methods applied in an academical framework (Cowen et al., 2002). The methods to estimate how much gene flow occurs in a natural population are categorized into two classes: "Direct methods" which is direct observations of movement, which can indicate the gene flow at a particular time, but do not show gene flow over a longer period of time. To indicate gene flow over longer periods of time "indirect methods" can be useful. Here, estimates of gene flow are based on the analyses of gene frequencies, determined using electrophoretic studies, polymorphisms, and DNA sequence data. The estimations on the extend of gene flow are done using Wright's statistic, F_{ST} (Slatkin, 1987; Wright, 1943).

Some of the common methods found in the toolbox used to study migration of marine species, such as sharks can be assessed using artificial, chemical, genetic tags (McMillan et al., 2017), and/or dietary studies (Besnard et al., 2022) to trace movements between populations. All these methods give valid information, but not all of them are suitable for deep-sea sharks such as *E. spinax*. Artificial tags method can be applied for deep-sea sharks, but challenging and inefficient, due to the need of catch and release for fastening the tag, which comes with the risk of mortality (IUCN et al., 2007). Chemical tags method use chemical signatures found in natural elements and isotope tags, which are found in calcified body parts, such as the vertebra in sharks to look on population structure (McMillan et al., 2017). Genetic tags are one of the most applied method, and it is good for defining long-term gene flow patterns across generations (genetic-connectivity) (Hellberg et al., 2002). The mitochondrial DNA (mtDNA) markers are commonly used as genetic tags in studies due to unique properties: evolving at a slower rate than nuclear markers, small sized, availability, maternal inheritance, and high mutation rate (Anderson et al., 2010; Duchêne et al., 2011; Wang, 2010). These properties make them good markers for studying population genetics and connectivity.

Common regions to use are the control region (CR) (Gubili et al., 2016; Veríssimo et al., 2011), the mitochondrial cytochrome C oxidase subunit I (COI) (Bors et al., 2012), or the mitochondrial nicotinamide dehydrogenase subunit 2 gene (NADH2) marker (Straube et al., 2015; Straube et al., 2021).

Using the tools and methods to find out if a population is genetically connected will help identifying if the populations can be considered "closed" or "open", the latter being more resilient to exploitation (Cowen et al., 2000). A closed population is fixed to a location and no new members are added or lost from the population due to migration, only through birth and death are individuals introduced and removed from the population. While open populations exchange individuals, at least periodically (Caley et al., 1996; Cowen et al., 2000). Most species have a degree of open population, with moderate connectivity due to geographical overlap or migration, and rarely a population of complete independence (Waples & Gaggiotti, 2006). If a population is thought to be "fully" open, this can lead to over estimation of population exchange (Cowen et al., 2000).

In the NE Atlantic, the only population genetic study on *E. spinax* which used mtDNA CR (Gubili et al., 2016) found population structure between the NE Atlantic and the Mediterranean, but no structure within the NE Atlantic. McMillan et al. (2017) suggested three potential populations using elemental chemistry: western Norway, southern Norway, and France, which indicates potential population structure. Study by Maaholm and Mihalitsis (2014) found population structure between the Norwegian Sea and the Celtic Sea (France) by the use of NADH2 sequences. This corresponds with the findings in McMillan et al. (2017). It will be interesting if this study can confirm either the high level of connectivity reported by Gubili et al. (2016) or find the same population structure in NE Atlantic as indicated by McMillan et al. (2017) and Maaholm and Mihalitsis (2014). NADH2 is not an extensively used marker on *E. spinax,* and no population level studies have been published yet (but see: Bachelor study by Maaholm and Mihalitsis (2014)). All these three studies cover few locations with few individuals in Norway, in addition to the other locations in the NE Atlantic. This study compared to those will include more locations along the whole coast of Norway, and not only the southern parts, in addition to more individuals per location. This increases the chances of confirming or denying the tendencies of population structure in Norway, as well as for the whole NE Atlantic.

2 Goals and expectations

Goals of the study:

(1) Are there any potential populations of *E. spinax* in the NE Atlantic?

(2) Are there any potential populations within Norway?

(3) Do the results of 1 and 2 have implications for management of *E. spinax* in the NE Atlantic?

Expectations in this study:

(1) Find difference between Norway and the rest of the NE Atlantic, due to expectation of larger genetic difference with increasing geographical distance.

(2) Within Norway there is an expectation of finding difference between fjord and coast population because the fjord threshold is expected to work as a barrier.

(3) How to manage *E. spinax* is expected to depend on if there is identified one or more potential populations, and how the connectivity is between them.

3 Methodology

3.1 Origin of samples and sequences

Tissue samples were collected by different scientific surveys conducted in the Norwegian Sea and North Sea by the Institute of Marine Research (IMR) and selected locations were chosen for this study. The individuals caught on board the vessel were either frozen as whole specimens (and processed later in the lab) or processed at sea. Measurements taken were weight, length, sex, maturity, and tissue samples stored in ethanol for later analysis. The total length is determined by measuring from snout to the end of the caudal fin, the maturity stage is determined according to WKMSEL report (ICES, 2018).

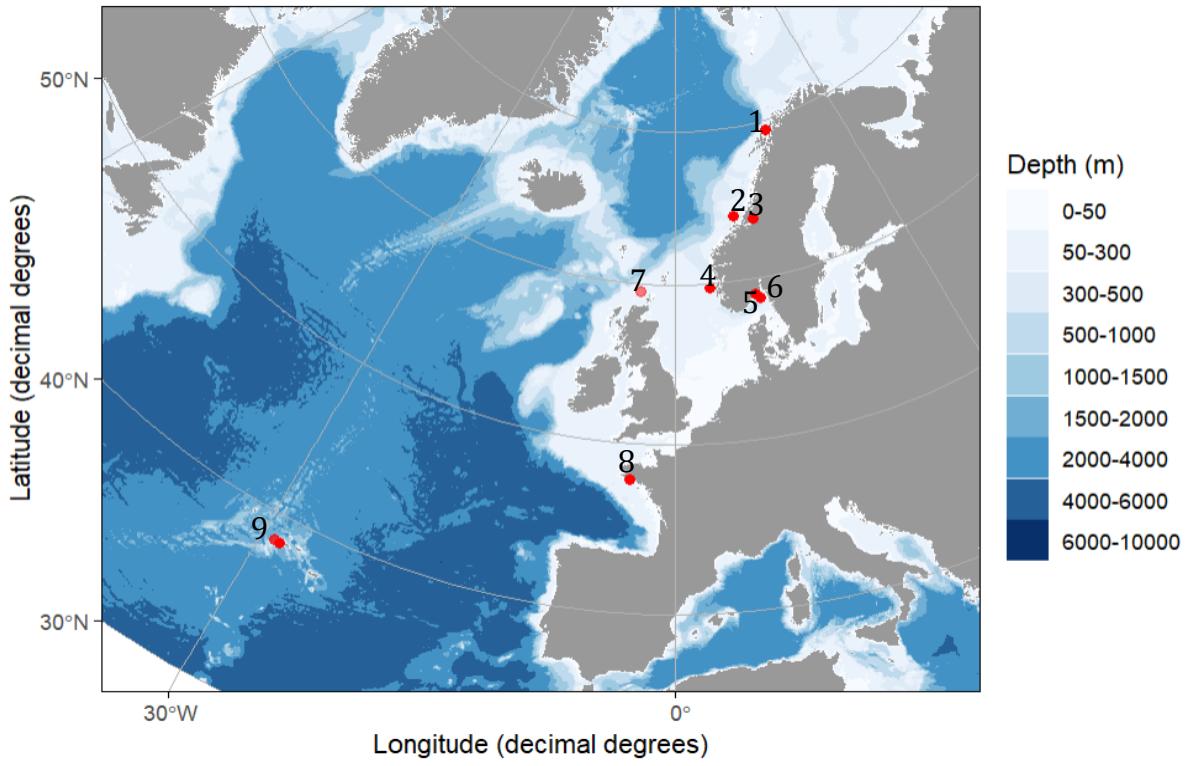

Figure 1: Locations of the 129 Etmopterus spinax individuals (red dots) analyzed in the present study. Study area consists of the locations; (1) NO_North, (2) NO_TRD_Coast, (3) NO_TRD_Fjord, (4) NO_BGO, (5) NO_Langesund, (6) NO_OSL, and (7) Scotland; SCOT, (8) Bay of Biscay in France; FR, and the (9) Azores. Positions of individuals from Bay of Biscay, Scotland and the Azores are approximated. Detailed information about the samples can be found in (Appendix 1).

Additional NADH2 sequences were obtained for 10 individuals from one fishing competition in southern Norway (NO_Langesund) and 30 associated with Naylor et al. (2012) augmented with additional data from the chondrichthyan Tree of Life project, sent directly from Naylor. Those 30 were from: 22 Azores, 6 FR, 1 Scotland, and 1 «Norway» (Appendix 1). The one sample from Norway (name: P-CH-0067, Appendix 1) is missing coordinates and therefore missing from the map, and difficult to use. The naming is somewhat confusing and therefore this single sequence will be referred to as «Norway».

With all the data collected (individuals collected between 2006 and 2019) there are 129 sequences shared between nine locations illustrated in Figure *1* conducted in R Core Team (2020), using the package ggOceanMap (Vihtakari, 2022), which we need three other codes to run; ggplot2 (Wickham, 2016), tidyvese (Wickham et al, 2019) and dplyr (Wickham et al., 2022) (code in Appendix 2). Five of the locations along the Norwegian coast are chosen from the research survey (location: 1-5), and one location is the fishing competition (location: 6). The individuals got names according to their location (SpeciesID_2-letter Country code_Region) (Appendix 1). Location 1-5 are chosen upon these criteria: (1) The locations have distance between them, (2) hypothesis of genetic difference between individuals in fjords and out by the coast, which is covered by the locations NO_TRD_Coast and NO_TRD_Fjord, (3) 20 individuals or more at the location.

3.2 Procedures

3.2.1 DNA extraction and sequencing

DNA extraction was done using DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufactured protocol with some minor modifications, such as a double elution step to increase the DNA concentration. Tools used under measuring tissue for extraction were tweezers, scalpel and weighing boats. To avoid cross-contamination under preparation for the DNA extraction, the tweezers were cleaned in bleach water, ethanol and burned between each sample. Scalpel blades and weighing boats were changed between each sample.

The concentrations of the eluted DNA were determined by using Qubit 4™ Fluorometer with dsDNA BR Assy Kit™ and with dsDNA HS Assy Kit™ (Thermo Fisher Scientific, USA). Difference between broad range (BR) and high sensitivity (HS) is that HS can detect even smaller concentrations of DNA then BR can (Thermo Fisher Scientific). The concentration of DNA is detected by fluorescent dyes that are specific to the target of interest, in this context the DNA. These fluorescent dyes emit only when bond to the target molecules, even at low concentrations. In addition, an agarose gel electrophoresis was conducted (0.8 %, 80 V, and 20 – 30 min), one time with the overall DNA and again with the PCR products. The agarose gel electrophoresis checks the size of the DNA fragments, as it separates them through the application of an electric current by size. Polymerase chain reaction (PCR) is run for amplification of the targeted region, NADH2, of mtDNA of *E. spinax*. The PCR is run by following Qiagen Multiplex PCR Kit (Qiagen), Quick-start protocol (Qiagen, 2016), with modifications to fit the samples. This kit was chosen due to its versatility, and easy usage due to the setup which can be done in room temperature and master-mix solution contains HotStarTaq DNA Polymerase (Qiagen, 2010). The primers are designed to target the complete coding sequence of NADH2. A single set of universal primers (Naylor et al., 2005) designed to bind to ASNM - AAC GCT TAG CTG TTA ATT AA Reverse primer (5’ -3’, R) and ILEM - AAG GAG CAG TTT GAT AGA GT Forward primer (5’ -3’, F) regions of the mitochondrial genome were used. The 20 µl reaction mix for the PCR consist of: 10 µl Qiagen Multiplex mastermix, 0.64 µl Primer (0.32 µl of each, ILEM and ASNM), 6.36 µl MilliQ water, and 3 µl DNA. The PCR tubes are placed in the SimpelAmp thermal cycler following four stages: (1) Holding to activate the enzyme at 95 °C for 15 minutes. (2) Cycling 35 times, divided into three steps per round: denature at 94 °C for 30 sec, anneal at 52 °C for 90 sec, and extend at 72 °C for 60 sec. (3) Extend at 72 °C for 10 min. (4) Holding at 4 °C for ∞, until picked up for storage at -20 until further analyzes.

To confirm that the PCR method was working, the protocol was assessed on seven random samples. This included running the PCR and a gel to verify the length of the PCR – product. The results (not provided) were valid, and therefore the protocol is executed on the remaining samples. Under the PCR the samples are in strips, and then transferred into a 96-well PCR plate for cleaning, using ExoSAP-IT™ PCR Product Cleanup Reagent (Applied Biosystems, 2017). Under this process 5 µl of PCR product were mixed with 2 µl Exo-SAP-IT. This

mixture was incubated at 37 °C for 15 minutes to depredate the primers and nucleotides. Then it is incubated at 80 °C for 15 minutes to inactivate the Exo-SAP-IT reagent.

Sanger sequencing reaction is run using BigDye™ Terminator v3.1 Cycle Sequencing Kit (BD v3.1) (Thermo fisher scientific, Applied Biosystems (2016)) on the cleaned PCR product. The kit has been formulated to deliver robust performance across a wide variety of DNA sequences while maximizing read lengths (Applied Biosystems, 2016). The mixture for the Sanger sequencing consists of 4 µl BD v 3.1, 1 µl of 3,2 µM concentration of the F and R primers, 3 µl Rnase free H_2O and 2 µl PCR product due to being a long sequence. The finished samples were stored at -20 °C until Sanger sequencing with Applied biosystems 3500xl Genetic Analyzers at University hospital of North Norway (UNN) Tromsø.

3.3 Data analyses

3.3.1 Alignment and phylogenetic analysis

All raw sequences from this study (n=94) together with additional sequences from NO_Langesund (n =12) and «Norway», Scotland, France, and Azores (n=30) where imported into the program 'Molecular Evolutionary Genetics Analyses' (MEGA X) (Kumar et al., 2018).

Quality check and alignment

Under the reading of the sequence, ambiguous sites were present (unknown sites). These can appear due to background noise. This noise should be small, and not bother the real peaks. Therefore, the base-calling was manually checked in the chromatograms, to check if the peaks representing the different nucleotides were correct. If peaks at the same position in multiple individuals were the same, and only one was irregular it was checked against corresponding sites in other individuals. If the nucleotide at the site was uniform across all individuals, it was assumed the same for the individual in question. These corrections made sure there was no errors in the sequence and made them more dependable. If there were too many errors the sequence was removed from the dataset. Scotland, «Norway», FR, and Azores sequences where already edited and controlled for sequencing errors and stop codons by analyzing the

translation into amino acids (Straube et al., 2015). This leaves a dataset of n = 129 sequences after seven samples were removed, two from NO_Langesund, and five from the collected sequences of this study.

The sequences were then aligned by Clustal W in MEGA X and trimmed to the same length of 1044 base pairs. The full dataset containing all samples covering all locations, is used in the phylogenetic analyses and haplotype network. But for the genetic analyses of AMOVA and pairwise F_{ST}, the «Norway» and SCOT location is not included, due to only containing one individual each.

Analyses

A model test based on maximum likelihood (Kumar et al., 2018; Nei & Kumar, 2000) analysis was run to find the best DNA/protein model in MEGA X, which was the Hasegawa-Kishino- Yano (HKY) model (Hasegawa et al., 1985). With the chosen model, a Maximum Likelihood phylogenetic tree (ML-tree) was conducted on the *E. spinax* sequences with a bootstrap of 2000. *Etmopterus pusillus* was used as an outgroup, downloaded from NCBI - GenBank (GN3771, Straube et al. (2015)), to have a point of comparison for the ingroup *E. spinax*.

In order to infer relationships between haplotypes, an Integer Neighbor-Joining (IntNJ) network (French et al., 2014; Leigh & Bryant, 2015), with a provided trait file coding for locality information of samples was conducted in PopART v1.7 (Population Analysis with Reticulate Trees) (Leigh & Bryant, 2015). As explained in Leigh and Bryant (2015) and related references, the IntNJ method computes a matrix of distance between unique haplotypes. The distance is used to presume a tree using NJ method (Saitou & Nei 1987). This tree is the backbone of the network. Nodes (haplotypes) and edges (representing mutations between haplotypes) from the tree are added to the network.

3.3.2 Haplotype and gene flow estimates

Haplotypes were assigned by GenAlEx 6.51b2 (Peakall & Smouse, 2006, 2012). The relative nucleotide composition, number of polymorphic sites, haplotype diversity (H_d), nucleotide

diversity (π), number of segregated sites (S), and the average number of nucleotide differences between pairs of sequences (K) were found using DnaSP6 (Rozasa et al., 2017) to estimate genetic variation. Interpretation of the degree of population subdivision was based on hierarchical Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992) implemented in Arlequin 3.5.2.2 (Excoffier & Lischer, 2010) to test for significant differences between groups, between locations within the groups, and within the locations. The locations were grouped on a broad scale by all the Norwegian locations as one group (Group 1 Norway) and the rest of NE Atlantic locations as another group (Group 2 Europe). A separate AMOVA test on a smaller scale was preformed, with only the Norwegian locations divided into three groups, based on geographical regions: Group NO_east (NO_OSL and NO_Langesund), Group NO_west (NO_BGO), and Group NO_mid-north (NO_North, NO_TRD_Coast, and NO_TRD_Fjord). A permutation test, pairwise $F_{ST,}$ which estimates the level of gene flow, was conducted containing all locations in Arlequin 3.5.2.2, with 10100 permutations. The additional sequences SCOT and «Norway» were excluded from both the AMOVA and F_{ST} analyses due to only containing one individual each.

4 Results

4.1 Molecular characteristics and genetic diversity

Of all samples sequenced in the lab (n=94), five of the NADH2 sequences were removed due to either being an invalid read (no sequence obtained), or too high uncertainty (too much background noise, potential contamination). Together with the other obtained sequences, overall, 129 sequences were successfully aligned, containing 1044 bp, and exhibiting 23 haplotypes and 28 polymorphic sites (Table 1). Average nucleotide frequencies were T = 30 %, C = 27.9 %, A = 31 %, and G = 11.1 %.

Of the 23 haplotypes, 10 occurred more than once (Appendix 3). The number of haplotypes per location (among 1 to 22 individuals sequenced) range from 1 to 8 (Table 1). There are 15 unique haplotypes shared between six of the ten locations. All locations have unique haplotypes, except from NO_OSL, NO_TRD_Coast, «Norway», and SCOT. The two last ones are due to only obtaining one individual at the locations. The haplotype diversity (H_d) was moderate (0.47) to high (0.93), with an average of 0.74. While the nucleotide diversity was low, with an average of 0.0023, and the mean number of pairwise differences is (K) 2.39 (Table 1). FR has the highest H_d and NO_Langesund have the highest π. NO_North has the lowest H_d and π.

Table 1: Measures of genetic diversity of NADH2 of *E. spinax*. Location, n_i; number of individuals (successfully aligned), n_h: number of haplotypes, Unique h: unique haplotypes for the locations, S: number of segregated sites, H_d: haplotype diversity, Pi (π): nucleotide diversity, K: average number of nucleotide difference between pairs of sequences.

Location	**n_i**	**n_h**	**Unique, h**	**S**	**H_d**	**π**	**K**
NO_North	18	4	H5	7	0.47	0.0012	1.22
NO_TRD_Fjord	19	6	H11, H13	11	0.60	0.0023	2.45
NO_TRD_Coast	13	5	-	9	0.78	0.0026	2.74
NO_BGO	19	6	H12	6	0.70	0.0016	1.65
NO_OSL	20	6	-	8	0.52	0.0013	1.40
NO_Langesund	10	6	H2, H3, H15, H20, H22	11	0.84	0.0032	3.33
«Norway»	1	1	-	-	-	-	-
SCOT	1	1	-	-	-	-	-
Azores	22	8	H1, H8. H18, H19 H23	11	0.77	0.0028	2.90
FR	6	5	H6	7	0.93	0.0026	2.73
Total/overall	129	23		28	0.74	0.0023	2.39

4.2 Phylogenetic analysis

The best model fit to the NADH2 data was HKY as determined with the model-test in MEGA X. It is the best suited model because it had the lowest Bayesian Information Criterion (BIC) which describes the substitution pattern the best, and the lowest Akaike Information Criterion, corrected (AICc) which ranks substitution models based on minimum corrected theoretical information criterion. For the models, there is minor difference between HKY and HKY+G (without and with gamma distribution). But based on BIC value the model without gamma distribution is lower in value (values not presented), and therefore this one is used.

The ML-tree of *E. spinax* with *E. pusillus* as an outgroup has a low bootstrap support (Figure 2). The outgroup is its own cluster, which verifies that *E. spinax* is a separate species. The topology can be divided into ten clusters, with two clusters being the tidiest clusters, but with bootstrap under 50 %. This would be the top cluster (from here on called Cluster 1) which consists of locations from within Norway with a few sequences from Azores blended in. The second cluster (from now on Cluster 2) to notice is in the bottom of the tree, with a collection of sequences from the Azores together with a few sequences from FR and the one sequence from Scotland, and one NO_OSL interrupting. The clusters in-between Cluster 1 and Cluster 2 have a higher mix of sequences from different locations. Two of the mixed clusters have bootstrap support over 50 %. These clusters (from now on Cluster 3 and Cluster 4) are Cluster 3 obtaining location NO_OSL, NO_TRD_Coast, NO_TRD_Fjord, and FR, and Cluster 4 obtaining location NO_TRD_Coast, NO_TRD_Fjord, and Azores.

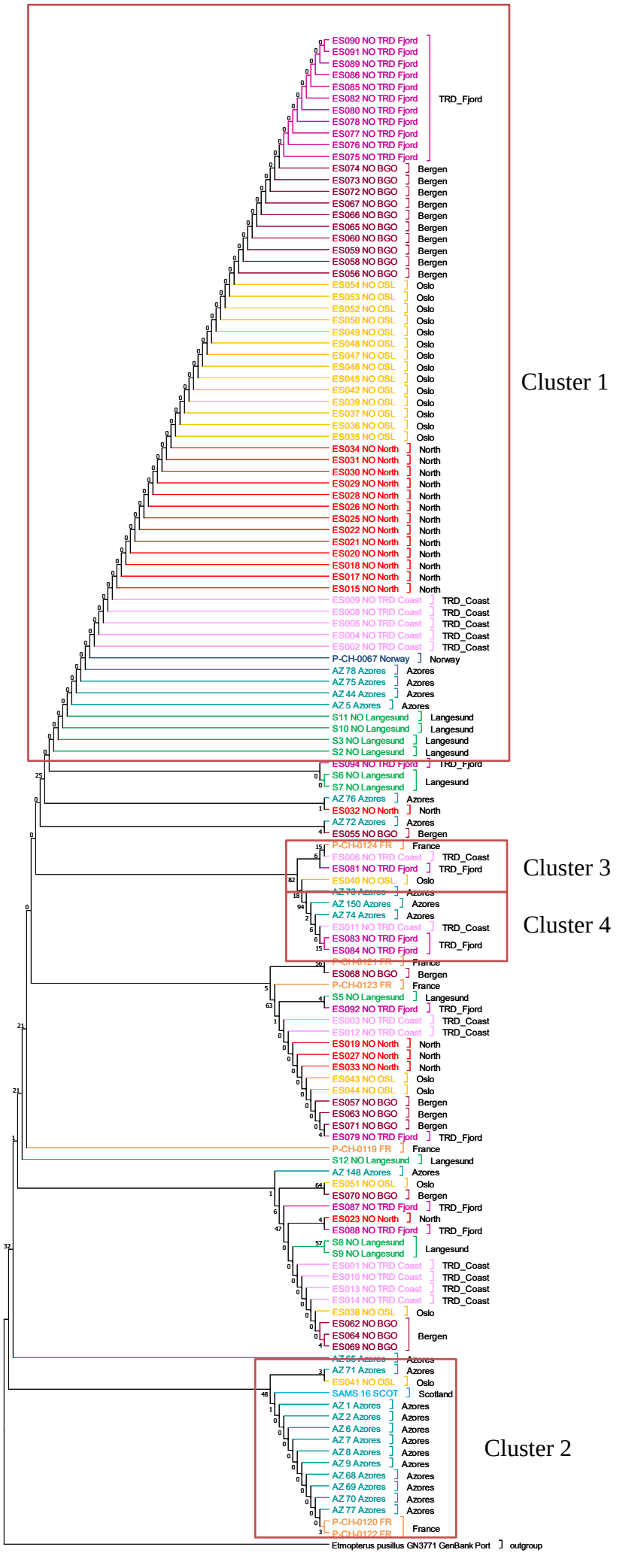
ES090 NO TRD Fjord
ES091 NO TRD Fjord
ES089 NO TRD Fjord
ES086 NO TRD Fjord
ES085 NO TRD Fjord
ES082 NO TRD Fjord
ES080 NO TRD Fjord
ES078 NO TRD Fjord
ES077 NO TRD Fjord
ES076 NO TRD Fjord
ES075 NO TRD Fjord
TRD_Fjord
ES074 NO BGO
ES073 NO BGO
ES072 NO BGO
ES067 NO BGO
ES066 NO BGO
ES065 NO BGO
ES060 NO BGO
ES059 NO BGO
ES058 NO BGO
ES056 NO BGO
Bergen
ES054 NO OSL
ES053 NO OSL
ES052 NO OSL
ES050 NO OSL
ES049 NO OSL
ES048 NO OSL
ES047 NO OSL
ES046 NO OSL
ES045 NO OSL
ES042 NO OSL
ES039 NO OSL
ES037 NO OSL
ES036 NO OSL
ES035 NO OSL
Oslo
ES034 NO North
ES031 NO North
ES030 NO North
ES029 NO North
ES028 NO North
ES026 NO North
ES025 NO North
ES022 NO North
ES021 NO North
ES020 NO North
ES018 NO North
ES017 NO North
ES015 NO North
North
ES009 NO TRD Coast
ES008 NO TRD Coast
ES005 NO TRD Coast
ES004 NO TRD Coast
ES002 NO TRD Coast
TRD_Coast
P-CH-0067 Norway
Norway
AZ 78 Azores
AZ 75 Azores
AZ 44 Azores
AZ 5 Azores
Azores
S11 NO Langesund
S10 NO Langesund
S3 NO Langesund
S2 NO Langesund
Langesund
ES094 NO TRD Fjord
TRD_Fjord
S6 NO Langesund
S7 NO Langesund
Langesund
AZ 76 Azores
Azores
ES032 NO North
North
AZ 72 Azores
Azores
ES055 NO BGO
Bergen
P-CH-0124 FR
France
ES006 NO TRD Coast
TRD_Coast
ES081 NO TRD Fjord
TRD_Fjord
ES040 NO OSL
Oslo
AZ 73 Azores
Azores
AZ 150 Azores
Azores
AZ 74 Azores
Azores
ES011 NO TRD Coast
TRD_Coast
ES083 NO TRD Fjord
ES084 NO TRD Fjord
TRD_Fjord
P-CH-0121 FR
France
ES068 NO BGO
Bergen
P-CH-0123 FR
France
S5 NO Langesund
Langesund
ES092 NO TRD Fjord
TRD_Fjord
ES003 NO TRD Coast
ES012 NO TRD Coast
TRD_Coast
ES019 NO North
ES027 NO North
ES033 NO North
North
ES043 NO OSL
ES044 NO OSL
Oslo
ES057 NO BGO
ES063 NO BGO
ES071 NO BGO
Bergen
ES079 NO TRD Fjord
TRD_Fjord
P-CH-0119 FR
France
S12 NO Langesund
Langesund
AZ 148 Azores
Azores
ES051 NO OSL
Oslo
ES070 NO BGO
Bergen
ES087 NO TRD Fjord
TRD_Fjord
ES023 NO North
North
ES088 NO TRD Fjord
TRD_Fjord
S8 NO Langesund
S9 NO Langesund
Langesund
ES001 NO TRD Coast
ES010 NO TRD Coast
ES013 NO TRD Coast
ES014 NO TRD Coast
TRD_Coast
ES038 NO OSL
Oslo
ES062 NO BGO
ES064 NO BGO
ES069 NO BGO
Bergen
AZ 65 Azores
Azores
AZ 71 Azores
Azores
ES041 NO OSL
Oslo
SAMS 16 SCOT
Scotland
AZ 1 Azores
AZ 2 Azores
AZ 6 Azores
AZ 7 Azores
AZ 8 Azores
AZ 9 Azores
AZ 68 Azores
AZ 69 Azores
AZ 70 Azores
AZ 77 Azores
Azores
P-CH-0120 FR
P-CH-0122 FR
France
Etmopterus pusillus GN3771 GenBank Port
outgroup
Cluster 1
Cluster 3
Cluster 4
Cluster 2

Figure 2: Maximum likelihood tree, where numbers indicate bootstrap values. Locations are colored, according to locations with the same color palette as in the haplotype network (Figure 3). Red boxes mark the clusters of interest (Cluster 1 to 4).

The IntNJ haplotype network gives a good illustration of the clustering of haplotypes (Figure 2). The most common, haplotype 4 (Cluster 1), occurs 63 times (Appendix 3), which includes individuals from all locations except FR. This is the same clustering as found in Cluster 1 in the ML – tree. Cluster 2 from the ML – tree is also clearly visualized in the haplotype network (illustrated by the red boxes on Figure 3). The other clusters of haplotypes in the network reflect the mixing found (as an example cluster 3 and 4, not illustrated on Figure 3) in the mid part of the ML – tree. The network shows Langesund as the location with the most unique haplotypes with 5 of the 6 haplotypes being unique, with 1 to 3 edges (mutations) difference from other haplotypes (Figure 3, Table 1).

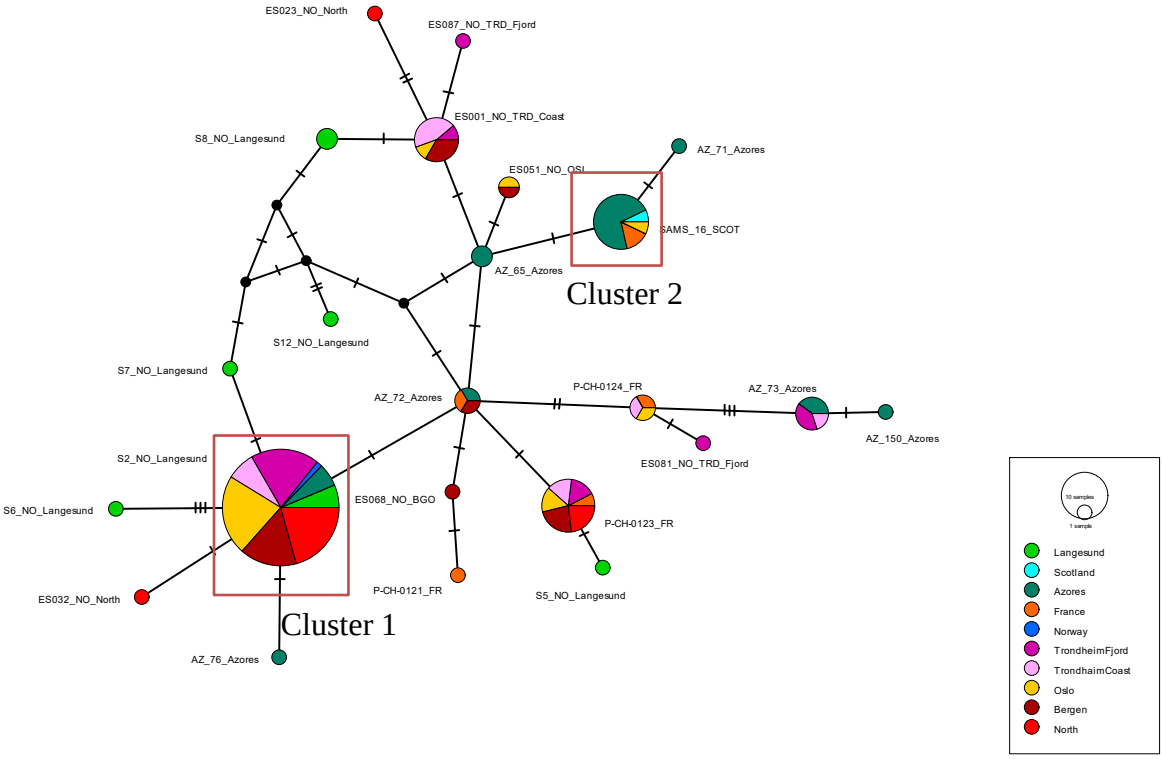

Figure 3: Unrooted Haplotype network, NJ network of the 23 different haplotypes in: NO_Langesund = Langesund, Scotland, Azores, FR = France, Norway, NO_TRD_Fjord = Trondheim Fjord, NO_TRD_Coast = Trondheim Coast, NO_OSL = Oslo, NO_BGO = Bergen, and NO_North = North locations. The size of the circle reflects the number of individuals sharing the same haplotype, with larger circle having more individuals, and the smallest is only one individual. Each circle is colored according to the respective locations (see legend). Black circles indicate hypothetical or unsampled haplotypes. The edges over the connection lines between the populations illustrate the number of mutations. And overall, there is commonly only one edge from one haplotype to the other one, with the exception being between haplotypes for Langesund with commonly 3-4 edges. The red boxes illustrate Clusters of interest matching with Figure 2.

4.3 Population structure and gene flow estimates

4.3.1 AMOVA

The AMOVA for all sampling locations (Table 2 indicated that genetic percentage (%) variation among groups (Group 1 Norway = all the Norwegian locations, Group 2 Europe = the remaining NE Atlantic locations; FR and Azores) is moderate but significant (19.47 %, $p < 0.05$). However, the % variation among locations within groups was very low, and not significant (1.38 %, $p > 0.05$). Most of the % variation was found within locations, and was highly significant (79.15 %, $p < 0.001$).

An AMOVA of only the Norwegian samples, excluding the additional Norwegian sample from location «Norway» without precise location information was conducted. None of the results were significant. Among groups (Group NO_mid-north = NO_North, NO_TRD_Coast, and NO_TRD_Fjord, Group NO_west = NO_BGO, Group NO_east = NO_Langesund and NO_OSL) the % variation is negative and therefore consider as no variation (-3.20 %, $p > 0.05$). Among the locations within groups the variation was extremely low, but the closest to being significant (4.73 %, $p >0.05$). The highest % variation was found within the locations (98.47 %, $p >0.05$).

Table 2: AMOVA results Among groups (Group 1 Norway: NO_North, NO_TRD_Coast, NO_TRD_Fjord, NO_BGO, NO_Langesund, and NO_OSL, and Group 2 Europe: FR and Azores), Among populations within locations, and within locations. d.f; degrees of freedom, SS; Sum of squares, VC; Variance components, % Var; Percentage variation, F; F-statistics (FST, FSC, and FCT), and P; P-value.

Source of variation	**d.f**	**SS**	**VC**	**% Var**	**F**	**P**
Among groups	1	13.109	0.267 Va	19.47	F_{CT} = 0.195	0.04
Among locations within groups	6	8.248	0.019 Vb	1.38	F_{SC} = 0.017	0.13
Within locations	119	129.376	1.087 Vc	79.15	F_{ST} = 0.208	0
Total	126	153.744	1.374			

4.3.2 Pairwise F_{ST}

In the pairwise F_{ST} test for all locations (Table 3) there were 28 pairwise comparisons with F_{ST}s ranging from -0.005 to 0.323. Of those, 13 were significantly different with p values ranging from $p < 0.05$ to $p < 0.001$, see Table 3. Those involved locations are all the Norwegian locations, which are genetically different from the rest of the NE Atlantic locations (FR, Azores), except for the pair FR and NO_TRD_Coast. The highest difference is detected between FR and NO_North (0.323, $p < 0.001$), with Azores and NO_North coming second (0.288, $p < 0.001$). For the pairwise F_{ST} values between the Norwegian locations (Group 1) there were 15 pairwise comparisons ranging from -0.005 to 0.112, of those two were significant ($p < 0.05$). Those were NO_TRD_Coast with NO_ North and NO_North with NO_Langesund (Table 3).

Table 3: Pairwise F_{ST} for all sample locations (Table 1, excluding «Norway» and SCOT). Significance obtained from 10100 permutations (*p < 0.05, **p < 0.01, ***, p < 0.001****).

Location	**NO_Langesund**	**NO_OSL**	**NO_BGO**	**NO_TRD_Coadt**	**NO_TRD_Fjord**	**NO_North**	**FR**
NO_Langesund	0						
NO_OSL	0.059	0					
NO_BGO	0.033	-0.013	0				
NO_TRD_Coast	0.029	0.057	-0.006	0			
NO_TRD_Fjord	0.047	-0.011	0.007	-0.005	0		
NO_North	0.084*	-0.024	0.024	0.112*	0.015	0	
FR	0.125*	0.235**	0.145*	0.033	0.136*	0.323****	0
Azores	0.178**	0.217****	0.184****	0.100*	0.171**	0.288****	-0.004

5 Discussion

5.1 Haplotype diversity

The mean haplotype diversity of *E. spinax* in this study is relatively high (0.74, Table 1) compared to other deep-sea shark species. Maaholm and Mihalitsis (2014) and Gubili et al. (2016) studied the same species and found similar haplotype diversity values. Maaholm and Mihalitsis (2014) found a mean H_d of 0.75 by using the same mtDNA marker as in this study, NADH2. In Gubili et al. (2016) they used mtDNA marker CR, and found H_d ranging from 0.500 to 0.959. The values from these studies support a high haplotype diversity in the Atlantic. Study on the Portuguese dogfish (*Centroscymnus coelolepis*) (Veríssimo et al., 2011) found a mean haplotype diversity of 0.65. But the different studies have different locations in the NE Atlantic they have samples from, which potentially can be a factor influencing the haplotype diversity.

The lowest haplotype and nucleotide diversity found in this study was at the NO_North location, and NO_OSL has the second lowest. Overall, at all locations the haplotype diversity is moderate to high, and the nucleotide diversity low. This can point to a relatively recent (in evolutionary terms) population expansion (Avise, 2000).

5.2 NE Atlantic population structure

There is identified population structure on a broad scale in this study, supported by the significant high pairwise F_{ST}, and AMOVA F_{CT} values (Table 2, Table 3). These populations being defined as to groups: Group 1 Norway (NO_North, NO_TRD_Coast, NO_TRD_Fjord, NO_BGO, NO_Langesund, and NO_OSL) and Group 2 Europe (Azores and FR). This population structure is indicated despite relatively short geographical distance between the studied locations (even if it is assumed the English Channel is not used for migration), compared to studies on similar species. If we look at the Portuguese dogfish (*Centroscymnus coelolepis*) (Veríssimo et al., 2011) or the longnose velvet dogfish (*Centroscymnus crepidater*) (Cunha et al., 2012), there is no genetic population structure found based on mtDNA analysis in these species in the Atlantic Ocean. Because they have similar life-history traits and found in the same geographical area as *E. spinax*, the findings in these species

support the finding in this study of locations within Group 2 being connected. This broad scale connectivity within Group 2 is also supported by dietary composition and isotopic variation study by Besnard et al. (2022), where overlap in bulk isotope niches occur between Portugal and Rockall (Scotland) samples of *E. spinax*. This supports the idea of gene flow on a larger scale of *E. spinax* in the Atlantic Ocean.

In the introduction three potential populations on a broad scale identified by McMillan et al. (2017) based on vertebral chemistry was presented. These potential populations were western Norway, southern Norway, and France. Which also is supported by the population structure found in Maaholm and Mihalitsis (2014) between North Sea and Celtic Sea (France) While in the study by Gubili et al. (2016) based on mtDNA and PCoA analyses, which covered locations both in the NE Atlantic and the Mediterranean Sea, they identified a clear separation between the NE Atlantic and Mediterranean locations. Within the NE Atlantic they found significant differences between locations, however when they adjusted the p-value for multiple testing to $p < 0.001$ no significant F_{ST} values remained. These differences based on p-value < 0.05 between the locations can be divided into two groups, which can be potential populations. One group covering locations in Norway, and another group covering location; Azores, Ireland, and Scotland.

The potential populations of *E. spinax* on a broad scale found in these three studies (Gubili et al., 2016; McMillan et al., 2017; Maaholm & Mihalitsis, 2014) overlap with the populations found in this study, and support a separation between Group 1 and Group 2 which confirm that they are separate populations. But in McMillan et al. (2017) there is three potential populations, and this study only supports two of them. The western Norway potential population, which overlap with the NO_BGO location cannot be separated as its own population from population 3 NO_west + east (overlapping with eastern Norway potential population) based on this genetic study with NADH2 sequences. Migration seems to be higher within regions on a small scale (between closely related locations) then between regions on a broad scale (between locations of Group 1 and Group 2), which can be a reason for not being able to separate out NO_BGO. Therefore, on a small scale (locations within Group 1 Norway) there is not expected to find any major populations structure based of that migration theory, and the results of the AMOVA run only on Group 1 Norway. But based of the pairwise F_{ST} values (Table 3) there is a possibility for three populations: Population 1

NO_North, Population 2 NO_TRD, and population 3 NO_west + east. Population 1 is furthest north, and the most "isolated" of the three potential populations. It is closest to population 2 that it is significantly different from, which was unexpected, and the reason for them to be together in Group NO_mid-north in the AMOVA analyses. But taking the distance and topography between the locations into consideration, it is not unlikely that they are different. The locations within population 2 (NO_TRD_Fjord and NO_TRD_Coast) was expected to be separated, due to being inside and outside a fjord. But due to no significant difference between the locations, they are found to be one population, NO_TRD. Population 2 is also in the area of the highest abundance of *E .spinax* (Jac et al., 2021), and the opening into the fjord is deep, which might be the reasons for not finding a genetic difference between the locations in population 2. In population 3, the expectation was NO_Langesund and NO_OSL to not show any difference, due to being so close to each other. Which turned out to be correct. A reason for the limited genetic variation between these two locations might be due to a potential breeding ground in this area (McMillan et al., 2017). NO_BGO was expected to separate out as its own population, as the study of McMillan et al. (2017) pointed out, but it did not. This could be due to all the locations in population 3 are in the Norwegian trench, and that the individuals easily migrate between the locations, due to no physical barriers in the trench.

5.3 Conservation of *E. spinax* in the NE Atlantic

Chondrichthyan fishes are highly vulnerable to fishing mortality due to their K-selected life strategy. Many deep-sea chondrichthyans species are even more vulnerable, because of even longer growth and maturation time then bigger pelagic chondrichthyan species (Simpfendorfer & Kyne, 2009). *Etmopterus spinax* is in the VU category with a declining trend. Based on the estimations from IUCN, the stability of the population will crash if the declining trend is not stopped (Kyne & Simpfendorfer, 2010). With a generation length of 7.8 years (Coelho & Erzini, 2008) and approximately 50% reduction in biomass of the global population in three generation lengths (23 years) (Finucci et al., 2021), the population can potentially crash relatively fast, considering evolutionary time. This is if no conservation and management measures are taken.

Population structure found on a broad scale in this study, with support from other studies, suggest that the two populations (Group 1 and Group 2) should be individually managed. Because, if Group 1 populations and Group 2 populations are taken into consideration as one population when considering management and conservation, it can lead to overestimation of population size (Cowen et al., 2000), and the implications made for conservation will be weaker than if they are managed separately. To give the correct management for these two populations the knowledge gap of not knowing enough of their ecology and distribution (which is larger on a regional scale) (Jac et al., 2021) needs to be filled in. The reproductive cycle of *E. spinax* will have significant implications on management and conservation. Therefore, combining study of life history and population structure is much needed to paint the full picture for giving correct management and conservation criteria.

In Norway fisheries are regulated, and these regulations are updated frequently based on scientific surveys and research findings. *Etmopterus spinax* is not a fisheries-targeted species in Norwegian waters due to no economic value, but they end as bycatch frequently. The results do not give any clear implications on what management should be prioritized for *E. spinax* in Norway. Potentially each of the three populations (NO_north, NO_TRD and NO_west + east within Group 1 need to have different management implemented. But this need further research to be confirmed.

When it comes to Group 2 Europe it gets a bit trickier. There is no population structure found between the locations FR and Azores, and therefore one common management and conservation plan need to be agreed upon between the countries. There are already a few restrictive management measures for the NE Atlantic implemented at separate times, to increase the protection of *E. spinax* and alike species. For example, the EU zero TAC, and a ban on use of trawls and gillnets in waters >200 m in the Azores, Madeira and Canary Islands and international waters regulated by ICES (Finucci et al., 2021). There is also a network of closed areas in the Azorean waters, where deep-water fishing is not allowed (Finucci et al., 2021). It will be a challenge to manage a cross – jurisdictional population as Group 2, due to the vast distances between the locations of known distribution of *E. spinax* and multiple involved countries.

To be able to make management and conservation estimations at all, better statistics of by-catch, discharge, and landings of *E. spinax* are needed. Also, species identification (Marandel et al., 2018) of *E. spinax* is a problem in certain parts of the Atlantic, due to overlapping with other *Etmopterus* species covering the same habitats. It is not an issue in Norway due to *E. spinax* being the only *Etmopterus* species.

5.4 Methodical limitations

There were problems with a few sequences which were of bad quality or contaminated. Reasons for this quality problem can be due to sampling at the research cruises. When taking the tissue samples for genetic analyses the same knife or scissors is used on all individuals with minimal cleaning in between. The same goes for if the individuals are handled after they have been frozen, in addition to the frozen tissue being more damaged than fresh tissue due to the freezing and then thawing process to get the tissue samples. But the risk of cross contamination is relatively low.

Under the process of analyzing the results, population structure limitations were observed. Which can be improved by including more locations with representative numbers of individuals. Another reason to not finding population structure on a small scale (focused on Norway) can be due to mitochondrial markers such as NADH2 used in this study or CR used in multiple other studies, alone fail to detect fine-scale population structure. The challenge with mtDNA is that it is maternally inherited, which can limit conclusions about gene flow in cases of sex-biased dispersal (Kraft et al., 2020). But the use of mtDNA markers can be beneficial in the case of male biased gene flow because the markers would notice the female population structure, but not a male-based one. Also, when only focusing on a single mtDNA marker and not including multiple and/or nuclear markers, lots of variation and resulting population structure may be missed (Kraft et al., 2020; and references within). Therefore, it could be beneficial to do additional sampling of SNPs across the genome to increase the resolution for *E. spinax*. Another result that should be noted is the NO_Langesund location, which stands out from the other locations. Off the six haplotypes found, five of them were unique. This can have affected the pairwise difference measures. As all those samples stem from a fishing competition off Langesund, one could speculate where exactly the individuals

have been fished. A follow-up study with exact locations would be useful to decipher what could explain the high level of haplotype diversity.

Last limitation noticed for this study is that there is a poor collection of *E. spinax* NADH2 sequences in the GenBank – NCBI database. Here, sequences for all types of species from around the world are collected, which is helpful to use as supplementary data for new research. But it is not helpful when no one has shared their data. For *E. spinax* there were three sequences of NADH2, but a higher selection of sequences for other nuclear and mitochondrial DNA markers. Improving databases such as this one can increase the value of new studies and give higher accuracy of genetic estimations of population structure. The NADH2 sequences obtained on *E. spinax* from this study will be uploaded to NCBI GenBank to build up a stronger database.

6 Conclusion

The question we wanted answers to were:

(1) Are there any potential populations in the NE Atlantic? Yes, there are two distinct populations in the NE Atlantic. And these populations are the same as expected, one population covering the Norwegian locations and one population covering the rest of the NE Atlantic locations (Azores and FR).

(2) Are there any potential populations within Norway? Here the answer is not as clear as a yes or no. The results might support three potential populations. The expectations were to find difference between coast and fjord location, which have been represented by NO_TRD_Coast and NO_TRD_Fjord. No genetic difference could be detected between these two locations, which can be due to the opening of the fjord being deep and giving little to no barrier between the coast and fjord location. Or it could be NADH2 marker is not the right marker to detect differences on this small of a scale. Regarding the rest of the locations in Norway there is weak support for three potential populations.

(3) Do the results of 1 and 2 have implications for management of *E. spinax* in the NE Atlantic? This question's answer depends on if there was found one or more potential

populations, and how the connectivity is between them. And as identified in question 1, two populations were identified on the broad scale, which implies that there should be different management plans for each of the populations. Regarding question 2, there is a need for further studies to say if the potential populations within Norway should be managed jointly or separately.

7 Future studies

Future studies on *E. spinax* population structure should be focused on small scales. More knowledge of population structure on a small scale will give more knowledge to include in the assessment of the regional population status and help inform the management of the species at appropriate scales. Proposals for further small-scale studies based in Norway, is to use multiple genetic markers of both mtDNA and genomic DNA. It could be interesting to combine using CR and NADH2 region, due to more studies have used CR region than NADH2. Other methods and techniques than mitochondrial DNA sequencing could also be useful. For example, single nucleotide polymorphisms (SNPS) across the genome, which work as chromosomal tags to specific regions of DNA and can function as biological markers. And next generation sequencing (NGS), can be beneficial to use with a large dataset with many genetic markers analyzed. This can be beneficial if larger parts of the genome of *E. spinax* is to be analyzed, because it has a large genome (Stingo et al., 1980). The fjord vs. coast population could have been better represented in this study. In future studies it would be interesting to include more than one fjord location. With larger sample sizes and more included locations within the European distribution area it would be remarkably interesting to also investigate isolation by distance (IBD). IBD is the concept of individuals in locations further apart from each other will be more genetically different, then the individuals in locations closer to each other (Wright, 1943).

And lastly, with the populations identified and hypothesized to be represented in Norway, it would be interesting to combine a genetical study with a life-history traits study on *E. spinax*. This could help identify breeding grounds, which can be valid information in management and conservation plans for the species (Heupel et al., 2007).

8 References

Anderson, C. D., Epperson, B. K., Fortin, M.-J., Holderegger, R., James, P. M. A., Rosenberg, M. S., Scribner, K. T., & Spear, S. (2010). Considering spatial and temporal scale in landscape-genetic studies of gene flow. *Molecular Ecology*, *19*(17), 3565-3575. https://doi.org/10.1111/j.1365-294x.2010.04757.x

Andrewartha, H. G., & Birch, L. C. (1986). *The ecological web: More on the distribution and abundance of animals*. University of Chicago Press. https://books.google.no/books?id=6OpmD0Oaz8QC

Applied Biosystems. (2016). BigDye™ Terminator v3.1 Cycle Sequencing Kit. In(pp. 2).

Applied Biosystems. (2017). ExoSAP-IT™ PCR Product Cleanup. In.

Aranha, A., Menezes, G., & Pinho, M. R. (2009). Biological aspects of the velvet belly lantern shark, *Etmopterus spinax* (Linnaeus, 1758) off the Azores, North East Atlantic. *Marine Biology Research*, *5*(3), 257-267. https://doi.org/10.1080/17451000802433175

Avise, J. C. (2000). *Phylogeography: The history and formation of species*. Harvard University Press.

Beaugrand, G., Brander, K. M., Alistair Lindley, J., Souissi, S., & Reid, P. C. (2003). Plankton effect on cod recruitment in the North Sea. *Nature*, *426*(6967), 661-664. https://doi.org/10.1038/nature02164

Besnard, L., Duchatelet, L., Bird, C. S., Croizier, G. L., Michel, L., Pinte, N., Lepoint, G., Schaal, G., Vieira, R. P., Gonçalves, J. M. S., Martin, U., & Mallefet, J. (2022). Diet consistency but large-scale isotopic variations in a deep-sea shark: the case of the velvet belly lantern shark, *Etmopterus spinax*, in the northeastern Atlantic region and Mediterranean Sea. *Deep-Sea Research Part I: Oceanographic Research Papers*, 182. https://doi.org/10.1016/j.dsr.2022.103708

Bors, E. K., Rowden, A. A., Maas, E. W., Clark, M. R., & Shank, T. M. (2012). Patterns of deep-sea genetic connectivity in the New Zealand region: Implications for management of benthic ecosystems. *PLoS One*, *7*(11), Article e49474. https://doi.org/10.1371/journal.pone.0049474

Cailliet, G. M., Musick, J. A., Simpfendorfer, C. A., & Stevens, J. D. (2005). Ecology and life history characteristics of chondrichthyan fish. In S. L. Fowler, R.D. Cavanagh, M.

Camhi, G.H. Burgess, S. V. F. G. Cailliet, C.A. Simpfendorfer & J. A. Musick (Eds.), *Sharks, rays and chimaeras: The status of the chondrichthyan fishes* (pp. 12-18). IUCN SSC Shark Specialist Group.

Caley, M. J., Carr, M. H., Hixon, M. A., Hughes, T. P., Jones, G. P., & Menge, B. A. (1996). Recruitment and the local dynamics of open marine populations. *Annual Review of Ecology and Systematics*, *27*(1), 477-500. https://doi.org/10.1146/annurev.ecolsys.27.1.477

Camhi, M., Fowler, S., Musick, J., Brautigam, A., & Frodham, S. (1998). *Sharks and their relatives: Ecology and conservation* (20 ed.). IUCN.

Capape, C., Bradai, M., Seck, A., Diata, Y., Tomasini, J., & Quignard, J. (2001). Aspects of the reproductive biology of the velvet belly, *Etmopterus spinax* (Elasmobranchii: Squalidae). *Bulletin de L'Institut National des Sciences et Technologies de la Mer*, *28*. http://hdl.handle.net/1834/4084

Coelho, R. (2007). *Biology, population dynamics, management and conservation of deep water lantern sharks, Etmopterus spinax and Etmopterus pusillus (Chondrichthyes: Etmopteridae) in southern Portugal (northeast Atlantic)* [PhD, Universidade Do Algarve]. Sapientia. http://hdl.handle.net/10400.1/485

Coelho, R., Blasdale, T., Mancusi, C., Serena, F., Guallart, J., Ungaro, N., Litvinov, F., Crozier, P., & Stenberg, C. (2009). *Etmopterus spinax*, Velvet Belly Lanternshark. *The IUCN Red List of Threatened Species* https://doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161388A5412576.en

Coelho, R., & Erzini, K. (2005). Length at first maturity of two species of lantern sharks *etmopterus spinax* and *etmopterus pusillus* off southern portugal. *Journal of the Marine Biological Association of the United Kingdom*, *85*(5), 1163-1165. https://doi.org/10.1017/s0025315405012245

Coelho, R., & Erzini, K. (2008). Life history of a wide-ranging deepwater lantern shark in the north-east Atlantic, *Etmopterus spinax* (Chondrichthyes: Etmopteridae), with implications for conservation. *Journal of Fish Biology*, *73*(6), 1419-1443. https://doi.org/doi:10.1111/j.1095-8649.2008.02021.x

Coelho, R., & Erzini, K. (2010). Depth distribution of the velvet belly, *Etmopterus spinax*, in relation to growth and reproductive cycle: The case study of a deep-water lantern

shark with a wide-ranging critical habitat. *Marine Biology Research*, *6*(4), 381-389. https://doi.org/10.1080/17451000802644706

Coelho, R., Rey, J., Gil de Sola, L., Fernandez de Carvalho, J., & Erzini, K. (2010). Comparing Atlantic and Mediterranean populations of the velvet belly lanternshark, *Etmopterus spinax*, with comments on the efficiency of density-dependent compensatory mechanisms. *Marine Biology Research*, *6*(4), 373-380. https://doi.org/10.1080/17451000903300885

Cowen, R. K., Gawarkiewicz, G., Pineda, J., Thorrold, S., & Werner, F. (2002). Population connectivity in marine systems. In *Report of a workshop to develop science recommendations for the National Science Foundation* (Vol. 84, pp. 119-119). NSF.

Cowen, R. K., Lwiza, K. M. M., Sponaugle, S., Paris, C. B., & Olson, D. B. (2000). Connectivity of marine populations: Open or closed? *Science*, *287*(5454), 857-859. https://doi.org/10.1126/science.287.5454.857

Cunha, R. L., Coscia, I., Madeira, C., Mariani, S., Stefanni, S., & Castilho, R. (2012). Ancient divergence in the trans-oceanic deep-sea shark centroscymnus crepidater. *PLoS One*, *7*(11), Article e49196. https://doi.org/10.1371/journal.pone.0049196

Duchêne, S., Archer, F. I., Vilstrup, J., Caballero, S., & Morin, P. A. (2011). Mitogenome phylogenetics: The impact of using single regions and partitioning schemes on topology, substitution rate and divergence time estimation. *PLoS One*, *6*(11), Article e27138. https://doi.org/10.1371/journal.pone.0027138

Dulvy, N. K., Pacoureau, N., Rigby, C. L., Pollom, R. A., Jabado, R. W., Ebert, D. A., Finucci, B., Pollock, C. M., Cheok, J., Derrick, D. H., Herman, K. B., Sherman, C. S., Vanderwright, W. J., Lawson, J. M., Walls, R. H. L., Carlson, J. K., Charvet, P., Bineesh, K. K., Fernando, D., Ralph, G. M., Matsushiba, J. H., Hilton-Taylor, C., Fordham, S. V., & Simpfendorfer, C. A. (2021). Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. *Current Biology*, *31*(21), 4773-4787.e4778. https://doi.org/10.1016/j.cub.2021.08.062

Ebert, D. A., Dando, M., & Fowler, S. (2021). *Sharks of the world: A complete guide*. Princeton University Press.

Ebert, D. A., Fowler, S. L., & Compagno, L. J. (2013). *Sharks of the world: A fully illustrated guide*. Wild Nature Press.

Ebert, D. A., & Stehmann, M. F. (2013). *Sharks, batoids and chimaeras of the North Atlantic*. Food and agriculture organization of the united nations, FAO, Roma.

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564-567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, *131*(2), 479-491. https://doi.org/10.1093/genetics/131.2.479

Finucci, B., Derrick, D., Dia, M., Ducrocq, M., Neat, F. C., Pacoureau, N., & Serena, F. V., W.J. (2021). *Etmopterus spinax*. The IUCN red list of threatened species 2021. Article e.T161388A124475610. https://doi.org/10.2305/IUCN.UK.2021-2.RLTS.T161388A124475610.en.

French, N., Yu, S., Biggs, P., Holland, B., Fearnhead, P., Binney, B., Fox, A., Grove-White, D., Leigh, J. W., Miller, W., Muellner, P., & Carter, P. (2014). Evolution of campylobacter species in New Zealand. In S. K. Sheppard & G. Méric (Eds.), *Campylobacter Ecology and Evolution* (pp. 221-240). Caister Academic Press.

Grubbs, R. D. (2010). Ontogenetic shifts in movements and habitat use. In J. C. Carrier, J. A. Musick, M. R. Heithaus & D. H. Evans (Eds.), *Sharks and their relatives II: biodiversity, adaptive physiology, and conservation* (pp. 319-350). Taylor & Francis. https://ebookcentral-proquest-com.mime.uit.no/lib/tromsoub-ebooks/detail.action?docID=565837

Gubili, C., Macleod, K., Perry, W., Hanel, P., Batzakas, I., Farrell, E. D., Lynghammar, A., Mancusi, C., Mariani, S., & Menezes, G. M. (2016). Connectivity in the deep: Phylogeography of the velvet belly lanternshark. *Deep Sea Research Part I: Oceanographic Research Papers*, *115*, 233-239. https://doi.org/10.1016/j.dsr.2016.07.002

Hasegawa, M., Kishino, H., & Yano, T.-A. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, *22*(2), 160-174. https://doi.org/10.1007/bf02101694

Hellberg, M. E. (2009). Gene flow and isolation among populations of marine animals. *Annual Review of Ecology, Evolution, and Systematics*, *40*(1), 291-310. https://doi.org/10.1146/annurev.ecolsys.110308.120223

Hellberg, M. E., Burton, R. S., Neigel, J. E., & Palumbi, S. R. (2002). Genetic assessment of connectivity among marine populations. *Bulletin of marine science*, *70*(1), 273-290.

Hesthagen, T., Wienerroither, R., Bjelland, O., Byrkjedal, I., Fiske, P., Lynghammar, A., Nedreaas, K., & Straube, N. (2021). Fisker: Vurdering av svarthå *Etmopterus spinax* for Norge. Rødlista for arter 2021. Artsdatabanken. https://www.artsdatabanken.no/lister/rodlisteforarter/2021/23384

Heupel, M., Carlson, J., & Simpfendorfer, C. (2007). Shark nursery areas: concepts, definition, characterization and assumptions. *Marine Ecology Progress Series*, *337*, 287-297. https://doi.org/10.3354/meps337287

Hirschfeld, M., Dudgeon, C., Sheaves, M., Barnett, A., & Macneil, A. (2021). Barriers in a sea of elasmobranchs: From fishing for populations to testing hypotheses in population genetics. *Global Ecology and Biogeography*, *30*(11), 2147-2163. https://doi.org/10.1111/geb.13379

Howell, K. L., Hilário, A., Allcock, A. L., Bailey, D., Baker, M., Clark, M. R., Colaço, A., Copley, J., Cordes, E. E., Danovaro, R., Dissanayake, A., Escobar, E., Esquete, P., Gallagher, A. J., Gates, A. R., Gaudron, S. M., German, C. R., Gjerde, K. M., Higgs, N. D., Le Bris, N., Levin, L. A., Manea, E., McClain, C., Menot, L., Mestre, N. C., Metaxas, A., Milligan, R., Muthumbi, A. W. N., Narayanaswamy, B. E., Ramalho, S. P., Ramirez-Llodra, E., Robson, L. M., Rogers, A. D., Sellanes, J., Sigwart, J. D., Sink, K., Snelgrove, P. V. R., Stefanoudis, P. V., Sumida, P. Y., Taylor, M. L., Thurber, A. R., Vieira, R., Watanabe, H. K., Woodall, L. C., & Xavier, J. R. (2021). A decade to study deep-sea life. *Nature Ecology & Evolution*, *5*(3), 265-267. https://doi.org/10.1038/s41559-020-01352-5

ICES. (2018). *Working group on elasmobranch fishes (WGEF)* (Lisbon, Portugal, Issue 16).

ICES. (2020). Working group on elasmobranch *ICES Scientific Report*, *2*(77), 789. https://doi.org/10.17895/ices.pub.7470

IUCN. (2012). *IUCN red list categories and criteria* (2nd ed.) [Version 3.1]. IUCN.

IUCN, S., Kyne, P. M., & Simpfendorfer, C. A. (2007). A collection and summarization of available data on deepwater chondrichthyans: Biodiversity, life history and fisheries.

IUCN, S. a. P. C. (2019). *Guidelines for using the IUCN red list categories and criteria* (Version 15). Prepared by the Standards and Petitions Committee. https://www.iucnredlist.org/resources/redlistguidelines

Jac, R. (2020). *Study of the life history traits of a deep-sea shark: Etmopterus spinax* [Master, Unevesitie de Bertagne Occidentale].

Jac, R., Höffle, H., Albretsen, J., Jakobsdóttir, K., Staby, A., Søvik, G., & Junge, C. (2021). Of three sharks and one chimaera: Varied habitat preferences across a latitudinal range revealed by coastal and offshore surveys. *Journal of Fish Biology*, 1-15. https://doi.org/10.1111/jfb.14979

Jakobsdóttir, K. B. (2001). Biological aspects of two deep-water squalid sharks: *Centroscyllium fabricii* (Reinhardt, 1825) and *Etmopterus princeps* (Collett, 1904) in Icelandic waters. *Fisheries Research*, *51*(2-3), 247-265. https://doi.org/10.1016/s0165-7836(01)00250-8

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547-1549. https://doi.org/10.1093/molbev/msy096

Kyne, P. M., & Simpfendorfer, C. A. (2010). Deepwater chondrichthyans. In J. C. Carrier, J. A. Musick, M. R. Heithaus & D. H. Evans (Eds.), *Sharks and their relatives II: biodiversity, adaptive physiology, and conservation* (pp. 37-114). Taylor & Francis. https://ebookcentral-proquest-com.mime.uit.no/lib/tromsoub-ebooks/detail.action?docID=565837

Leigh, J. W., & Bryant, D. (2015). Popart : Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110-1116. https://doi.org/10.1111/2041-210x.12410

Lowe, W. H., & Allendorf, F. W. (2010). What can genetics tell us about population connectivity? *Molecular Ecology*, *19*(15), 3038-3051. https://doi.org/10.1111/j.1365-294x.2010.04688.x

Lynghammar, A., Christiansen, J. S., Mecklenburg, C. W., Karamushko, O. V., Møller, P. R., & Gallucci, V. F. (2013). Species richness and distribution of chondrichthyan fishes in the Arctic Ocean and adjacent seas. *Biodiversity*, *14*(1), 57-66. https://doi.org/10.1080/14888386.2012.706198

Marandel, F., Lorance, P., Andrello, M., Charrier, G., Cam, S. L., Lehuta, S., & Trenkel, V. M. (2018). Insights from genetic and demographic connectivity for the management of rays and skates. *Canadian Journal of Fisheries and Aquatic Sciences, 75*(8), 1291-1302. https://doi.org/10.1139/cjfas-2017-0291

McMillan, M. N., Izzo, C., Junge, C., Albert, O. T., Jung, A., & Gillanders, B. M. (2017). Analysis of vertebral chemistry to assess stock structure in a deep-sea shark, *Etmopterus spinax*. *ICES Journal of Marine Science, 74*(3), 793-803. https://doi.org/10.1093/icesjms/fsw176

Monteiro, P., Araújo, A., Erzini, K., & Castro, M. (2001). Discards of the Algarve (southern Portugal) crustacean trawl fishery. *Hydrobiologia*, *449*(1), 267-277. https://doi.org/10.1023/A:1017575429808

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*(5820), 1846-1850. https://doi.org/10.1126/science.1138657

Maaholm, D. J., & Mihalitsis, M. (2014). *Are fisheries going too deep? An assessment of the genetic diversity, phylogeography, and possible dangers from deepsea fishing on the velvet belly lantern shark (Etmopterus spinax).* [Bachelor, Uneversety of Cambrage, Denmark].

Naylor, G. J., Ryburn, J., Fedrigo, O., & Lopez, J. (2005). Phylogenetic relationships among the major lineages of modern elasmobranchs. *Reproductive biology and phylogeny*, *3*(1), 25.

Naylor, G. J. P., Caira, J. N., Jensen, K., Rosana, K. A. M., White, W. T., & Last, P. R. (2012). A DNA sequenceвђ“based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. *Bulletin of the American Museum of Natural History*, *367*(367), 1-262. https://doi.org/10.1206/754.1

Nei, M., & Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford University Press, USA.

Neiva, J. o., Coelho, R., & Erzini, K. (2006). *Feeding habits of the velvet belly lanternshark Etmopterus spinax (Chondrichthyes: Etmopteridae) off the Algarve, southern Portugal* [835-841]. Cambridge :.

Nielsen, J., Hedeholm, R. B., Heinemeier, J., Bushnell, P. G., Christiansen, J. S., Olsen, J., Ramsey, C. B., Brill, R. W., Simon, M., & Steffensen, K. F. (2016). Eye lens radiocarbon reveals centuries of longevity in the Greenland shark (*Somniosus microcephalus*). *Science*, *353*(6300), 702-704. https://doi.org/10.1126/science.aaf1703

Peakall, R., & Smouse, P. E. (2006). Genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, *6*(1), 288-295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Peakall, R., & Smouse, P. E. (2012). Genalex 6.5: Genetic analysis in excel. Population genetic software for teaching and research-an update. . *Bioinformatics 28*, 2537-2539.

Qiagen. (2010). *QIAGEN® multiplex PCR handbook*. https://www.qiagen.com/no/resources/resourcedetail?id=a541a49c-cd06-40ca-b1d2-563d0324ad6c&lang=en

Qiagen. (2016). *Quick-Start protocol QIAGEN® multiplex PCR kit*.

R Core Team. (2020). *R: A language and environment for statistical computing* R Foundation for Statistical Computing. https://www.R-project.org/

Ramírez-Amaro, S., Ordines, F., Esteban, A., García, C., Guijarro, B., Salmerón, F., Terrasa, B., & Massutí, E. (2020). The diversity of recent trends for chondrichthyans in the Mediterranean reflects fishing exploitation and a potential evolutionary pressure towards early maturation. *Scientific reports*, *10*(1). https://doi.org/10.1038/s41598-019-56818-9

Riginos, C., & Liggins, L. (2013). Seascape genetics: Populations, individuals, and genes marooned and adrift. *Geography Compass*, *7*(3), 197-216. https://doi.org/10.1111/gec3.12032

Rodhe, J. (1989). The large-scale mixing and the estuarine circulation in the Skagerrak; calculations from observations of the salinity and velocity fields. *Tellus A: Dynamic Meteorology and Oceanography*, *41*(5), 436-446. https://doi.org/10.3402/tellusa.v41i5.11851

Ronce, O. (2007). How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annual Review of Ecology, Evolution, and Systematics*, *38*(1), 231-253. https://doi.org/10.1146/annurev.ecolsys.38.091206.095611

Rozasa, J., Ferrer-Mata, A., Sánchez-DelBarrioo, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP v6: DNA sequence polymorphism analysis of large datasets. *34*, 3299-3302.

Runge, J. P., Hines, J. E., & Nichols, J. D. (2007). Estimating species-specific survival and movement when species identification is uncertain. *Ecology*, *88*(2), 282-288. https://doi.org/10.1890/0012-9658(2007)88[282:ESSAMW]2.0.CO;2

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. *Global Change Biology*, *22*(8), 2729-2743. https://doi.org/10.1111/gcb.13316

Simpfendorfer, C. A., & Kyne, P. M. (2009). Limited potential to recover from overfishing raises concerns for deep-sea sharks, rays and chimaeras. *Environmental Conservation*, *36*(2), 97-103. https://doi.org/10.1017/s0376892909990191

Sion, L., Bozzano, A., D'Onghia, G., Capezzuto, F., & Panza, M. (2004). Chondrichthyes species in deep waters of the Mediterranean Sea. *Scientia Marina*, *68*(S3), 153-162. https://doi.org/10.3989/scimar.2004.68s3153

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science*, *236*(4803), 787-792. http://www.jstor.org.mime.uit.no/stable/1699930

Stevens, J., Bonfil, R., Dulvy, N. K., & Walker, P. (2000). The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES Journal of Marine Science*, *57*(3), 476-494.

Stingo, V., Du Buit, M.-H., & Odierna, G. (1980). Genome size of some selachian fishes. *Bolletino di zoologia*, *47*(1-2), 129-137. https://doi.org/10.1080/11250008009440330

Straube, N., Leslie, R. W., Clerkin, P. J., Ebert, D. A., Rochel, E., Corrigan, S., Li, C., & Naylor, G. J. (2015). On the occurrence of the Southern Lanternshark, *Etmopterus granulosus*, off South Africa, with comments on the validity of *E. compagnoi*. *Deep Sea Research Part II: Topical Studies in Oceanography*, *115*, 11-17. https://doi.org/10.1016/j.dsr2.2014.04.004.

Straube, N., Preick, M., Naylor, G. J. P., & Hofreiter, M. (2021). Mitochondrial DNA sequencing of a wet-collection syntype demonstrates the importance of type material as genetic resource for lantern shark taxonomy (Chondrichthyes: Etmopteridae). *Royal Society Open Science*, *8*(9), 210474. https://doi.org/10.1098/rsos.210474

Thermo Fisher Scientific.). Qubit™ dsDNA BR Assay Kit. In(pp. 8).

Veríssimo, A., McDowell, J. R., & Graves, J. E. (2011). Population structure of a deep-water squaloid shark, the Portuguese dogfish (Centroscymnus coelolepis). *ICES Journal of Marine Science*, *68*(3), 555-563.

Vihtakari, M. (2022). *ggOceanMaps: Plot Data on Oceanographic Maps using 'ggplot2'* (Version 1.2.12). https://mikkovihtakari.github.io/ggOceanMaps/

Wang, I. J. (2010). Recognizing the temporal distinctions between landscape genetics and phylogeography. *Molecular Ecology*, *19*(13), 2605-2608. https://doi.org/10.1111/j.1365-294x.2010.04715.x

Waples, R. S., & Gaggiotti, O. (2006). What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, *15*(6), 1419-1439. https://doi.org/10.1111/j.1365-294x.2006.02890.x

Wickham et al. (2019). Welcome to the tidyverse. Journal of open source software. *4(43)*, 1686. https://doi.org/10.21105/joss.01686

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis.* Springer-Verlag, New Yourk

Wickham, H., François, R., Henry, L., & Müller, K. (2022). *dplyr: A grammar of data manipulation* (Version 1.0.8). https://CRAN.R-project.org/package=dplyr

Wienerroither, R., Hesthagen, T., Bjelland, O., Byrkjedal, I., Fiske, P., Lynghammar, A., Nedreaas, K., & Straube, N. (2021). *Artsgruppeomtale fisker (Myxini, Petromyzontiformes, Chondrichthyes, Osteichthyes). Norsk rødliste for arter 2021*. Artsdatabanken. Retrieved 27.04.2022, from https://www.artsdatabanken.no/rodlisteforarter2021/Artsgruppene/Fisker

Wright, S. (1943). Isolation by distance. *Genetics*, *28*(2), 114.

Appendix 1

Details of sample collection. Name; location name, Serialnumber; station identification, Individuals; specimen identification, Cruise; IMR survey used for sampling, Sea; sampling sea, Date; date of catch; number; Capture location in Lat; latitude, Long; longitude, and Depth; depth caught in m, Sex; 1 = Female, 2 = Male.

Name	Serial number	Individuals	Cruise	Sea	Date	Lat	Long	Depth	sex
ES015_NO_North	55253	1	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	1
ES017_NO_North	55253	2	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	2
ES018_NO_North	55253	3	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	2
ES019_NO_North	55253	4	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	1
ES020_NO_North	55253	5	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	1
ES021_NO_North	55253	6	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	2
ES022_NO_North	55253	7	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	2
ES023_NO_North	55253	8	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	2
ES025_NO_North	55253	9	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	1
ES026_NO_North	55253	10	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	2
ES027_NO_North	55253	11	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	1

ES028_NO_North	55253	12	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	1
ES029_NO_North	55253	13	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	2
ES030_NO_North	55253	14	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	2
ES031_NO_North	55253	15	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	1
ES032_NO_North	55253	16	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	1
ES033_NO_North	55253	17	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	2
ES034_NO_North	55253	18	coastal survey	Norwegian Sea	17.10.2019	69.32	16.67	218.65	1
ES075_NO_TRD_Fjord	55309	1	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES076_NO_TRD_ Fjord	55309	2	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES077_NO_TRD_ Fjord	55309	3	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	2
ES078_NO_TRD_ Fjord	55309	4	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES079_NO_TRD_ Fjord	55309	5	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES080_NO_TRD_ Fjord	55309	6	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES081_NO_TRD_ Fjord	55309	7	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES082_NO_TRD_ Fjord	55309	8	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES083_NO_TRD_ Fjord	55309	9	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES084_NO_TRD_ Fjord	55309	10	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES085_NO_TRD_ Fjord	55309	11	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1

ES086_NO_TRD_ Fjord	55309	12	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	2
ES087_NO_TRD_ Fjord	55309	13	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	2
ES088_NO_TRD_ Fjord	55309	14	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES089_NO_TRD_ Fjord	55309	15	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES090_NO_TRD_ Fjord	55309	16	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES091_NO_TRD_ Fjord	55309	17	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES092_NO_TRD_ Fjord	55309	18	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	2
ES094_NO_TRD_ Fjord	55309	19	coastal survey	Norwegian Sea	02.11.2019	63.81	11.35	122.25	1
ES001_NO_TRD_Coast	73006	1	egga sør	Norwegian Sea	23.03.2018	64.26	8.60	428.36	1
ES002_NO_TRD_ Coast	73006	2	egga sør	Norwegian Sea	23.03.2018	64.26	8.60	428.36	2
ES003_NO_TRD_ Coast	73006	3	egga sør	Norwegian Sea	23.03.2018	64.26	8.60	428.36	1
ES004_NO_TRD_ Coast	73006	4	egga sør	Norwegian Sea	23.03.2018	64.26	8.60	428.36	1
ES005_NO_TRD_ Coast	73006	5	egga sør	Norwegian Sea	23.03.2018	64.26	8.60	428.36	1
ES006_NO_TRD_ Coast	73006	6	egga sør	Norwegian Sea	23.03.2018	64.26	8.60	428.36	1
ES008_NO_TRD_ Coast	73006	7	egga sør	Norwegian Sea	23.03.2018	64.26	8.60	428.36	1
ES009_NO_TRD_ Coast	73006	8	egga sør	Norwegian Sea	23.03.2018	64.26	8.60	428.36	1
ES010_NO_TRD_ Coast	73006	9	egga sør	Norwegian Sea	23.03.2018	64.26	8.60	428.36	1
ES011_NO_TRD_ Coast	73006	10	egga sør	Norwegian Sea	23.03.2018	64.26	8.60	428.36	1

ES012_NO_TRD_ Coast	73006	11	egga sør	Norwegian Sea	23.03.2018	64.26	8.60	428.36	1
ES013_NO_TRD_ Coast	73006	12	egga sør	Norwegian Sea	23.03.2018	64.26	8.60	428.36	2
ES014_NO_TRD_ Coast	73006	13	egga sør	Norwegian Sea	23.03.2018	64.26	8.60	428.36	NA
ES055_NO_BGO	22012	1	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	1
ES056_NO_BGO	22012	2	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	1
ES057_NO_BGO	22012	3	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	1
ES058_NO_BGO	22012	4	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	2
ES059_NO_BGO	22012	5	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	1
ES060_NO_BGO	22012	6	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	1
ES062_NO_BGO	22012	7	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	2
ES063_NO_BGO	22012	8	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	1
ES064_NO_BGO	22012	9	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	2
ES065_NO_BGO	22012	10	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	1
ES066_NO_BGO	22012	11	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	1
ES067_NO_BGO	22012	12	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	2
ES068_NO_BGO	22012	13	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	1
ES069_NO_BGO	22012	14	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	1
ES070_NO_BGO	22012	15	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	2

ES071_NO_BGO	22012	16	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	2
ES072_NO_BGO	22012	17	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	1
ES073_NO_BGO	22012	18	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	2
ES074_NO_BGO	22012	19	shrimp survey	North Sea	09.01.2019	59.78	4.32	280.91	2
ES035_NO_OSL	22107	1	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	2
ES036_NO_OSL	22107	2	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	1
ES037_NO_OSL	22107	3	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	1
ES038_NO_OSL	22107	4	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	2
ES039_NO_OSL	22107	5	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	1
ES040_NO_OSL	22107	6	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	2
ES041_NO_OSL	22107	7	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	2
ES042_NO_OSL	22107	8	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	1
ES043_NO_OSL	22107	9	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	2
ES044_NO_OSL	22107	10	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	2
ES045_NO_OSL	22107	11	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	2
ES046_NO_OSL	22107	12	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	1
ES047_NO_OSL	22107	13	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	1
ES048_NO_OSL	22107	14	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	1

ES049_NO_OSL	22107	15	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	2
ES050_NO_OSL	22107	16	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	1
ES051_NO_OSL	22107	17	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	2
ES052_NO_OSL	22107	18	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	1
ES053_NO_OSL	22107	19	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	2
ES054_NO_OSL	22107	20	shrimp survey	North Sea	22.01.2019	58.73	10.21	224.44	2
SAMS 16	1486	1	Scotland	North Sea	06.16.2006	59.56	-4,19		1
AZ_1	5157	1	Azores Islands	Atlantic Ocean	10.23.2008	38.51	-28.61		1
AZ_2	5158	2	Azores Islands	Atlantic Ocean	10.23.2008	38.51	-28.61		1
AZ_6	5161	3	Azores Islands	Atlantic Ocean	10.23.2008	38.51	-28.61		1
AZ_7	5162	4	Azores Islands	Atlantic Ocean	10.23.2008	38.51	-28.61		1
AZ_8	5163	5	Azores Islands	Atlantic Ocean	10.23.2008	38.51	-28.61		2
AZ_9	5164	6	Azores Islands	Atlantic Ocean	10.23.2008	38.51	-28.61		1
AZ_5	6460	7	Azores Islands	Atlantic Ocean	11.25.2008	38.51	-28.61		1
AZ_44	6542	8	Azores Islands	Atlantic Ocean	06.01.2009	38.51	-28.61		1
AZ_65	6562	9	Azores Islands	Atlantic Ocean	06.01.2009	38.51	-28.61		1
AZ_68	6565	10	Azores Islands	Atlantic Ocean	06.01.2009	38.51	-28.61		1
AZ_69	6566	11	Azores Islands	Atlantic Ocean	06.01.2009	38.51	-28.61		1

AZ_70	6567	12	Azores Islands	Atlantic Ocean	06.01.2009	38.51	-28.61	1
AZ_71	6568	13	Azores Islands	Atlantic Ocean	06.01.2009	38.51	-28.61	1
AZ_72	6569	14	Azores Islands	Atlantic Ocean	06.01.2009	38.51	-28.61	1
AZ_73	6570	15	Azores Islands	Atlantic Ocean	06.01.2009	38.51	-28.61	1
AZ_74	6571	16	Azores Islands	Atlantic Ocean	06.01.2009	38.51	-28.61	1
AZ_75	6572	17	Azores Islands	Atlantic Ocean	06.01.2009	38.51	-28.61	1
AZ_76	6573	18	Azores Islands	Atlantic Ocean	06.01.2009	38.51	-28.61	1
AZ_77	6574	19	Azores Islands	Atlantic Ocean	06.01.2009	38.51	-28.61	1
AZ_78	6575	20	Azores Islands	Atlantic Ocean	06.01.2009	38.51	-28.61	1
P-CH-0067	7923	1	North Sea	North Sea	03.21.2012			
P-CH-0119	7956	1	Bay of Biscay	Atlantic Ocean	03.21.2012	47.84	-3.94	2
P-CH-0120	7957	2	Bay of Biscay	Atlantic Ocean	03.21.2012	47.84	-3.941	
P-CH-0121	7958	3	Bay of Biscay	Atlantic Ocean	03.21.2012	47.84	-3.942	2
P-CH-0122	7959	4	Bay of Biscay	Atlantic Ocean	03.21.2012	47.84	-3.943	2
P-CH-0123	7960	5	Bay of Biscay	Atlantic Ocean	03.21.2012	47.84	-3.944	2
P-CH-0124	7961	6	Bay of Biscay	Atlantic Ocean	03.21.2012	47.84	-3.945	1
AZ_148	13745	1	Bay of Biscay	Atlantic Ocean	10.01.2012	38.54	-29.05	1
AZ_150	13747	2	Bay of Biscay	Atlantic Ocean	10.01.2012	38.54	-29.05	1

S2_NO_Langesund	1	Langesund	North Sea	aug.11	58.98	09.76
S3_NO_Langesund	2	Langesund	North Sea	aug.11	58.98	9.76
S5_NO_Langesund	3	Langesund	North Sea	aug.11	58.98	9.76
S6_NO_Langesund	4	Langesund	North Sea	aug.11	58.98	9.76
S7_NO_Langesund	5	Langesund	North Sea	aug.11	58.98	9.76
S8_NO_Langesund	6	Langesund	North Sea	aug.11	58.98	9.76
S9_NO_Langesund	7	Langesund	North Sea	aug.11	58.98	9.76
S10_NO_Langesund	8	Langesund	North Sea	aug.11	58.98	9.76
S11_NO_Langesund	9	Langesund	North Sea	aug.11	58.98	9.76
S12_NO_Langesund	10	Langesund	North Sea	aug.11	58.98	9.76

Appendix 2

```
---

title: "R Notebook"

output: html_notebook

---

#Map of all study locations:


#Libraryes:

library(dplyr)

library(tidyverse)

library(ggplot2)

library(ggOceanMaps)


#Code:

map_all<-basemap(limits = c(-40, 20, 35, 75), bathymetry = TRUE,
land.border.col = NA) +

  geom_spatial_point(data = Espinax, shape = 20, alpha = 0.5, aes(x =
longitudes, y = latitude), color="red", size = 3)
```

map_all

Appendix 3

The 23 haplotypes found among all 129 individuals, only showing the 28 polymorphic nucleotide positions (locations where the mutations are). N: how many times each haplotype appears among all sequenses.

Haplotype	N	9	29	95	183	190	216	293	342	351	447	477	507	508	624	727	757	762	803	806	810	829	878	906	921	981	1004	1008	1036
H 1	1	A	C	A	G	G	T	C	G	G	T	T	T	T	T	G	G	A	A	A	A	C	A	A	C	A	T	T	T
H 2	1	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	G	T	.	.	.	.	.	.	.	.
H 3	1	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
H 4	63	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 5	1	.	.	G	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 6	1	.	T	G	A	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 7	2	.	T	G	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
H 8	1	.	T	G	.	.	C	.	A	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	G	.	.	.
H 9	5	.	T	G	.	.	C	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	G	.	G	.	.	.
H 10	14	.	T	G	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
H 11	1	.	T	G	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	.	.	.	.	T	.	.	.	.
H 12	1	.	T	G	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 13	1	.	T	G	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	.	.	G	.	.	.	.	.

H 14	3	.	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	.
H 15	2	.	T	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	T	.	A	.	.
H 16	9	.	T	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	T	.	.	.	.
H 17	1	.	T	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	T	.	T	.	.	.	C
H 18	3	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 19	2	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
H 20	1	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	A	.	A	C	.
H 21	13	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.
H 22	1	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	G	C	.	.	.	.	.	.	.	.	.	.	.
H 23	1	G	T	G	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.