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*from marine conservation to population genetic structure*

# Applications of eDNA-based methods: from marine conservation to population genetic structure

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**Abstract**

Environmental DNA (eDNA)-based methods enable the detection of organisms from genetic material in environmental samples (e.g. water, soil, sediment) and are emerging as promising biomonitoring tools. In the research presented in this thesis, I explore the utility of eDNA-based analyses in elasmobranch conservation, in investigations of mesopelagic fish, and in fish population genetics, across three different aquatic ecosystems. In Chapter 1, I review the persistence and dispersal of aquatic eDNA, evaluate the advantages of eDNA compared to traditional aquatic survey methods, and discuss the potential of eDNA in marine conservation and population genetics. In Chapter 2, I present a study of eDNA metabarcoding of elasmobranch diversity in a temperate marine ecosystem. The study provides evidence of the ability of eDNA to reveal species richness, and spatial and temporal differences in community structure elasmobranch community, over a spatial scale of 30km. The eDNA-derived data are compared to historic trawl data, and demonstrate the ability of eDNA metabarcoding to serve as a semi-quantitative biomonitoring approach. In Chapter 3, I present an eDNA metabarcoding study of the mesopelagic fish composition of the Scotia Sea region of Southern Ocean, across a depth gradient from the surface to 1000m. The study showed a decoupling between eDNA read abundance and the abundance of fish in survey trawls. In addition, the distribution of marine mammal species encountered in eDNA "molecular bycatch" was used to investigate their diversity and distribution. In Chapter 4, I present a study using eDNA to quantify population genetic structure of the Eastern happy cichlid fish (*Astatotilapia calliptera*) across a thermo-oxycline in crater Lake Masoko, Tanzania. This research demonstrated that eDNA could reveal differences in allele frequencies within aquatic environment across a small spatial scale (<30 m). In Chapter 5, I summarize these findings and discuss knowledge gaps. Overall, the research presented here shows that aquatic eDNA-based can be used to establish the patterns of biodiversity, both within and across species, and in freshwater and marine ecosystems.

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**Authors declaration**

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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**Table of contents**

**List of tables**

**List of figures**

# Chapter 1

# General Introduction

## 1.1 Overview

Environmental DNA (eDNA)-based methods are emerging as promising biomonitoring tools with the capability to advance many research fields from conservation biology to paleoclimatology (Boussarie et al., 2018; Armbrecht et al., 2019). To date, a high proportion of eDNA-based research has focused on aquatic systems, with applications including fisheries management, marine conservation, and invasive species detection, among others. The methods have now developed to a stage where they are being used for monitoring of aquatic ecosystems and informing environmental impact assessment (Mathieu et al., 2020; Banerjee et al., 2021; Hinz et al., 2022). However, the protocols for eDNA sampling and laboratory processing of samples do not yet have global standards. Moreover, appropriate interpretation of eDNA-derived data remains unclear, given uncertainty about the rates of persistence and dispersal of eDNA (Senapati et al., 2019; Ely et al., 2021). This introduction focusses on the practical applications of aquatic eDNA-based methods, reviews evidence related to the persistence and dispersal of eDNA in aquatic environments, considers uses of eDNA-based methods as quantitative tools for measuring abundance of species, and explores potential for eDNA to be used as a substrate for population genetic analysis. The potential benefits of eDNA-based surveys are evaluated in relation to traditional survey approaches, and advantages of coupling eDNA-based approaches with emerging technologies to support marine conservation are emphasised.

## 1.2 The definition, persistence, and dispersal of eDNA

### *1.2.1 Why eDNA is important for aquatic studies?*

Over the last century global biodiversity has decreased dramatically, and conservation of endangered species and ecosystems require new tools for rapidly assessing biodiversity and informing conservation interventions (Johnson et al., 2017). Marine and freshwater ecosystems are particularly notable in this regard due to the scale of risks threats they face from human activities, but also because of our relatively poor understanding of the composition and distribution of their biodiversity (He & Silliman, 2019; Smale et al., 2019; Pawlowski et al., 2020). Effective monitoring is required to provide key evidence for aquatic management and conservation, but conventional biomonitoring methods can be limited in their capacity to deliver timely and cost-effective information (Sigsgaard et al., 2020a; Ip et

al., 2021). To rapidly obtain precise biodiversity data, novel biomonitoring methods are needed. Recent developments in acoustic and visual monitoring technologies are useful advances. In addition, molecular techniques based on non-invasively collected environmental DNA are proving to be sufficiently sensitive to investigate biodiversity and abundance of aquatic organisms (Jerde et al., 2011; Takahara et al., 2012; Thomsen et al., 2012).

An early application of eDNA research on aquatic vertebrates focusses on the detection of American bullfrog (*Rana catesbeiana*) in both controlled environments and natural ponds. This study clearly demonstrated that DNA isolated from water can be used to detect the species present in the immediate environment, even at low densities (Ficetola et al., 2008). Subsequently, there has been a rapid advance in the methods being used for eDNA-based research, and increased interest among biodiversity-focussed researchers (Jerde et al., 2019; Nagler et al., 2022; Qian et al., 2022). Coupled with the availability of next-generation sequencing technology (von Bubnoff, 2008), eDNA-based studies have been able to provide unprecedented knowledge about the species present in logistically challenging environments, such as polar and deep-sea ecosystems (Canals et al., 2021; Howell et al., 2021). Moreover, applications of eDNA have been extended from detecting the presence or absence of species, to providing semi-quantitative or even quantitative estimates of species abundance (Ficetola et al., 2008; Boussarie et al., 2018; Levi et al., 2019; Liu et al., 2022). There are many issues, however, that may limit the accuracy of eDNA-based biodiversity inferences, including eDNA extraction techniques and sampling strategies, as well as rates of persistence and dispersal (Collins et al., 2018; Carraro et al., 2021; Cooper et al., 2022).

### *1.2.2 What is environmental DNA?*

With growing interest in eDNA-based methods for both pure and applied research purposes, it is useful to clearly define what eDNA represents, enabling both clear information exchange among researchers, as well as communication with environmental stakeholders (Pawlowski et al., 2020). The term “Environmental DNA” (eDNA) was firstly used by Ogram et al. (1987) referring to DNA extracted from sediment samples without isolating the microorganisms they were studying. Previously, DNA from environmental samples used in studies that targeted microorganisms was called “extracellular DNA”, a term widely used in

microbiology and referred to as the pioneer of “environmental DNA (eDNA)” (Taberlet et al., 2018). It was more than two decades later that Ficetola et al. (2008) used the term “environmental DNA” to refer to vertebrate DNA extracted directly from water to detect an invasive bullfrog (*Rana catesbeiana*) in ponds. Subsequently, environmental DNA (eDNA) has been defined as “DNA that can be extracted from environmental samples (such as soil, water or air), without first isolating any target organisms (Taberlet et al., 2012)”. Such eDNA can be both cellular or extracellular DNA, and can also include community samples or bulk samples such as plankton from netting, or the contents of digestive systems (Taberlet et al., 2012).

Alternative definitions have been provided. For example, Turner et al., (2014) suggested the term eDNA should be restricted to “cells or DNA fragments left in environments by organisms which are absent in the environmental samples”. This definition is not widely accepted due to requirements for verifying or testing the absence of whole organisms, which may include the fertilised eggs or larval stages of organisms. Another definition was provided by Thomsen and Willerslev (2015), who define eDNA as “genetic material obtained directly from environmental samples (soil, sediment, water etc.) without any obvious signs of biological source material”. This definition ruled out the use of the term to encompass bulk samples, either of collected organisms from traps or nets, or from gut contents.

Practically, those definitions that broadly consider eDNA to describe isolated DNA of target species from the environment, without direct sampling those target species, are perhaps the most useful to the widest range of researchers. Therefore, Pawlowski et al. (2020) recently proposed that eDNA is described as “a pool of genomic DNA from any form of environmental samples, including intra/extracellular and intra/extra organismal”. To further clarify the term Pawlowski et al. (2020) also proposed a two-level terminology: 1) define the environmental media where the DNA templates are from (e.g. freshwater DNA, marine eDNA), 2) specify the targeted taxonomic group in qPCR or metabarcoding (e.g. insect eDNA, elasmobranch eDNA). This suggestion to use a standard two-step terminology may increase the difficulty of describing studies (Rodriguez-Ezpeleta et al., 2021), where those studies include DNA from more than one medium, and/or where they target more than one targeted taxonomic groups (Zhang et al., 2020; Ragot & Villemur, 2022). Therefore, broadly referring eDNA as diverse kinds of DNA isolated from all environmental media and providing

clear statements about DNA sources and target species in methods statements, seems to be appropriate and widely accepted measures (Deiner et al., 2017; Rodriguez-Ezpeleta et al., 2021).

### *1.2.3 Persistence eDNA in aquatic ecosystems*

The dynamics of eDNA, including persistence and dispersal, and how they are related to extrinsic environmental factors, remains one of the biggest challenges when interpreting eDNA-derived biodiversity information (Ely et al., 2021; Joseph et al., 2022). Determining the timescale that eDNA is detectable after being released by source organism will be critical for interpreting the results of eDNA detection protocols. It is now clear that eDNA degrades at an exponential rate in aquatic environments, with most being degraded soon after release. It has been estimated that the half-life time of aquatic eDNA can, however, vary between 0.7 to 70 hours in natural environments (Collins et al 2018), with a meta-analysis of published experimental work identifying faster decay rates in warmer temperatures, and in seawater relative to freshwater (Jo and Minamoto 2021). Additionally, we know persistence may be determined by other factors, including water pH and exposure to UV-B light (Strickler et al., 2015). Biological factors such as the presence of biofilms (Shogren et al., 2018) and the density of source organisms (Harper et al., 2018) will also significantly influence the persistence time of eDNA.

### *1.2.4 The dispersal of eDNA in aquatic environment*

In dynamic aquatic ecosystems, flows, tides and currents can all carry organic particles leading to dispersal over large spatial scales, and over rapid timescales. Tides can have significant effects on communities of marine organisms, with tidal cycles strongly determining the movements and habitat uses of species (Tsujii et al., 2022; Wang et al., 2018). To date, eDNA-based studies of nearshore marine ecosystems have found only limited influence of tides on the species compositions recovered (Kelly et al., 2018; Ely et al., 2021; Lafferty et al., 2021; Harris et al., 2022). By contrast significant changes in community structure have been identified using eDNA in estuarine environments, coincident with salinity shifts (Schwentner et al., 2021). Currents may also be predicted to transport eDNA over great distances (Jeunen et al., 2019). In one of the few direct studies of this phenomenon, Andruszkiewicz et al. (2019) studied eDNA movement in Monterey Bay from

a point source and concluded that eDNA transport was only in the order of tens of kilometres over a few days. Another field-based study of fish similarly concluded at best a modest effect of currents on eDNA distributions (Inoue et al., 2022). The complexity of oceanographic patterns means that more studies linking models of particle movement in different oceanographic environments to dispersal of eDNA are needed. It seems probable that strong flow regimes, like those that influence eDNA composition in rivers and estuaries (Shogren et al., 2017; Shogren et al., 2019), will similarly influence eDNA in fully marine systems. However, it is notable that even under conditions of strong water flow (in the vicinity of Otago Harbor, New Zealand) eDNA-based methods can still capture variation in the community composition of marine species over modest spatial scales of <5km (Jeunen et al., 2019).

The distribution of aquatic eDNA can also be influenced by water stratification, upwelling and downwelling. Thermal stratification in Canadian lakes can strongly affect fish assemblage structure due to thermal preferences, and this pattern has been recovered in analyses of eDNA collected from across depth gradients (Littlefair et al., 2021). Vertical eDNA stratification has also been reported in deep-sea fish community (Canals et al., 2021) and this kind of eDNA stratification can show a seasonal pattern (Stoeckle et al., 2021). Upwelling or downwelling may also, in principle, cause vertical spreading and dilution of environmental DNA (Andruszkiewicz et al., 2019). It has been demonstrated that stronger upwelling conditions in coastal California can led to greater differences in fish composition among sites that are recovered using eDNA-based methods (Closek et al., 2019).

## 1.3 Working with eDNA: considerations and practicalities

### *1.3.1 eDNA Sampling*

Water samples for aquatic eDNA studies come from strikingly different environments, such as aquariums, pools, ponds, reservoirs, rivers and oceans (Longsnaw et al. 2012; Fernandes et al., 2018). Sampling challenges may therefore vary considerably, and the sampling strategies, including numbers of replicates, water volume, water preservation and filtering methods can vary accordingly (Goldberg et al., 2016; Cantera et al., 2019).

Although in some circumstances eDNA can be concentrated from a water sample using centrifugation, environmental DNA is typically collected using “dead end” filtering methods, where pressure is applied, collecting the complex suspended particulate matter (SPM) containing genetic materials on a filter membrane (Harrison et al., 2019). The volume of water that can be passed through filter depends on the pore size and the particle size distribution in this water body (Hunter et al., 2019; Cooper et al., 2022). Therefore, the volume of samples obtainable can be strongly influenced by the turbidity of the water. Sampling methods may also vary depending on the expected density of targeted organisms within the water body. For instance, sampling eDNA of fish in small ponds may require only small samples and obtaining the eDNA from as little as 15 mL of water may be sufficient to detect species of interest (Ficetola et al., 2008), while only 120 mL may be sufficient to reveal broader patterns of biodiversity (Minamoto et al., 2012). By contrast, sampling marine environments is typically done using higher volumes of water, often between 1 and 30 L (Westfall et al., 2021; Sanchez et al., 2022). Target organisms are an important factor to consider when designing sampling strategies, as low-density organisms will require higher volumes of water to be filtered (Sepulveda et al., 2019). On-site filtering is generally easier for long-distance transportation, however contamination may be hard to control in the field - on a moving vessel for example. Filtering in a clean laboratory is recommended where possible. Afterwards, filters can be preserved in salt-solution at room temperatures for short periods of time allowing transportation, or they can be frozen directly, most commonly at either -20 °C or -80 °C.

### *1.3.2 The quantitative PCR (qPCR) approach*

Environmental DNA-based research focused on detecting and/or quantifying single taxa typically uses qPCR-based assays. Here, the target sequences of the focal species are used to design the primers and probe required for the assay (Wood et al., 2020). These assays are widely used and have proven to be sufficiently reliable to enable commercial applications, such as surveys for great crested newt (*Triturus cristatus*), a species protected by UK legislation (Harper et al. 2018). Using a qPCR approach, it is possible to rapidly assay tens or hundreds of samples in a single run and detect DNA fragments with low number of copies (Blattner et al., 2021).

### *1.3.3 Sequencing technologies*

One of the earliest Next Generation Sequencing (NGS) platforms was the Roche 454 pyrosequencer, developed in 2005 (Kulski, 2016), and used by Thomsen et al. (2012) to sequence *cytochrome b* eDNA fragments of fish from seawater. The relatively high price of 454 pyrosequencing (~$10 per million bases) resulted in more use of the lower cost Illumina technology (HiSeq and MiSeq), which arrived on the market in 2007. Currently the majority (> 70%) of users of NGS technology use Illumina sequencing (Liu et al., 2012). With a falling per base price for sequencing (Pareek et al., 2011) these technologies have provided new opportunities for metagenomic and metabarcoding approaches to be widely employed in environmental biodiversity-focussed studies. More recently, third-generation long-read sequencing platforms have gained prominence, namely single-molecule real-time sequencing (Pacific Biosciences) and Nanopore (Oxford Nanopore Technologies). While these methods currently suffer from relatively high error rates, they can uniquely and reliably provide reads of 10-50 kb in size (Sunagawa et al., 2015), which may be valuable for some analyses of environmental DNA samples, for example reconstruction of genomes from novel microbial taxa.

### *1.3.4 Metagenomics (shotgun sequencing)*

Shotgun sequencing of environmental DNA is the generation of genomic sequences from all taxa that have deposited any DNA in the environment and is commonly referred to as “metagenomics”. This approach can be valuable where target organisms are likely to comprise a sufficiently high proportion of the reads. It also offers the advantage of avoiding a PCR-based target amplification step, and therefore the preparation of the library may be more straightforward. Typically, those studies using metagenomic approaches on eDNA samples (including bulk samples or gut contents) target one or several groups of taxa but may consider the whole tree of life. Mostly, however, metagenomic methods are used to capture diversity information on microbial species that often comprise the bulk of the environmental DNA, for example bacteria and fungi (Lim et al., 2022; Stat et al., 2018).

### *1.3.5 Metabarcoding (sequencing of PCR-amplified barcode regions)*

DNA barcodes are short DNA fragments (often 100-700 bp) that are typically chosen because they are present across a range of species in a taxonomic group, can be readily PCR amplified using a single primer pair, and although they usually have a highly heterogeneous region within them, they typically possess conservative flanking regions. Typically, a good DNA barcode will be unique to a single species in any sampled area, and there will be well curated reference library to support any identification made when the presence of these fragment is identified in a sample. DNA barcoding is typically referred to as the case where DNA from a single individual is sequenced and compared to a reference library. By contrast metabarcoding refers to the scenario where multiple sequences are generated from a single environmental DNA sample (including bulk samples or gut contents) and compared to the reference library (Taberlet et al., 2018). Typically, eDNA metabarcoding is used to identify the species composition of a sample, and in principle metabarcoding data can be generated on any taxonomic group for which reference sequences are available (Zinger et al., 2019; Liu et al., 2022).

Since environmental DNA metabarcoding is based on PCR, it has the advantage of being able to detect rare or endangered species with few copies of DNA fragments within an environmental DNA sample. However, this use of PCR amplification can introduce multiple issues, including PCR bias (where some sequences preferentially amplify), PCR error (introduction of novel nucleotide combinations), PCR homogeneity (which can reduce the efficiency of Illumina sequencing) and tag jumping (where unique sample identifiers are switched during PCR). Due to these biases, eDNA metabarcoding data has been considered to be either semi-quantitative or non-quantitative with respect to species abundance, unlike metagenomic or qPCR-based eDNA methods where results often reflect the abundance of the eDNA in the environment more accurately. Nevertheless, protocols have been optimised to minimise errors during metabarcoding. For example, introducing a variable number of leading Ns ahead of primers can increase sequence variability to avoid sequence homogeneity and improve Illumina output.

The most obvious advantage of eDNA metabarcoding is that the method enables the detection of a broad range of species in a focal taxonomic lineage from a single sample

(Pawlowski et al., 2022; Pont et al., 2022). This is particularly useful when investigating the biodiversity of rare or neglected taxa, or when studying poorly sampled habitats. Currently, metabarcoding is the most popular approach used by environmental DNA researchers to study community-level biodiversity. Given the potential biases and errors that can be brought forward during PCR amplification and library preparation stages, however, there is a need for multiple mitigation steps to be undertaken in both the field and laboratory (Table 1.1).

**Table 1.1.** Considerations when designing eDNA-based metabarcoding.

| **Stages** | **Consideration** | **Bias/Errors** |
|---|---|---|
| Sampling | Sampling volume | Small volumes will generate false negatives, while larger volumes can lead to filter clogging. Uneven sampling across sampling sites may lead to biases in results. |
| | Replication | Few replicates may lead to false negatives, while too many replicates will extend laboratory processing required. At least 3 replicates is typically recommended. |
| | Field controls | Required to provide information on potential contamination of samples during collection and processing, specifically false positives. |
| eDNA extraction | Laboratory environment | Prior to handling samples, ensure space is free from contamination, or samples may yield false positives. |
| | PCR inhibitors | Use of a PCR inhibitor removal kit can improve the quality of PCR |
| | DNA extraction controls | Required to provide information on potential contamination of samples from the extraction step onwards, specifically false positives. |
| PCR | PCR annealing temperature | Low PCR temperatures will lead to amplification of non-target DNA fragments, while high temperatures can lead to overly selective or failed PCR. |
| | Primer volume | Excess primer will lead to formation of dimers, while insufficient primer will lead to failed PCRs and false negatives. |
| | PCR cycling | Too few cycles will lead to low amplicon concentrations, and false negatives. |
| | PCR replicates | Ideally each PCR will be repeated on the same sample multiple times, to maximise chances of the species presence being amplified. |
| | PCR negative controls | Required to provide information on potential contamination of samples from the PCR step onwards, specifically false positives. |
| Library preparation | Standardise input DNA from sample amplicons | Use of a standard concentration of cleaned PCR amplicon enables consistency in read number across samples in the final dataset. |

### *1.3.6 Environmental DNA sampling considerations*

Sampling design for environmental DNA-based work requires an initial understanding of the factors that could influence the rate of production, transport, or persistence of environmental DNA. For example, if the sampling area is a marine ecosystem, researchers need to consider oceanographic factors, such as temperature, tides, and currents. It may also be worth considering anthropogenic influences, such as the activities inside and outside of MPAs, which may affect the production of eDNA from focal species groups. Modelling may also help to optimise the spatial positioning of sampling locations, for example rates of eDNA transport can in principle be modelled *a-priori* (e.g. Andruszkiewicz et al. 2019).

While sampling methods should be decided based on population densities of targeted organisms (Carraro et al., 2021), they are also dependent on practicalities. To avoid clogging of filters, pre-filtering using disposal meshes or gradient filtering can be used. Pre-testing filtering speeds to decide sampling volumes is highly recommended. Sampling timespans should also be considered. It may be preferable in some environments to collect water samples at low frequency and filter them later in a dedicated laboratory, for example when working on survey vessels (Aguzzi et al., 2019; Westfall et al., 2021; Suzuki et al. 2022). In ideal monitoring schemes, samples would be taken regularly at pre-designated frequencies (Levi et al., 2019; Jo & Minamoto, 2021).

## 1.4 Challenges in the management of marine systems

Globally, the marine environment is changing because of human activities, including overfishing, pollution discharge, climate change and infrastructural development (McCauley et al., 2015). To tackle the challenges of conserving and restoring marine ecosystems, one of the key strategies is the establishment of marine protected areas (MPAs) (McCauley et al., 2015), and sound decisions on the placement, size, and management strategies of MPAs need to be based on monitoring data that accurately describes the biodiversity present. However, collecting marine biodiversity data across different habitat types, and across different groups of marine organisms, remains highly challenging. This can be particularly the case for highly mobile marine megafauna that may be intrinsically uncommon. Moreover, ensuring reliable time series to determine the effectiveness of MPAs can be an even greater challenge.

### *1.4.1 Conservation and biomonitoring of marine megafauna*

Marine megafauna includes a variety of species groups, including mammals (e.g. cetaceans, pinnipeds, sirenians), fish (e.g. sharks, rays, billfishes, tuna), reptiles (e.g. marine turtles) and seabirds (e.g. auks, gulls, albatrosses). The body length of species can vary from ~140cm (giant clam) to ~24m (sperm whale) (Todd et al., 2020). Marine megafauna species serve important roles in marine ecosystems, as predators and prey, and in ecosystem-level processes such as nutrient cycling (Pimiento et al., 2020). However, more than 30% of all marine megafauna species are threatened with extinction (IUCN, 2022). It has been predicted that approximately 18% of marine megafauna species will be extinct in the next 100 years under continuing practices, although a worst-case scenario has been envisaged where up to 40% of these species will go extinct (IUCN, 2022).

Despite continuing threats for many species, conservation efforts to protect and restore populations of marine megafauna species have been successful in many regions, following global cooperation, legislation, and establishment of MPAs (Manel et al., 2019). Such effective management of megafauna species benefits from the availability of timely information on their distribution and abundance of species within the ecosystem. Many species of marine megafauna are, however, pelagic mobile predators, including elasmobranchs and cetaceans, and monitoring these species can be highly challenging using traditional survey methods. Visual surveys, for example, can underestimate the diversity of species present, while capture-based methods for surveying fish, such as angling and midwater trawling, may be inefficient and be considered unethical within a conservation or biodiversity management context.

Over recent years there has been growing interest in applying novel survey methods for evaluating the abundance and distributions of the marine megafauna. These include baited remote underwater video (BRUVs), unmanned aerial systems or vehicles (UASs or UAVs), passive acoustic monitoring (PAM) and satellite tracking of tagged individuals (Martin et al., 2020). All these methods rely on novel technologies, requiring devices that are costly to deploy (particularly over larger spatial scales), and capturing data that can be technically challenging to analyse. For example, video-based analyses require considerable researcher expertise to identify taxa, and many species can be overlooked on images leading to

incomplete community information (Zhou et al. 2022). UAVs have similar problems with taxonomic identification and are also highly dependent on weather conditions (Ventura et al., 2018). Meanwhile, passive acoustic monitoring and satellite tracking of tagged individuals can provide extremely detailed information on space use but, typically, they can only be used for a small number of individuals within a population (van Zinnicq Bergmann et al., 2022). Moreover, passive acoustic monitoring of tagged individuals can only be used within the context of a proximate hydrophone array (Lea et al. 2016).

The challenges of obtaining marine biodiversity information are not only because of animal behaviour, but also related to extreme and unpredictable environmental conditions. Deep water habitats, including the mesopelagic "twilight zone" (200-1000m depth), are poorly known relative to surface waters. This is particularly the case for deep water habitats within Antarctic and Arctic polar ecosystems (Griffiths, 2010; Martin et al., 2020; Canals et al., 2021; Howell et al., 2021). There are now concerns that these relatively unexplored deep-water habitats may be under threat from human activities, such as the development of new fisheries, deep-sea mining, and climate change (Dornan et al. 2019; Levin et al., 2019; Martin et al., 2020). In response, the Intergovernmental Oceanographic Commission (IOC), Census of Marine Life (CoML), the Ocean Biodiversity Information System (OBIS) and the Deep-Ocean Stewardship Initiative (DOSI) are focusing on global collaboration and cooperation to enable the collection and sharing of marine biological and environmental monitoring data, under the Deep-Ocean Stewardship Initiative (DOSI) (Levin et al., 2019; Cavanagh et al., 2021; Estes et al., 2021).

### *1.4.2 Advantages of eDNA-based methods for studying distribution and abundance of marine fauna.*

All marine biodiversity monitoring methods have key advantages and shortfalls, but eDNA-based methods are emerging as excellent alternative or supplementary approaches. Environmental DNA has now been used for study the distribution and abundance of many megafauna species (including sharks, rays, dolphins, whales) in multiple marine habitats. Methods based on eDNA have also proved successful at being able to locate spatial and temporal community structure within and among habitats with a high degree of accuracy and sensitivity (Qu & Stewart, 2019; Liu et al., 2022). Increasingly, research gaps are being

filled using eDNA metabarcoding, qPCR or shotgun sequencing, and collectively these are helping to resolve patterns of risk to marine species from environmental stressors (e.g. Richards et al., 2022).

For many community-level sampling applications, eDNA-based methods may be more sensitive and accurate than other technologies. For example, Boussarie et al. (2018) report a study on tropical sharks from New Caledonia, comparing species occurrence data from underwater visual censuses (dive surveys), BRUVs and eDNA metabarcoding. The authors found that eDNA metabarcoding detected 44% more shark species than visual censuses.

Observations that eDNA-based methods can provide quantitative or semi-quantitative assessments of the abundance of focal organisms also add to their value. Evidence supporting the semi-quantitative capacity of eDNA metabarcoding has been found in multiple studies. For example, a positive association was found between eDNA read abundance and fish biomass from a coincident trawling survey in subarctic waters off Greenland (Thomsen et al., 2016). Similarly, there was positive association between eDNA read abundance and total active acoustic fish biomass estimates near Tokyo Bay in Japan (Sato et al., 2021), although unlike eDNA, the acoustic survey was not able to provide species specific information.

An unexpected finding from eDNA-based metabarcoding studies is that primers developed specifically for one group of taxa may additionally amplify multiple species in other groups of taxa. These additional biodiversity records have been termed “molecular bycatch” (Mariani et al. 2021) or “metabarcoding bycatch” (Ritter et al., 2022). An example comes from a study of fishes around three estuaries in the Northeast coast of England, that also generated data on the community composition of riparian birds and mammals of the region. (Mariani et al., 2021).

To conclude, eDNA has been proved to be a non-invasive and highly sensitive method for studying the abundance and diversity of species in marine ecosystems.

(1) *Non-invasive* and *low stress.* Sampling can be undertaken from water or sediment samples, without any significant environmental impacts that would endanger sensitive species. Collection methods are not typically invasive and do not cause physical harm

to non-microscopic organisms. There may be indirect stress caused from approaching sampling sites. For example, if flying drones were used to collect samples, their impacts on fauna (for example nesting seabirds) may need to be considered (see Weimerskirch et al. 2018).

(2) *Fast* and *easy sampling.* The relative ease of sampling is one of the clearest advantages of eDNA-based survey methods (Rees et al. 2014; Howell et al., 2021). Although collection of water from samples from the deep sea is still logistically challenging, it may prove possible to obtain depth and time-integrated biodiversity samples from surface waters, or for samples to be collected autonomously.

(3) *Low-cost*. Currently the cost of eDNA-based methods is high, if sample collection, sampling processing, data analysis and data interpretation are fully accounted for (Smart et al., 2016). Nevertheless, where assays are developed and tested, they may become less expensive than conventional sampling methods. It is possible that eDNA-based methods will become competitively priced complementary tools to other more direct methods of acquiring biodiversity information, including remotely operated vehicles (ROVs), autonomous underwater vehicles (AUVs), passive acoustic monitoring and satellite tracking.

(4) *High sensitivity.* A clear benefit of eDNA-based methods is their high sensitivity to even low concentrations of DNA of focal species in the environment. This has been most clearly demonstrated in invasive species detection (Jerde et al., 2013; Larson et al., 2020).

### 1.5 The potential for environmental DNA-based population genetic inference

Environmental DNA-based methods have typically focussed on the detection and quantification of taxa in the environment, very often with species-level resolution. However, there is strong potential for environmental DNA to provide a more detailed understanding of population structure within species, enabling tests of evolutionary theories, and inference of population genetic processes such as gene flow. Currently, population genetic studies on animals typically obtain genetic information from DNA from captured individuals, for example using blood and tissue samples, but such sampling methods can be technically difficult as well as ethically questionable for some species groups (Bearzi, 2000; Kellar et al.,

2015). Therefore, application of eDNA-based non-invasive, highly sensitive population genetic methods may have a role in future evolutionary-ecology focussed research.

### *1.5.1 Current applications of eDNA on population genetics*

Initial explorations of the potential for eDNA in population genetic research focused on the identification of mitochondrial haplotypes of invasive species, including zebra mussels, quagga mussels and silver carps in North America (Marshall & Stepien, 2019; Stepien et al. 2019). In these cases, the population-genetic differences were initially investigated using approaches based on tissue samples, but the authors were able to show that eDNA was able to detect intraspecific variation in the same mitochondrial loci. Similar results have been achieved by studying whale sharks in Western Australia. Here, mitochondrial DNA sequences derived from eDNA collected in close proximity to 28 individual sharks were 100% matches to sequences from tissue samples sourced from those individuals (Dugal et al., 2022).

In an extension of these observations, it has been shown that using shotgun sequencing of eDNA collected from sand in the proximity of turtles (either nesting adults or juveniles), it was possible to reconstruct mitochondrial genomes suitable for population genetic analyses. (Farrell et al., 2022). A further milestone study was reported by Andres et al. (2021) who constructed amplicons containing microsatellite loci of round goby (*Neogobius melanostomus*) from environmental DNA samples collected from both mesocosms and the field. They were also able to demonstrate that allele frequencies obtained from eDNA were a close match to those obtained from tissue samples. However, the authors did not explicitly test for population genetic divergence using eDNA from the natural environment.

### *1.5.2 The challenges of eDNA in population genetics*

To date, most eDNA-based biodiversity research on animals has focussed upon mitochondrial loci. This is because: i) reference sequences available on global databases allow for matching reads to species (Langlois et al., 2021); ii) there is sufficient intrinsic sequence variation to enable species-level identifications; and iii) there are often conservative regions of sequence flanking the variable target regions, facilitating reliable primer design. However, mitochondrial DNA is typically maternally inherited and non-

recombining. The whole mtDNA genome acts as a single locus, providing information on material species history (Galtier et al. 2009). Ideally, we would employ nuclear eDNA-variation in population genetics, but this requires comprehensive lists of allelic variation based on a-priori sequencing or genotyping of many individuals, which may require a considerable research investment for species of interest (Sigsgaard et al., 2020b). We will also need to have a good understanding of the variation present in heterospecific species that may be present in the sampling region and be sequenced during assays.

Similar to eDNA-based studies aiming to detect or quantify individual species, population-genetic studies will be subject to PCR amplification errors, sequencing errors and laboratory contamination. Notably the software used in typical eDNA metabarcoding bioinformatic pipelines tends to overlook single mutations and cluster them into OTUs/ASVs, which may lead to a loss of population genetic data and inaccurate allele ratios (Macé et al., 2022). Any analysis strategy is therefore likely to depend on methods of handling data where reads are mapped to reference genome data, and allele frequencies of known variants are called. To do this reliably, even for mtDNA based studies, it may be preferably to fully understand the variation in the studied populations prior to inference of population structure using eDNA. To fully develop the potential for eDNA methods of population-genetic inference, it seems likely that new bioinformatic software will need to be developed that accounts for error, and is able to remove sequences matching heterospecific species likely to be in a sampling area. If challenges can be overcome, then eDNA may help to solve more complicated biological and ecological questions than can be addressed using species occurrence or abundance data alone.

### 1.6 The aims of research presented in this thesis

The central aim of the research presented here has been to extend our knowledge and potential applicability of eDNA-based methods for investigating the species and communities within aquatic environments. **Chapter 2** investigates the elasmobranch community in the Western English Channel using an eDNA metabarcoding approach. Seawater samples were taken from the three stations (surface and bottom) monthly for 14 months. The study demonstrates the capacity of eDNA-based metabarcoding to help characterise the composition of an elasmobranch community and provides insight into the

spatial and temporal pattern of these highly mobile marine predators. **Chapter 3** investigates the use of environmental DNA to study the mesopelagic fish diversity of the Scotia Sea region of Southern Ocean. A set of samples were collected from a range of depth enabling a comparison of species composition by depth, while further samples were collected across a broad latitudinal gradient enabling a larger scale perspective. The study demonstrates how eDNA-metabarcoding can capture differences in fish communities across depth gradients, but these patterns were not necessarily linked to the abundance of adult mesopelagic species. The study also shows how eDNA metabarcoding could be a useful tool for mapping both fish and mammals – the latter encountered in the samples as "molecular bycatch". **Chapter 4** evaluates the use of eDNA for population genetics, by quantifying and comparing allele frequencies present in eDNA and fish samples. A case study is reported from Lake Masoko, a small crater lake in Africa, where a single cichlid fish species shows population genetic structure either side of a thermo-oxycline. This demonstrates potential for the development of eDNA-based population-genetic methods that could be used more widely. **Chapter 5** provides a summary of the results presented earlier in the thesis, alongside discussion of future directions for aquatic eDNA research.

# Chapter 2

# Environmental DNA captures elasmobranch diversity in a temperate marine ecosystem, the Western English Channel

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**Abstract**

Many sharks, skates and rays (elasmobranchs) are highly threatened by the activities of commercial fisheries, and a clear understanding of their distributions, diversity and abundance can guide protective measures. However, surveying and monitoring elasmobranch species can be highly invasive or resource intensive, and utilisation of non-invasive environmental DNA based methods may overcome these problems. Here we studied spatial and seasonal variation in the elasmobranch community of the Western English Channel using environmental DNA (eDNA) collected from surface and bottom waters periodically over an annual cycle (2017-2018). In total we recovered 13 elasmobranch species within eDNA samples, and the number of eDNA reads was positively associated with the abundance of these species resolved from 105-year time series of the region (1914-2018). Notably, eDNA recorded a greater number of species per sampling event than a conventional trawl survey in the same area over the same sampling years (2017-2018). Several threatened species were recovered within the eDNA, including undulate ray, porbeagle shark and thresher shark. Using eDNA, we found differences in elasmobranch communities among sampling stations and between seasons, but not between sampling depths. Collectively, our results suggest that non-invasive eDNA-based methods can be used to study the spatial and seasonal changes in the diversity and abundance of whole elasmobranch communities within temperate shelf habitats. Given the threatened status of many elasmobranchs in human-impacted marine environments, eDNA analysis is poised to provide key information on their diversity and distributions to inform conservation-focussed monitoring and management.

## 2.1 Introduction

Globally, elasmobranch diversity is threatened by human activities, and particularly by unsustainable fisheries. They are the most at-risk vertebrate class, after amphibians, with over one third threatened with extinction (Dulvy et al. 2021; Pacoureau et al. 2021). Specifically, of the 1192 known elasmobranch species (Stein et al. 2018), 64 are considered by the IUCN to be at a high risk of extinction (Critically Endangered or Endangered), with a further 249 considered Vulnerable or Near Threatened (IUCN, 2022). Given the increasing vulnerability of many elasmobranch species to unsustainable harvesting, there is pressing need for management focussed on conservation of these species (Baum et al. 2003, Birkmanis et al. 2020; Pacoureau et al. 2021).

Effective management of elasmobranch species requires information on their distributions and abundance within ecosystems. Areas with high species richness or unique species compositions are often identified as conservation priority areas (Derrick et al. 2020), but the benefit of establishing marine protected areas (MPAs) for mobile or wide-ranging elasmobranch species can be unclear if their abundance cannot be reliably monitored. Non-invasive survey methods capable of capturing multispecies abundance data such as underwater visual censuses and baited remote underwater video (Juhel et al. 2018), as well as satellite imagery (Williamson et al. 2019), may overlook components of the full elasmobranch assemblage. Moreover, capture-based fisheries survey methods can have strong intrinsic vulnerability biases to survey gears (Young et al. 2019), and their use as survey methods are highly impactful and therefore unsuitable for protected areas. Meanwhile, passive acoustic monitoring (e.g. Rider et al. 2021) and satellite tracking of tagged individuals (e.g. Queiroz et al. 2019; Vedor et al. 2021) are capable of providing extremely detailed information on space use but, typically, they can only be used for a small number of individuals within a population. In the case of passive acoustic monitoring, it is only possible within the spatial context of a proximate hydrophone (acoustic receiver) array (Lea et al. 2016).

A potentially efficient and non-invasive method of capturing information on the whole community of elasmobranchs is to study their environmental DNA (eDNA). In the case of marine fishes, eDNA will be derived from multiple sources, including faeces, urine, gametes,

mucous or decomposing tissues. There are two main approaches employed to study the eDNA of fishes. The first approach is to design species-specific quantitative PCR (qPCR) assays for target DNA fragments, which enable the number of copies of that fragment in the eDNA sample to be determined. This can be an effective tool for studies focussed on a small number of species, but it would be challenging to design complementary assays for a whole marine species assemblage. The second approach is to use a metabarcoding approach, where "barcode" regions of multiple species are PCR amplified from eDNA templates, sequenced using high-throughput technologies, and resultant sequences assigned to species using reference databases (Bohmann et al. 2014; Andruszkiewicz et al. 2017; Bista et al. 2017; Deiner et al. 2017). This approach has the advantage of being able to characterise the composition of whole communities, but the precise number of copies of a target DNA of individual species within the eDNA samples are not directly assayed, in part because of differences in amplification efficiencies of primers on different target DNA templates. Hence, metabarcoding methods are broadly considered to be only semi-quantitative approaches to assay target eDNA (Blaboli et. al 2021).

Several studies suggest that the quantity of the eDNA in marine environments, measured either through target DNA copy number (qPCR) or target read number (metabarcoding) can generally reflect the abundance of source individuals in the environment (Salter et al. 2020; Sato et al. 2021; Stoeckle et al. 2021; Rourke et al. 2022). This relationship is often weak, however, due to multiple contributing factors (Lamb et al. 2019). For example, there is evidence that fish breeding behaviour can elevate the amount of eDNA in water (Bylemans et al. 2017), and that the direction and strength currents will influence the detectability at the site of production (Andruszkiewicz et al. 2019). Nevertheless, given then relatively rapid rate of eDNA degradation (Holman et al. 2022), with half-life of eDNA in marine systems ranging from 18.2 to 71.1 hours in seawater (Collins et al. 2018), there is confidence that the locations and times where eDNA is detected is likely to reflect the occurrence of species, and that eDNA quantities measured using qPCR or metabarcode read numbers can at least be partially indicative of fish abundance. Consequently, there is growing advocacy for the use of marine environmental DNA-based methods for routine monitoring, and for the derived data to inform marine management and policy decisions (Gilbey et al. 2021).

To date, work on surveying marine elasmobranch communities using eDNA has primarily focussed on subtropical or tropical environments (Bakker et al. 2017; Boussarie et al. 2018; Laffety et al. 2018; West et al. 2020; Mariani et al. 2021). The ability of eDNA-based methods to detect and monitor abundance of elasmobranch communities in temperate waters has received less attention (see Weltz et al. 2017 for an example). This is notable, because many threatened elasmobranch species are present in heavily-fished temperate waters. In northeast Atlantic shelf seas these include the blue skate *Dipturus batis*, tope *Galeorhinus galeus*, angelshark *Squatina squatina*, undulate ray *Raja undulata*, spiny dogfish *Squalus acanthias* and porbeagle shark *Lamna nasus* (Heessen et al. 2015; Lawson et al. 2020). Therefore, eDNA-based methods could offer a much-needed enhancement of spatial and temporal monitoring practices for elasmobranch communities in temperate seas.

In this study, we used environmental DNA metabarcoding to describe the species composition of the elasmobranch community of the Western English Channel, near Plymouth, United Kingdom. The location was selected because the marine fish community of the region has been well characterised by over a century of survey trawls conducted by the Marine Biological Association of the UK, giving us knowledge of the rarer species in the assemblage, and enabling us to test the ability of eDNA-based methods to detect them. By periodically sampling eDNA from surface and bottom waters at three stations over a full annual cycle, we were able to determine the diversity of species present, and the extent of spatial and temporal variation in the assemblage. The results are discussed with reference to the biology of focal species, and the potential for eDNA-based surveys to inform conservation-focussed marine management.

## 2.2 Materials and Methods

### *2.2.1 Choice of sampling strategy*

To limit contamination on board the vessel, water samples were collected and placed on ice for later sampling within a clean laboratory at the Marine Biological Association in Plymouth. Although some studies sampled large volume of seawater, such as 10 L and 30 L (Westfall et al., 2021; Sanchez et al., 2022), other studies have used 1.5-2 L of seawater to study fish communities (Dugal et al., 2021; McClenaghan et al., 2020). In this case, the use of 2 L of water enabled the efficient collection and processing of samples as part of a regular

monitoring programme. Biological replicates are important to establish the variability across eDNA samples (Taberlet et al., 2018), and here, we used three biological replicates. The pore size of filters can affect sampling efficiency and results, with smaller pore size retaining more DNA but clogging more rapidly (Coster et al., 2021). One recommended sampling strategy is to use 0.22 µm Sterivex-GP filters (Merck Millipore) for a small volume of water, or 1 µm filters for a large volume of water (Taberlet et al., 2018). In our case, we chose to use 0.22 µm filters to maximise yield from our 2L samples. We used the DNeasy PowerWater Kit (Qiagen), as this is a proven reliable method for eDNA extraction from water samples (Tsuji et al., 2019).

*2.2.2 Sampling*

Environmental DNA samples were collected using Niskin bottles from three stations in the Western English Channel (L4, L5, E1; Fig. 1), from both the surface and bottom, approximately once per month from February 2017 to April 2018. Surface water was collected at <1 m from the surface, to avoid detritus directly on the surface, while benthic water was collected close to the substrate without disturbing sediment. Water was first strained through a 250 µm nylon mesh to remove large plankton and debris, before being transferred to Nalgene HDPE collection bottles pre-sterilised with a 10% bleach solution, and the samples were then placed on ice. On each sampling event, triplicate 2 L samples of seawater were collected at each depth. A complete list of samples collected is presented in Supporting Information Table S2.1.

Within five hours of collection, each 2 L sample was filtered through an 0.22 µm Sterivex-GP PES filter (SVGP01050; Merck Millipore,) using a peristaltic pump. Across approximately 10% of events, 2 L of distilled water was taken into the field as a field negative control, and

otherwise treated identically to the seawater samples.

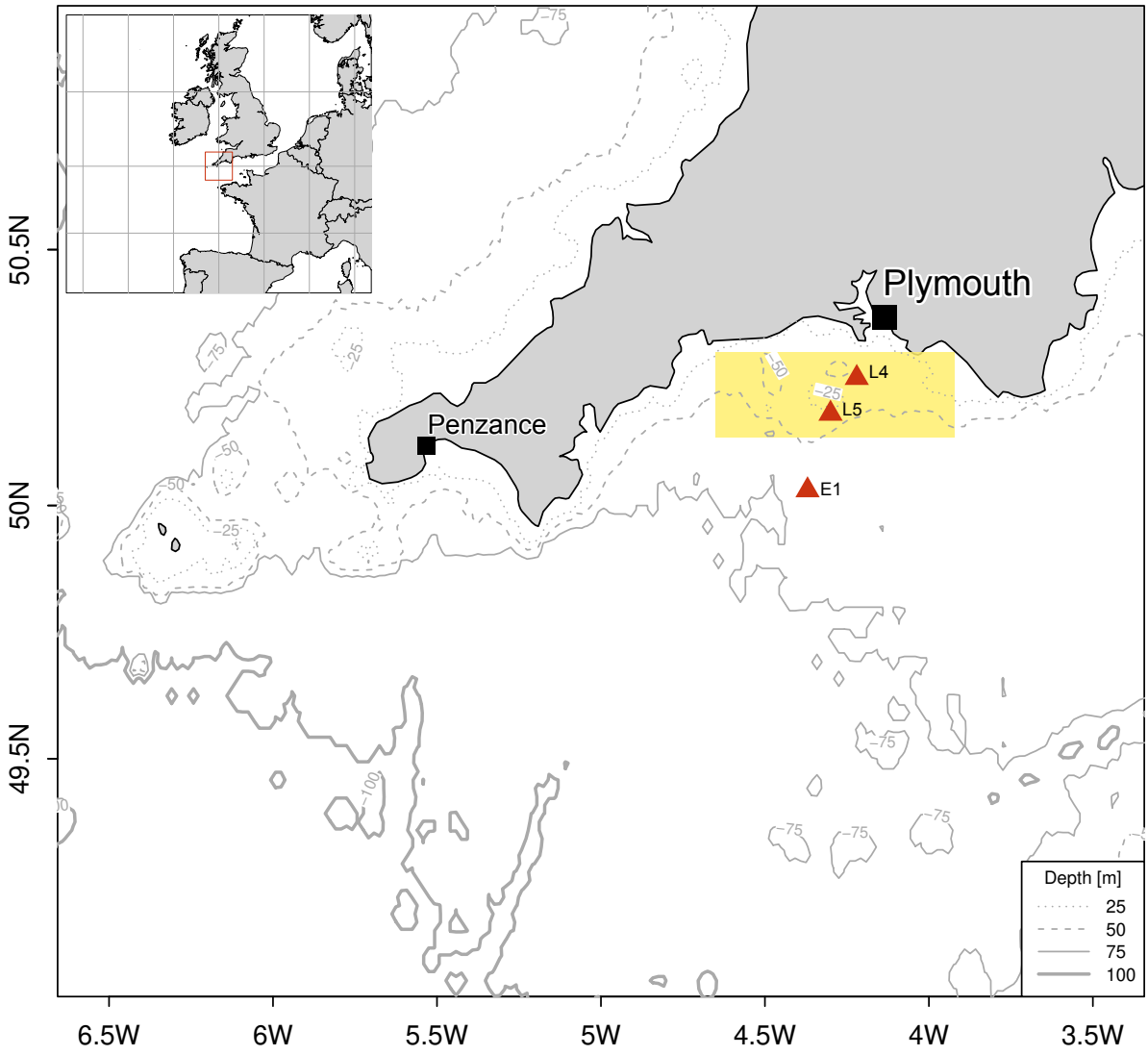



**Figure 2.1.** Sampling stations in the English Channel. eDNA samples were taken from each station (L4, L5 and E1) in surface waters and bottom waters (L4, 50.25°N 4.22°W, 51-56 m depth; L5, 50.18°N 4.30°W, 60-65 m depth; E1, 50.03°N 4.37°W, 70-72 m depth). The yellow box indicates the distribution of the long-term survey trawls conducted by the Marine Biological Association of the UK (50.13 to 50.30°N and 3.92 to 4.65°W).

### *2.2.3 DNA extraction, PCR amplification and Illumina sequencing*

DNA was extracted from filters using the DNeasy PowerWater Kit (Qiagen). The candidate metabarcoding primers were MiFish-U/E primers (Miya et al., 2015) and Elas02 elasmobranch primer pair (Taberlet et al. 2018) which is modified version of MiFish-U/E primers. We first compare the mismatched base pairs in the result of PCR in *silico*. Then, a

list of the PCR efficiencies of these primers against target sequences of species in the regional elasmobranch community, as determined by the decipher v2.22.0 package (Wright, 2016) is provided (Supplementary Information Table S2.2). According to the result, we decided to amplify a ~182 bp fragment of the mitochondrial 12S gene using the Elas02 elasmobranch primer pair (Taberlet et al. 2018). These PCR primers were adapted with unique 8-mer sample-identifying barcode tags identical on both the forward and reverse primer and incorporating 2-4 random 5` bases to increase sequencing heterogeneity. A total of eight PCRs were performed on each extracted eDNA template. Each PCR was in a 20 µL volume comprising: 10 µL AmpliTaq Gold 360 Master Mix (4398876; Applied Biosystems); 0.16 µL Bovine Serum Albumin (B14; ThermoFisher); 1 µL forward primer (5 µM); 1 µL reverse primer (5 µM); 5.84 µL molecular grade water; and 2 µL eDNA template. Thermocycling parameters comprised: polymerase activation at 95 °C for 10 mins; 40 cycles of 95 °C for 30 s, 59 °C for 30 s, 72 °C for 60 s; and a final extension of 72 °C for 7 mins. Alongside the extracted 209 samples (Supporting Information Table S1) we included seven filtration negative controls, eight extraction negative controls, 18 negative no-template PCR controls, and three positive PCR controls using 0.04-9.1 ng genomic DNA extracted from two non-UK species (fin tissues, spinner shark Carcharhinus brevipinna, and rough ray Raja radula) (Supplementary Information Table S2.3). The eDNA extractions, pre-PCR preparations and post-PCR procedures were carried out in separate rooms.

PCR products were checked by gel, and then pooled and purified using the MinElute PCR Purification Kit (28004; Qiagen) following the manufacturer's protocol. Illumina sequencing adapters were attached to the amplicons using the NEXTflex PCR-Free kit (5142-01; PerkinElmer) following the manufacturer's protocol. A total of 3 libraries using unique indexes were created. Libraries were then quantified using a NEBNext (E7630S; New England Biolabs) qPCR assay and sequenced on an Illumina NextSeq using v2 (2 × 150 bp paired-end) chemistry and 10% phiX spike-in. Sequence data are available at the Sequence Read Archive BioProject PRJNA808852.

*2.2.4 Bioinformatic analyses*

Raw sequencing reads were processed using the meta-fish-pipe v1.0 bioinformatics module (https://doi.org/10.5281/zenodo.5083336), following Collins et al. (2019). In brief, the

following steps were carried out: (i) reorientation and demultiplexing of reads using cutadapt v3.4 (Martin, 2011); (ii) denoising, merging, removal of chimaeric reads, and dereplication using dada2 v1.20 (Callahan, 2016); (iii) homology filtering using hmmer v3.1 (Eddy, 1998); (iv) first pass taxonomic assignment using sintax (Edgar, 2016) and NCBI RefSeq v206 reference library obtained using refseq-reflib v1.0 (https://doi.org/10.5281/zenodo.5083346); and (v) exhaustive taxonomic assignment using the meta-fish-lib v243 custom UK fish reference library (Collins et al., 2021), blastn v2.11.0 (https://blast.ncbi.nlm.nih.gov), and EPA-ng v0.3.8 (Barbera et al., 2019). Resulting amplicon sequence variants (ASVs) were cross-referenced with those from concurrent lab projects to control for laboratory contamination, and all non-elasmobranch species were removed for downstream statistical analyses.

*2.2.5 Trawl survey*

Our eDNA collections took place in a marine region where the demersal fish assemblage has been intensively surveyed by the Marine Biological Association of the UK (MBA) since 1911. Hence, we used these quantitative survey data to describe the known elasmobranch of the marine region, enabling us to determine if the prevalence of species in the eDNA sample we collected was broadly reflective of the known prevalence of species in the trawl survey data. Because of the vastly differing timescales of the eDNA and trawl surveys, we do use the trawl survey data to translate eDNA read abundance into metrics of actual fish abundance in the region.

The MBA trawl survey has taken place between 50.13 to 50.30°N and 3.92 to 4.65°W during the years 1911, 1913–14, 1919–22, 1950–58, 1967–79, 1983–94, 2001–10, 2016-2018. For much of this period the focal location has been the L4 sampling site. In total eight vessels have been used for sampling (1911–1919 SS Oithona, 1920–1922 RV Salpa, 1950–1952 RV Sabella, 1952–1973 RV Sula, 1974–2003 RV Squilla, 1979 RV Sarsia, 2004–2015 RV Plymouth Quest, 2015–2018 RV Sepia). Records suggest that the survey trawls have been broadly comparable throughout the series, being conducted at the same speed (ca. 4 knots), with gear of similar dimensions (headline length range, 16.2–19.8 m; groundrope length range, 19.8–27.4 m; main net stretched mesh diameter, 75–270 mm, and all vessels used a fine-mesh cod end or a cod-end cover). We conducted our analyses on the survey years where

elasmobranch individuals were reliably identified to species level in the data, namely 1914, 1919-22, 1953-58, 1976-79, 1983-94, 2001-2018. Within this subset of data, the average number of hauls during sampling years has been 23 (range 1 to 45), and the average duration of each haul has been 49 minutes (range 14 to 180 minutes). Records of smooth-hound *Mustelus mustelus* in the trawl data were considered as starry smooth-hound *Mustelus asterias*, given genetic analyses suggesting all *Mustelus* in this region are starry smooth-hound (Farrell et al. 2009). Average catch per unit effort (CPUE) was calculated as the average catch per hour trawling. Using these same data, the frequency of occurrence of species in hauls was also calculated.

*2.2.6 Analyses of eDNA data*

Community-level analyses of eDNA samples were conducted in R v3.6.2 (R Core Team 2019). We removed all samples where elasmobranchs were absent, leaving 174 of the 209 samples, and two dataframes were generated using data transformations previously proposed as appropriate for eDNA-derived metabarcode data. First, we generated a matrix comprising Wisconsin transformed data (following Kelly et al. 2019), using the "wisconsin" function in vegan v2.5.7 (Oksanen et al. 2020). Second, we generated a matrix comprising Hellinger transformed data (following Laporte et al. 2021), using the "hellinger" function in vegan.

To test for differences among stations, between sampling depth and between sampling months, we used PERMANOVA with the "adonis2" function in vegan, with 10000 permutations. To test for post-hoc differences between sample groups, we used the "pairwise.adonis" function, with 100000 permutations (Martinez Arbizu 2020). To identify species associated with statistically significant differences among sampling stations and depths we used the multilevel patten analysis "multipatt" function in indicspecies v1.7.9 (De Caceres & Legendre 2009) on the transformed data. To ordinate differences among samples, we used Principal Coordinates Analysis implemented with the "pcoa" function in ape v5.0 (Paradis & Schliep 2019) in each of the two matrices, using the resulting primary axes of variation to visualise differences among sampling stations and between sampling months.

To explore seasonal changes in abundance at the species level, we calculated the Hellinger standardised number of reads per sampling month across stations, and generated a heatmap of abundance. Then, again using Hellinger standardised data, we quantified seasonal variation by fitting generalised additive models (GAMs) to the data for the six species that were most abundant in the eDNA metabarcode reads (small spotted catshark *Scyliorhinus canicula*, starry smoothhound *M. asterias*, small-eyed ray *Raja microocellata*, thornback ray *Raja clavata*, spotted ray *Raja montagui* and blonde ray *Raja brachyura*). Models were generated using mgcv v1.8.33 (Wood et al. 2011), using the following predictors: the smooth factor sampling month (k=5), and fixed factors of sampling year, sampling depth and sampling station. Response data were assumed to have a negative-binomial distribution, and models were fitted using the REML smoothing parameter estimation method.

### *2.2.7 Comparison of eDNA data to trawl data*

To compare the total eDNA read abundance across the 209 samples, and the composition of the elasmobranch communities using the trawl surveys (CPUE and frequency of occurrence), we used linear regression. To compare the numbers of species encountered as a function of sample number (either trawl or eDNA) we calculated sample-level species accumulation curves using the "specaccum" function in vegan. We undertook this analysis for all survey trawls across all time periods where individuals in the trawl were identified to species level, and for the 22 survey trawls that took place during the eDNA sampling period (February 2017 to April 2018). Although these 22 survey trawls temporally overlapped with eDNA survey period, they were not conducted on the same days, and since only three elasmobranch species were caught across 22 survey trawls, they were not suitable for use to undertake analyses attempting to calibrate eDNA metabarcode read number against abundance measurements. Finally, we compared the species richness resolved through eDNA and the survey trawls to the diversity to all species of elasmobranchs encountered in proximity to Plymouth (Start Point in Devon to Looe in Cornwall, southward to the outer Channel grounds) using the Plymouth Marine Fauna (Marine Biological Association, 1957).

## 2.3 Results

### *2.3.1 eDNA metabarcoding*

In total, 209 samples from the three locations (L4: 66 samples, E1: 71 samples, L5: 72 samples) were collected during 2017-2018. A total of 161,183,652 raw sequencing reads were generated for the three libraries across the samples and controls. After quality-control filtering and taxonomic assignment, 58,684,923 reads were remaining (Supplementary Information Table S2.4). Then, following removal of non-elasmobranch and control species from samples, a total of 38,615,907 reads were assigned to native elasmobranch species within our 209 samples.

### *2.3.2 Contamination in negative and positive controls*

Contamination of elasmobranch species in field, extraction and PCR controls was typically very low (<150 reads, Supplementary Information Figure S2.1), although one field negative control contained a high number of reads (1511) of small-spotted catshark (Scyliorhinus canicula) - a common species in the sampling area. This abnormal sample was removed in Supplementary Information Figure S2.1. Most negatives had a very low number of reads compared to the large number of reads we recovered from standard field samples. Notably our positive controls contained non-target species. We suspect this may be contamination of tissue used for DNA extraction, as the tissue samples may have been handled using non-sterile instruments at the time of collection. Therefore, positive controls were not considered to have provided meaningful information.

### *2.3.3 Community composition*

In total 13 species were recovered in the eDNA samples from 2017-2018, and included seven sharks and six skate species (Fig. 2.2a). Shark species were thresher shark (*Alopias vulpinus*), tope, small-spotted catshark, nursehound (*Scyliorhinus stellaris*), starry smooth-hound, spiny dogfish and porbeagle shark. Skate species recovered were spotted ray, thornback ray, small-eyed ray, blonde ray, cuckoo ray (*Leucoraja naevus*) and undulate ray (Fig. 2.2a). By contrast only three elasmobranch species were encountered in survey trawls that took place during the 2017-2018 sampling eDNA sampling period, specifically small-spotted catshark, spotted ray and thornback ray.

In total, in the 1037 survey trawls of the region between 1914 and 2018 that we analysed, 14 taxa have been recorded, including 12 of the 13 species recovered in the eDNA. The only species present in the eDNA but absent in the trawl survey was thresher shark. Meanwhile, the taxa absent from eDNA, but present in long-term trawl time series were shagreen ray (*Leucoraja fullonica*) and angelshark. These two taxa were extremely uncommon in the trawl survey; shagreen ray (captured in three survey hauls in 1921) and angelshark (captured in two survey trawls, one in 1921 and one in 1957). Catch per unit effort of taxa in trawl surveys of the region between 1914 and 2018 was significantly positively associated with the numbers of eDNA reads recovered in the 2017-2018 sampling across the 209 eDNA samples (linear model, n = 15, $F_{1,13} = 7.258$, P = 0.035, $r^2 = 0.298$, Fig. 2.2b), but frequency of occurrence across within the trawl surveys was not significantly associated with the numbers of eDNA reads, n = 15, $F_{1,13} = 4.493$, P = 0.054, $r^2 = 0.257$, Fig. 2.2c).

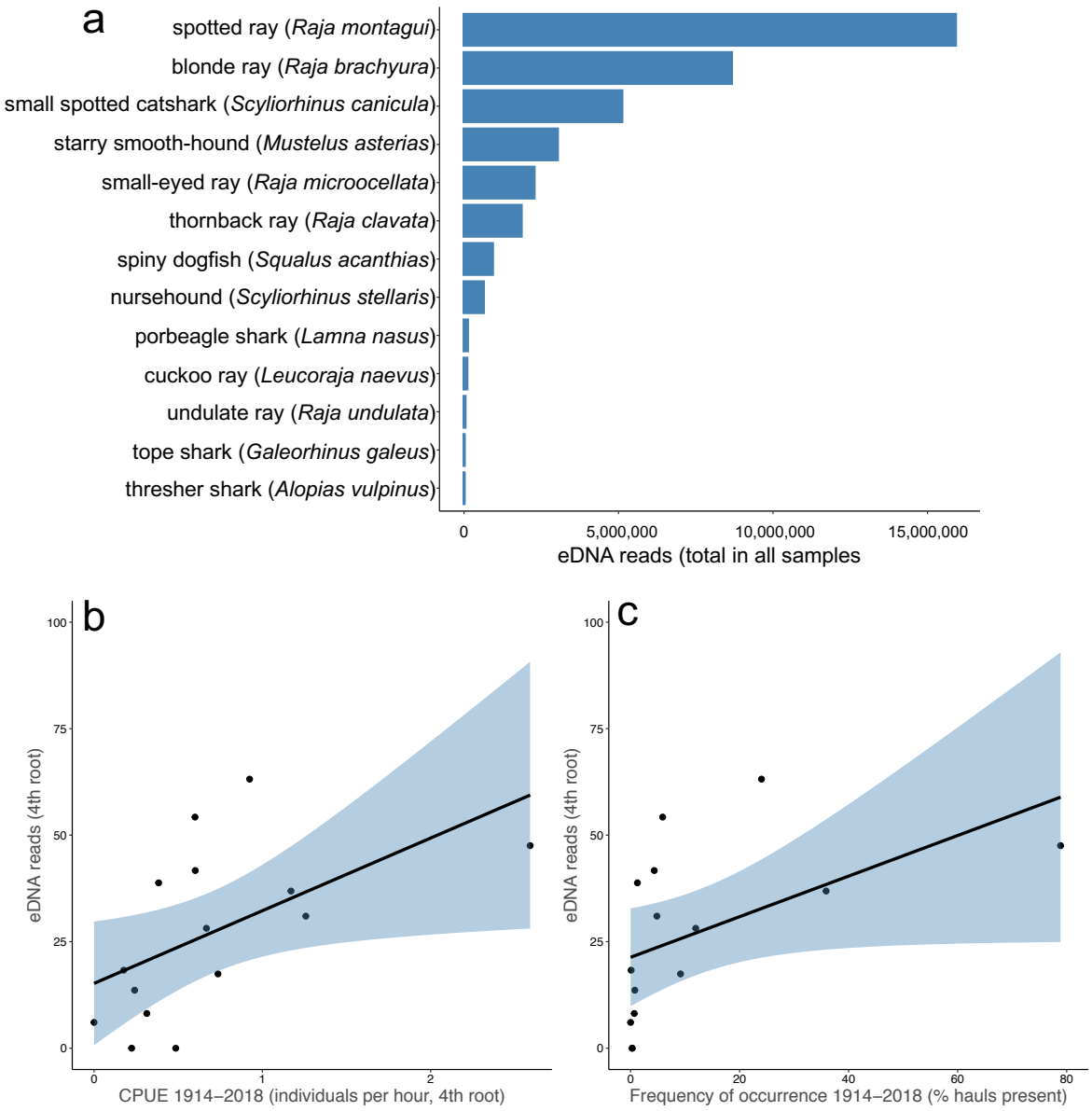



**Figure 2.2.** a) Total read abundance of the 13 species of elasmobranch identified across all 209 field samples. b) Association between the number of eDNA reads in samples (y-axis, 4th root transformed) and the catch per unit effort of 16 taxa recovered in survey hauls (1911-2018). c) Association between the number of eDNA reads in samples (y-axis, 4th root transformed) and the frequency of occurrence of 16 taxa recovered in survey hauls (1911-2018).

### *2.3.4 Spatial and temporal patterns of community structure*

Overall, there were significant differences among sampling stations, and between sampling depths (Fig. 2.3; Table 2.1). In post-hoc comparisons, the spatial differences were most

striking between the inshore site L4 and the two sites further offshore (L5 and E1), while there was no evidence of any spatial differentiation between L5 and E1 (Fig. 2.3; Table 2.2). From indicator analyses, we found that the only significant differences among sites corresponded only to a greater abundance of small-eyed ray and nursehound at L4 relative to L5 and E1. (Supplementary Information Table S2.5). Indicator analyses provided no evidence of significant abundance differences between the sampling depths (Supporting Information Table S2.5).

We found highly significant differences in community structure among sampling months (Table 2.1; Fig. 2.5a). Focussing on the primary axes of variation in the Principal Coordinates Analysis (PCoA1 or PCoA2), a seasonal cyclic pattern was present irrespective of data transformation method (Fig. 2.4). Exploring temporal changes in eDNA abundance using GAM models indicated strong differences among species in seasonal read abundance, and evidence of significant seasonal (between month) variation was present in four of the six species we considered (Supplementary Information Table S2.6). Specifically, small spotted catshark showed read abundance peaks in March-May, thornback ray peaked in September-November, and small-eyed ray and starry smooth-hound peaked in November-April (Fig. 2.5b-g). We found no significant seasonal (between month) variation in blonde ray or spotted ray. Temporal differences were also apparent in reads of rarer species, with porbeagle shark, thresher shark and tope shark present in July and November, while spiny dogfish was present between October and April (Fig. 2.5a).

**Table 2.1.** Statistical significance of differences in elasmobranch community structure from eDNA reads among sampling stations, between sampling depths and among sampling months, as resolved using PERMANOVA.

| Data | Factor | *Df* | *SS* | $r^2$ | *F* | *P* |
|---|---|---|---|---|---|---|
| Wisconsin transformed | Station | 2 | 1.308 | 0.0193 | 2.264 | **0.006** |
| | Depth | 1 | 0.622 | 0.009 | 2.152 | **0.035** |
| | Month | 9 | 12.439 | 0.183 | 4.784 | **< 0.001** |
| | Station * Depth | 2 | 0.403 | 0.006 | 0.698 | 0.809 |
| | Station * Month | 15 | 10.465 | 0.154 | 2.415 | **< 0.001** |
| | Depth * Month | 9 | 2.853 | 0.042 | 1.097 | 0.278 |
| | Station * Depth * Month | 14 | 4.746 | 0.070 | 1.173 | 0.115 |
| | Residual | 121 | 34.961 | 0.512 | | |
| | Total | 173 | 67.797 | 1 | | |
| Hellinger transformed | Station | 2 | 1.002 | 0.017 | 1.993 | **0.024** |
| | Depth | 1 | 0.541 | 0.009 | 2.154 | **0.042** |
| | Month | 9 | 11.817 | 0.197 | 5.226 | **< 0.001** |
| | Station * Depth | 2 | 0.344 | 0.006 | 0.684 | 0.799 |
| | Station * Month | 15 | 8.944 | 0.149 | 2.373 | **< 0.001** |
| | Depth * Month | 9 | 2.614 | 0.044 | 1.156 | 0.196 |
| | Station * Depth * Month | 14 | 4.199 | 0.070 | 1.194 | 0.107 |
| | Residual | 121 | 30.403 | 0.508 | | |
| | Total | 173 | 59.864 | 1 | | |

**Table 2.2.** Statistical significance of differences in elasmobranch community structure from eDNA reads between pairs of sampling stations, using pairwise PERMANOVA.

| Dataset | Site | *F* | $r^2$ | *P* |
|---|---|---|---|---|
| Wisconsin | L4 vs E1 | 2.496 | 0.021 | **0.013** |
| | L4 vs. L5 | 2.120 | 0.018 | **0.033** |
| | E1 vs L5 | 0.309 | 0.003 | 0.973 |
| Hellinger | L4 vs E1 | 2.201 | 0.019 | **0.036** |
| | L4vs. L5 | 1.938 | 0.016 | 0.064 |
| | L5 vs L1 | 0.152 | 0.001 | 0.998 |

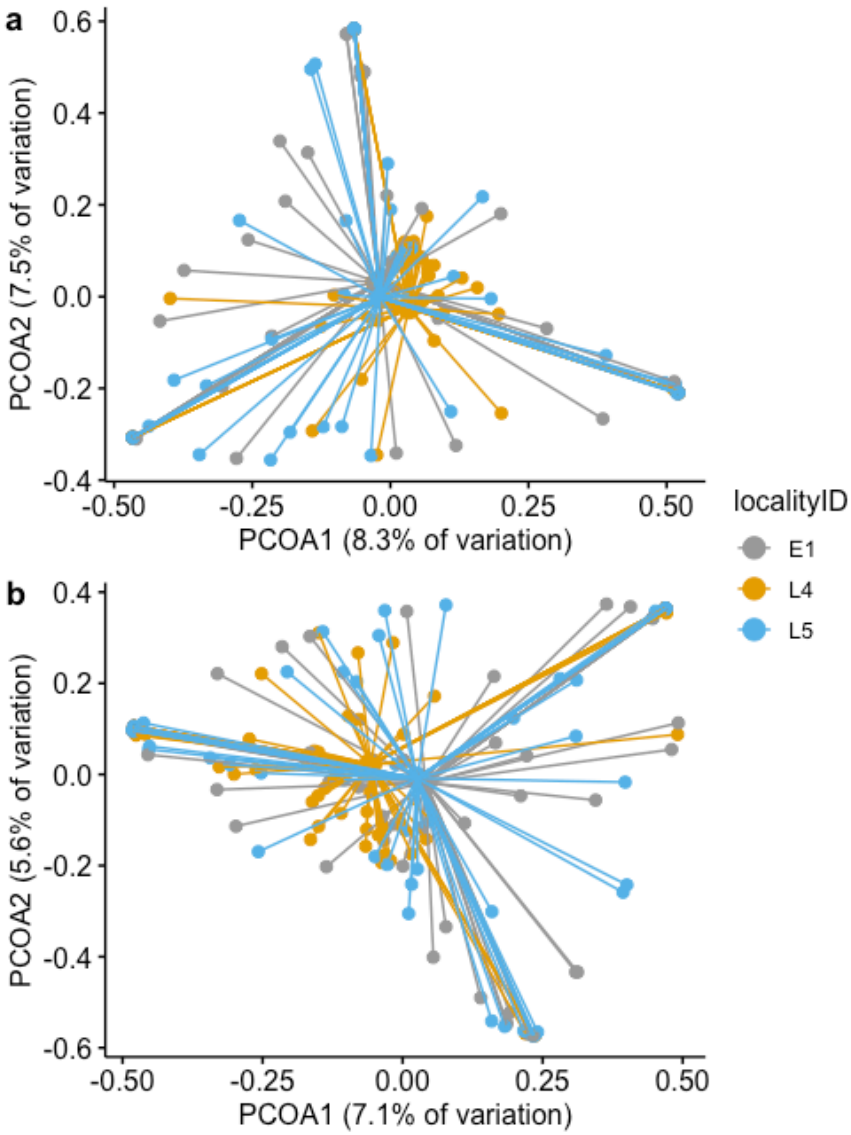



**Figure 2.3.** Differences in elasmobranch community structure between the three sampling stations as resolved from environmental DNA reads. a) Wisconsin-transformed data, b) Hellinger-transformed data.

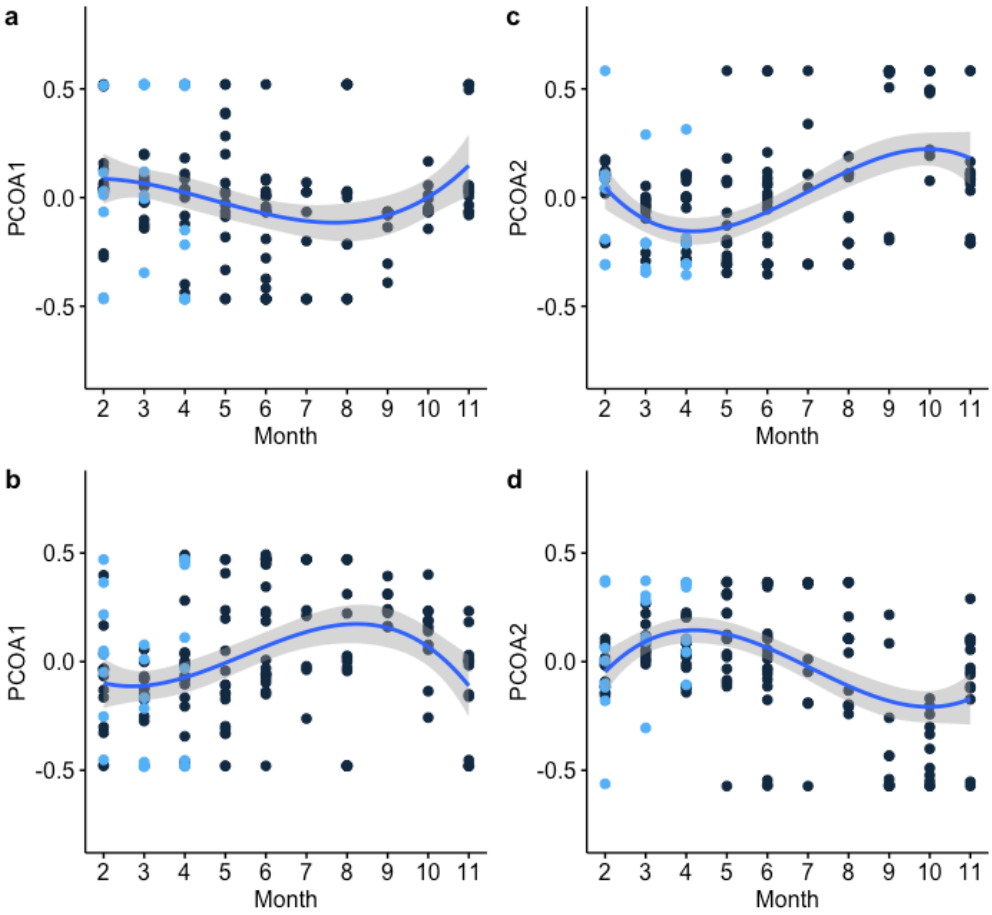



**Figure 2.4.** Principal Coordinates Analysis scores arranged by sampling month, during 2017 (dark blue) and 2018 (light blue). Illustrated are the scores from the primary axes of variation PCoA1(a-b) and PCoA2 (c-d), for each data transformation Wisconsin standardised (a,c), Hellinger standardised (b,d). Superimposed are 3rd order polynomial curves with the shaded area illustrating one standard error. Variance captured by each PCoA axis is reported in Figure 2.3.

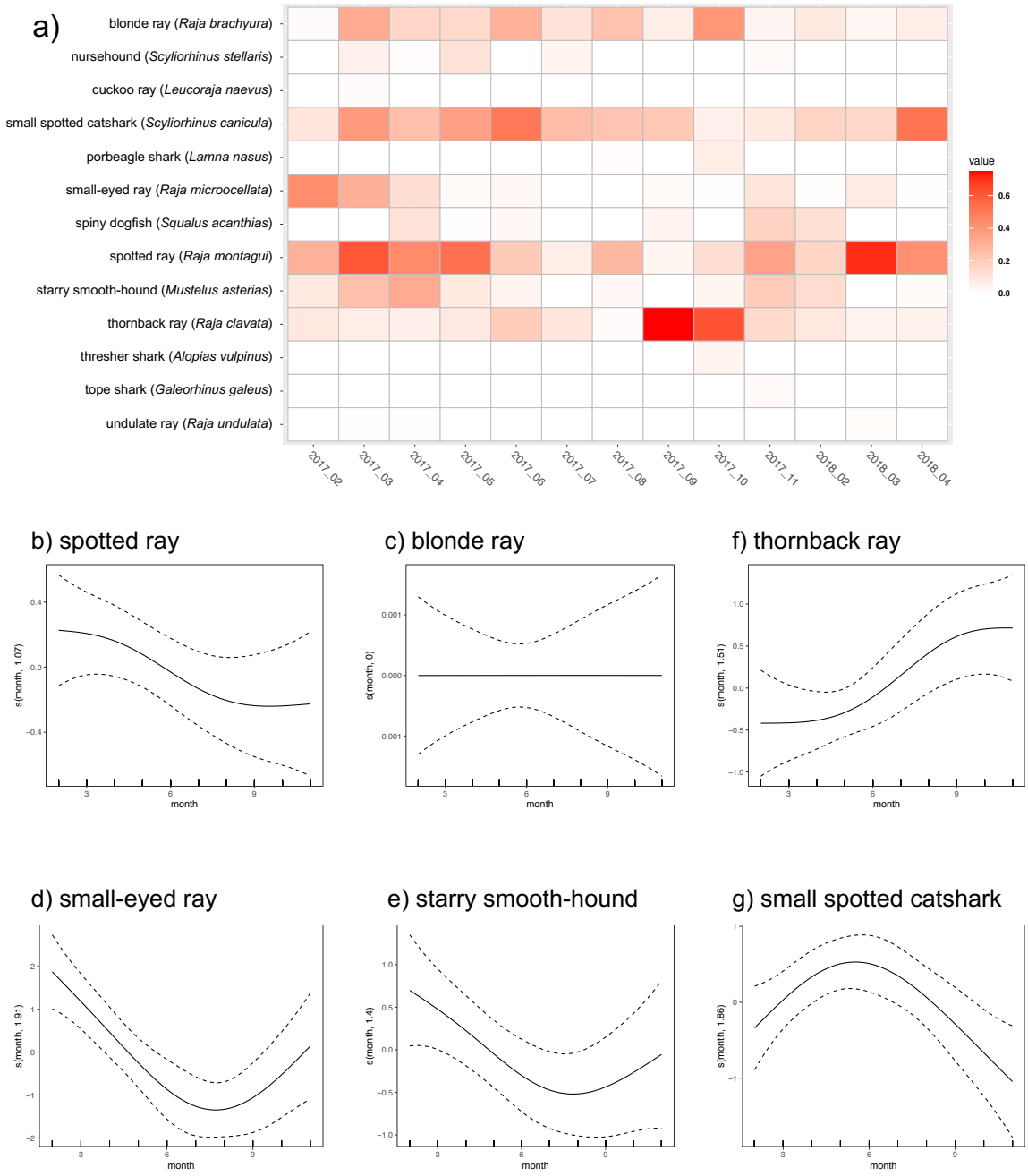



**Figure 2.5.** a) Reads per sample per sampled month (Hellinger transformed) between February 2017 (2017_02) and April 2018 (2018_04). b-g) Generalised additive model plots illustrating associations of Hellinger transformed species-level read abundance (standardised, y-axis) in relation to sampling month (x-axis).

## 2.4 Discussion

This study provides strong evidence of the ability of eDNA-based methods to generate information on the structure of both pelagic and demersal components of a temperate marine elasmobranch assemblage. In total, eDNA samples collected monthly over one year, detected nearly all species recovered in demersal trawl surveys over a century-long scale. The exceptions were species that are typically rare in the region, and have only been caught by trawl surveys on a small number of occasions. We did not aim to calibrate eDNA as tool for fully quantitative assessment of elasmobranch communities. Such a validation would require a very substantial amount of trawl effort, as the 22 trawls undertaken over the same timescale as the eDNA sampling caught individuals of only three species. Nevertheless, it was notable that the total number of reads we recovered of species was significantly positively associated with measures of abundance in the region derived from the full trawl survey data collected over the previous century. Thus, our results support the concept that eDNA metabarcoding can provide semi-quantitative information that may help to map distributions and primary habitat of elasmobranch species across marine regions. This is of importance, as current knowledge of elasmobranch assemblages is often based on visual or capture-based survey methods that can be strongly biassed towards the species that are more abundant, more easily captured and/or less cryptic (Boussarie et al. 2018).

### *2.4.1 Spatial structure*

Spatial differences in the abundance of elasmobranchs can be explained by differences in core ecological niches of the species (Humphries et al. 2016), as well as vulnerability to local fisheries (Brander 1981). We found evidence of significant differences in the spatial distribution of elasmobranch species, over a spatial scale of 30 km. Contrasts were most apparent between the inshore site (L4), relative to offshore sites (L5, E1), with the inshore sites being characterised by a greater abundance of small-eyed ray and nursehound in particular. This is supportive of eDNA being capable of resolving some of the fine-scale differences in depth and substrate preferences of the UK elasmobranch fauna, that have previously been resolved through trawl surveys (Kaiser et al. 2004) and tracking of tagged individuals (e.g. Kaiser et al 2004; Humphries et al. 2016; Simpson et al. 2018).

In marine systems, eDNA has been able to resolve spatial differences in fish community structure over small (<5 km; Jeunen et al. 2018; West et al. 2020), moderate (20-100 km; Lafferty et al. 2020; Mariani et al. 2021) and broader spatial scales (>100 km; Sigsgaard et al. 2020; Fraija-Fernandez et al. 2020; Holman et al. 2021; Valdivia-Carrillo et al. 2021; West et al. 2021). However, the extent of resolution is likely to depend partly on the degree of eDNA transport, which in turn depends on both the rates of eDNA persistence and the amount of horizontal advection of the eDNA (Andruszkiewicz et al. 2019). Therefore, the observed heterogeneity in spatial patterns of eDNA abundance may be related to oceanographic differences among the three sampling locations, as well as the relative rates of eDNA persistence at locations. Notably, all locations have similar oceanographic properties, including seasonal stratification and seasonal nutrient profiles (Smyth et al. 2010). Nevertheless, there are some differences linked to the proximity of L4 to the coast, most notably seasonal surface freshening, linked to freshwater input (Smyth et al. 2010). Thus, L4 may be more heavily influenced by eDNA from proximate shallow water inshore sites than the further offshore L5 and E1, potentially explaining some differences in eDNA composition. Experimental work around Plymouth has indicated eDNA to be detectable for around 48 hours (Collins et al. 2018), however the rate of decay was 1.6x faster at inshore waters (Sutton Harbour in Plymouth Sound) than the offshore waters (E1). Therefore, offshore sites may be more homogenous, perhaps due to longer eDNA persistence providing more opportunity for mixing.

Differences in marine communities resolved using eDNA-based methods over depth gradients have been reported, for example Jeunen et al. (2020) who studied a depth-temperature-salinity gradient in a New Zealand fjord, and Canals et al. (2021) who studied a 2000m open ocean depth gradient, encompassing both the epipelagic and mesopelagic zones. By contrast we found no clear-cut differences between elasmobranch communities resolved using eDNA from the surface and bottom waters over a distance of 50 m, which may be related to the mobility of the focal species. Several of the shark species are pelagic and therefore have the capability to move rapidly between surface and bottom waters and indeed have been tracked doing so in the southwest UK region (e.g. porbeagle; Pade et al. 2009). Moreover, benthic species, including the thornback ray, blonde ray, spotted ray and small eyed ray undertake diel vertical migrations from deep benthic habitat during the day

to shallow benthic habitat during the night (Humphries et al. 2017). However, it may also simply reflect intrinsic mixing of waters in the sampling region. Irrespective of the causes, our results are notable as they suggest reliable information on shelf sea pelagic, benthopelagic and benthic faunas may be sourced from eDNA collected from surface waters of temperate shelf seas, even when those species do not have pelagic larval dispersal phases.

### *2.4.2 Temporal structure*

Seasonal shifts in fish community structure have been reported using marine and estuarine eDNA (Stoeckle *et al.* 2017; Djurhuus *et al.* 2020; Stoeckle *et al.* 2021). Similarly, our data were characterised by temporal shifts in read abundance, likely linked to seasonal differences in habitat use. Seasonal occurrence of pelagic shark species in our data can be explained by seasonal migration. Porbeagle shark were present in our data in October, and Biais *et al.* (2017) report movements of satellite-tagged porbeagle northwards into UK waters during warmer summer-autumn months, before moving further north and west in autumn-winter and returning to southerly waters of the Iberian Peninsula during the coldest winter periods. Thresher shark was recorded in the eDNA in October, and these are typically recorded in UK waters in summer months (Stevens 1976). Populations on the western Atlantic undertake north-south migrations, being further north in summer-autumn, and returning south in winter-spring (Kneebone *et al.* 2020). Spiny dogfish were recorded in eDNA in November and February. This is a species known to spend winter in the Western English Channel and move northwards into the more northerly European shelf waters during summer (Vince 1991). We also found the starry smooth-hound to be most abundant in the winter months. Tagging studies have shown this is a highly migratory species, and consistent with our results, it has a general pattern of overwintering in the English Channel and Bay of Biscay, where pupping takes place, before spending summer months in the North Sea (Brevé *et al.* 2016; Brevé *et al.* 2020; Griffiths *et al.* 2020).

Benthic shark and skate species tended to be more consistently present in the data throughout the year, but with some seasonal peaks in abundance. For these species, it is less clear if migrations can explain the variation observed, as although seasonal migrations in benthic elasmobranchs are known (e.g. thornback ray; Hunter et al. 2006), most tagged

individuals exhibit strong philopatry, being recaptured within 50 km of the immediate release site (Rodriguez-Cabello *et al.* 2004; Bird *et al.* 2020; Simpson *et al*. 2020). Thus, if seasonal migrations do explain the variation, then they must be reflecting more modest within-region shifts in habitat use, perhaps related to reproduction. This explanation is supported by the movement patterns of species and within-species sexes recorded by long-term acoustic tracking of skates in the Western English Channel off Plymouth (Simpson *et al*. 2021). Equally, it is possible that eDNA abundance is linked to activity levels (de Souza *et al.* 2016; Thalinger *et al.* 2021), perhaps associated with reproduction. For example, we found a peak in abundance of the small spotted catshark during late spring (March-May), corresponding to peak breeding season in Plymouth in April (Sumpter & Dodd 1979). The spotted ray, small-eyed ray and thornback ray all showed peak eDNA abundances in late winter and spring, during which time these species have been recorded to start egg laying in northern European waters (Clark 1922; Holden 1975; Koop 2005).

*2.4.3 Detection of threatened species.*

Of the 13 species recovered within eDNA reads, several are rare species of particular conservation concern. Tope shark is listed as Critically Endangered by the IUCN, and was recovered in one eDNA sample (total 4404 reads). This is a benthopelagic species that is widespread in the Eastern Atlantic, undertakes long migrations throughout the region (Holden & Horrod 1979), but is rarely caught in trawl surveys (Heeseen et al. 2015). In MBA survey trawls they have been encountered in seven sampling events between 1921 and 2005, consistent with occasional presence in the region. The undulate ray was also present in multiple eDNA samples yet was represented by a relatively low number of reads (total 34254 reads) in comparison to other skate species. The undulate ray is IUCN listed as Endangered, and in northern European waters is abundant in parts of the English Channel away from Plymouth (e.g. Jersey), at depths less than 100 m (Heeseen et al. 2015). In Plymouth, the species was historically “not uncommon” at depths of “20 m or more S. of Eddystone” (Marine Biological Association, 1957), but has only rarely been caught in MBA survey hauls, consistent with the species having been sporadically present, but also suggesting our sampled region is not core habitat for the species.

Notably, the eDNA analyses failed to record two species that historically have been encountered in MBA survey trawls, but not during recent decades. The first species is the shagreen ray, which is most abundant in deep water trawls >70 m (Heessen et al. 2015), so may be expected to be absent from our sampling locations. The second species is the angelshark, listed as Critically Endangered by the IUCN. This is a species that was formerly common in Plymouth waters (Marine Biological Association 1957), but last sampled in an MBA trawl survey in 1957. The absence of angelshark in our eDNA samples is compatible with records suggesting the species is now extirpated from the English Channel region, with the nearest extant population in inshore waters at Cardigan Bay, West Wales (Hiddink et al. 2019; Ellis et al. 2020).

Collectively, our results show that eDNA metabarcoding can reliably capture the diversity of the proximate elasmobranch assemblage. By contrast trawl surveys undertaken over the same timescale were only able to capture a small number of species, most likely due the intrinsic rarity of most elasmobranch species in the survey area. Trawl surveys are the most commonly used – and destructive – methods to survey fish assemblages in temperate seas. We have shown that eDNA-based methods have potential to reveal part of the assemblage that would otherwise be unrepresented within contemporary surveys (Fig. 6). However, there are additional species that have historically been recorded within Plymouth waters that were not encountered in eDNA or survey trawls, and these species remain as "dark diversity" (following Boussarie et al. 2018), In the Western English Channel dark diversity would include pelagic species such as basking shark *Cetorhinus maximus* and blue shark *Prionace glauca*, as well as benthic feeding species that have been occasionally recorded, such as common eagle ray *Myliobatis aquila*, common stingray *Dasyatis pastinaca* and blue skate *Dipturus batis* (Fig. 6). Although most plausibly the absence of these species from our results is that they are either absent or intrinsically rare in the year we sampled (e.g. in contrast, basking shark were common off Plymouth between 1995 and 2006; Sims 2008), there remains the additional possibility that their ability to be detected may in part be related to volumes of water sampled, number of PCR replicates, depth of sequencing, or technical aspects of the assay, for example primer matching and bioinformatic filters (Diaz-Ferguson and Moyer 2014, Pilliod et al. 2014).

To conclude, this study has shown the ability of eDNA to illuminate the species richness of a temperate elasmobranch community, and its spatial and temporal structure, which fits with expectations from habitat features and species life histories. The results suggest that eDNA could be used for mapping and routine monitoring of elasmobranch assemblages, enabling semi-quantitative assessments of the effectiveness of marine management objectives. Further refinement of methodological aspects, especially pertaining to eDNA transport and the associations between eDNA metabarcoding read number and organismal abundance, will play a major part in facilitating the transition of eDNA monitoring from emerging tool to established practice in marine science.

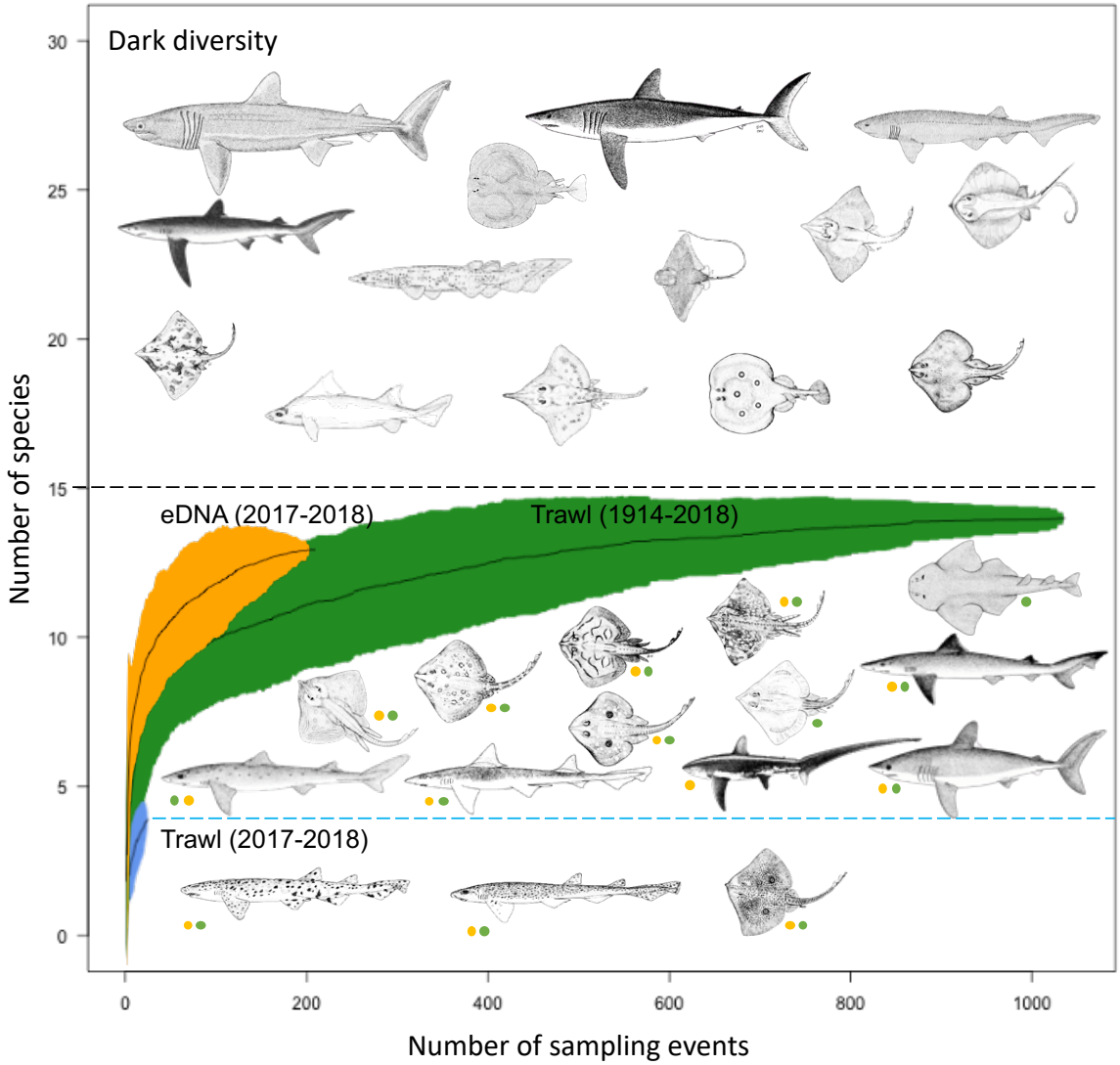
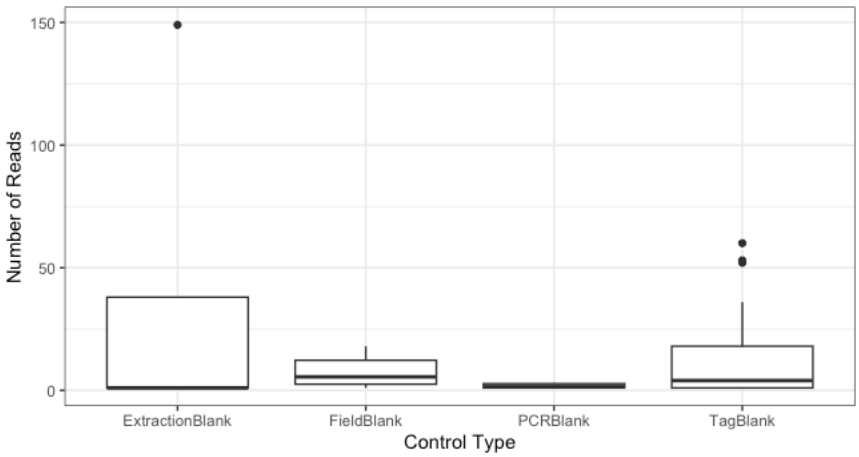



**Figure 2.6.** The elasmobranch community of the Western English Channel near Plymouth. Shown are a rarefied sampling curves for species found in the 2017-2018 eDNA survey (orange, total 13 species, 209 sampling events), for species caught in the trawl survey from 1914-2018 (green, total 14 species, 1037 sampling events), and for species caught in the trawl survey only during 2017-2018 (blue, total 3 species, 22 sampling events). Orange circles indicate present in eDNA, green circles indicate present in survey trawls. Also shown is the "dark diversity" (sensu Boussarie et al. 2018), which are species that have been recorded in the Western English Channel near Plymouth (Marine Biological Association, 1957), but were not recovered in either trawl records analysed or eDNA (14 species). Images from FAO, and are not to scale.

**Supporting Information**




**Supplementary Information Figure S 2.1.** Distribution of read counts of elasmobranch species in four types of negative controls.

**Supplementary Information Table S 2.1.** Sampling details.

| eventID | Hash | Day | month | year | localityID | Depth (1 surface, 2 bottom) |
|---|---|---|---|---|---|---|
| MBA-2017.02.08-L4-01 | 10feb54092e5 | 8 | 2 | 2017 | L4 | 1 |
| MBA-2017.02.08-L4-01 | 2d77a3f8f750 | 8 | 2 | 2017 | L4 | 1 |
| MBA-2017.02.08-L4-01 | 48177879cee9 | 8 | 2 | 2017 | L4 | 1 |
| MBA-2017.02.08-L4-02 | 74f8552fa706 | 8 | 2 | 2017 | L4 | 2 |
| MBA-2017.02.08-L4-02 | dafdc803afcd | 8 | 2 | 2017 | L4 | 2 |
| MBA-2017.02.08-L4-02 | dc622c208d71 | 8 | 2 | 2017 | L4 | 2 |
| MBA-2017.02.16-E1-01 | a6ae7a42ca58 | 16 | 2 | 2017 | E1 | 1 |
| MBA-2017.02.16-E1-01 | e3c92698d23e | 16 | 2 | 2017 | E1 | 1 |
| MBA-2017.02.16-E1-01 | ebe659236e82 | 16 | 2 | 2017 | E1 | 1 |
| MBA-2017.02.16-E1-02 | 4e9b0848d7c1 | 16 | 2 | 2017 | E1 | 2 |
| MBA-2017.02.16-E1-02 | c3d4b02ef16d | 16 | 2 | 2017 | E1 | 2 |
| MBA-2017.02.16-E1-02 | f510d0e8b37c | 16 | 2 | 2017 | E1 | 2 |
| MBA-2017.02.16-L5-01 | 3aa0948a6502 | 16 | 2 | 2017 | L5 | 1 |
| MBA-2017.02.16-L5-01 | fe26f9a74110 | 16 | 2 | 2017 | L5 | 1 |
| MBA-2017.02.16-L5-02 | 052622ba66cf | 16 | 2 | 2017 | L5 | 2 |
| MBA-2017.02.16-L5-02 | 94165942b14e | 16 | 2 | 2017 | L5 | 2 |
| MBA-2017.02.16-L5-02 | d687846d7da2 | 16 | 2 | 2017 | L5 | 2 |
| MBA-2017.03.09-L4-01 | 6692561e6349 | 9 | 3 | 2017 | L4 | 1 |
| MBA-2017.03.09-L4-01 | b0fda0e2fbdc | 9 | 3 | 2017 | L4 | 1 |
| MBA-2017.03.09-L4-01 | e989cf802ccc | 9 | 3 | 2017 | L4 | 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| MBA-2017.03.09-L4-02 | 37afdf9dfb8d | 9 | 3 | 2017 | L4 | 2 |
| MBA-2017.03.09-L4-02 | d888614e5b50 | 9 | 3 | 2017 | L4 | 2 |
| MBA-2017.03.09-L4-02 | f32ed90a44e5 | 9 | 3 | 2017 | L4 | 2 |
| MBA-2017.03.15-L4-01 | 1c05135337e9 | 15 | 3 | 2017 | L4 | 1 |
| MBA-2017.03.15-L4-01 | 2671901b3533 | 15 | 3 | 2017 | L4 | 1 |
| MBA-2017.03.15-L4-01 | 54950f0590b2 | 15 | 3 | 2017 | L4 | 1 |
| MBA-2017.03.15-L4-02 | 973c49d9ce4a | 15 | 3 | 2017 | L4 | 2 |
| MBA-2017.03.15-L4-02 | bf426834f432 | 15 | 3 | 2017 | L4 | 2 |
| MBA-2017.03.15-L4-02 | e71a641d4f89 | 15 | 3 | 2017 | L4 | 2 |
| MBA-2017.04.05-L4-01 | 08519a65fe17 | 5 | 4 | 2017 | L4 | 1 |
| MBA-2017.04.05-L4-01 | 21a68e8db4ca | 5 | 4 | 2017 | L4 | 1 |
| MBA-2017.04.05-L4-01 | 787fb7ed1a65 | 5 | 4 | 2017 | L4 | 1 |
| MBA-2017.04.05-L4-02 | 2.9135E+11 | 5 | 4 | 2017 | L4 | 2 |
| MBA-2017.04.05-L4-02 | 96f76a357c64 | 5 | 4 | 2017 | L4 | 2 |
| MBA-2017.04.05-L4-02 | dab919cd0d8d | 5 | 4 | 2017 | L4 | 2 |
| MBA-2017.04.10-E1-01 | 4c6e3481944b | 10 | 4 | 2017 | E1 | 1 |
| MBA-2017.04.10-E1-01 | 8fc43abfd1db | 10 | 4 | 2017 | E1 | 1 |
| MBA-2017.04.10-E1-01 | d88f4d17729d | 10 | 4 | 2017 | E1 | 1 |
| MBA-2017.04.10-E1-02 | 3151dc671a35 | 10 | 4 | 2017 | E1 | 2 |
| MBA-2017.04.10-E1-02 | 5a38338dbc33 | 10 | 4 | 2017 | E1 | 2 |
| MBA-2017.04.10-E1-02 | c4568aa1f29d | 10 | 4 | 2017 | E1 | 2 |
| MBA-2017.04.10-L5-01 | 7037be5c3afc | 10 | 4 | 2017 | L5 | 1 |
| MBA-2017.04.10-L5-01 | f1e65ed26083 | 10 | 4 | 2017 | L5 | 1 |
| MBA-2017.04.10-L5-01 | f453ee84c7fc | 10 | 4 | 2017 | L5 | 1 |
| MBA-2017.04.10-L5-02 | 1499748262d9 | 10 | 4 | 2017 | L5 | 2 |
| MBA-2017.04.10-L5-02 | 5cef05e94f77 | 10 | 4 | 2017 | L5 | 2 |
| MBA-2017.04.10-L5-02 | 70f4f25a0ac4 | 10 | 4 | 2017 | L5 | 2 |
| MBA-2017.05.18-L4-01 | 7f5e17e7c77c | 18 | 5 | 2017 | L4 | 1 |
| MBA-2017.05.18-L4-01 | b27ca8a13af4 | 18 | 5 | 2017 | L4 | 1 |
| MBA-2017.05.18-L4-01 | f4f5070698ea | 18 | 5 | 2017 | L4 | 1 |
| MBA-2017.05.18-L4-02 | 00ca64b6fbd5 | 18 | 5 | 2017 | L4 | 2 |
| MBA-2017.05.18-L4-02 | 10e5beae5994 | 18 | 5 | 2017 | L4 | 2 |
| MBA-2017.05.18-L4-02 | fffc65898254 | 18 | 5 | 2017 | L4 | 2 |
| MBA-2017.05.31-E1-01 | 0cfb6dede470 | 31 | 5 | 2017 | E1 | 1 |
| MBA-2017.05.31-E1-01 | d80fe015bc52 | 31 | 5 | 2017 | E1 | 1 |
| MBA-2017.05.31-E1-01 | d89f9a9aea5f | 31 | 5 | 2017 | E1 | 1 |
| MBA-2017.05.31-E1-02 | 9fca939a008a | 31 | 5 | 2017 | E1 | 2 |
| MBA-2017.05.31-E1-02 | ad7d365f88eb | 31 | 5 | 2017 | E1 | 2 |
| MBA-2017.05.31-E1-02 | fa3bda207699 | 31 | 5 | 2017 | E1 | 2 |
| MBA-2017.05.31-L5-01 | 5bc4b15698e1 | 31 | 5 | 2017 | L5 | 1 |
| MBA-2017.05.31-L5-01 | 6d3fb92a1b90 | 31 | 5 | 2017 | L5 | 1 |
| MBA-2017.05.31-L5-01 | bb2dc8d784fd | 31 | 5 | 2017 | L5 | 1 |
| MBA-2017.05.31-L5-02 | 70baeb363d96 | 31 | 5 | 2017 | L5 | 2 |
| MBA-2017.05.31-L5-02 | f2cc49f0a73a | 31 | 5 | 2017 | L5 | 2 |
| MBA-2017.05.31-L5-02 | f9fa9b11ce23 | 31 | 5 | 2017 | L5 | 2 |

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|---|---|---|---|---|---|---|
| MBA-2017.06.13-E1-01 | 4e19647a733e | 13 | 6 | 2017 | E1 | 1 |
| MBA-2017.06.13-E1-01 | 5d8d638daea4 | 13 | 6 | 2017 | E1 | 1 |
| MBA-2017.06.13-E1-01 | 672e9506b3f8 | 13 | 6 | 2017 | E1 | 1 |
| MBA-2017.06.13-E1-02 | 076089d69fb0 | 13 | 6 | 2017 | E1 | 2 |
| MBA-2017.06.13-E1-02 | 82da3e078169 | 13 | 6 | 2017 | E1 | 2 |
| MBA-2017.06.13-E1-02 | a917676e2602 | 13 | 6 | 2017 | E1 | 2 |
| MBA-2017.06.13-L5-01 | 064fedfe4e8d | 13 | 6 | 2017 | L5 | 1 |
| MBA-2017.06.13-L5-01 | 0bc0e7918fff | 13 | 6 | 2017 | L5 | 1 |
| MBA-2017.06.13-L5-01 | 415de3e17757 | 13 | 6 | 2017 | L5 | 1 |
| MBA-2017.06.13-L5-02 | 2b2824ab07f2 | 13 | 6 | 2017 | L5 | 2 |
| MBA-2017.06.13-L5-02 | 746858b3dfbc | 13 | 6 | 2017 | L5 | 2 |
| MBA-2017.06.13-L5-02 | e67e454121ee | 13 | 6 | 2017 | L5 | 2 |
| MBA-2017.06.15-L4-01 | 6623c72087ec | 15 | 6 | 2017 | L4 | 1 |
| MBA-2017.06.15-L4-01 | c4190a944231 | 15 | 6 | 2017 | L4 | 1 |
| MBA-2017.06.15-L4-01 | dcebd98d86a1 | 15 | 6 | 2017 | L4 | 1 |
| MBA-2017.06.15-L4-02 | 5470e9f509f2 | 15 | 6 | 2017 | L4 | 2 |
| MBA-2017.06.15-L4-02 | 71c497293b63 | 15 | 6 | 2017 | L4 | 2 |
| MBA-2017.06.15-L4-02 | 96096f3f383a | 15 | 6 | 2017 | L4 | 2 |
| MBA-2017.06.28-L4-01 | 4850b99d91d6 | 28 | 6 | 2017 | L4 | 1 |
| MBA-2017.06.28-L4-01 | 4e29dd2d2881 | 28 | 6 | 2017 | L4 | 1 |
| MBA-2017.06.28-L4-01 | 85e0ba22dbb8 | 28 | 6 | 2017 | L4 | 1 |
| MBA-2017.06.28-L4-02 | 4f2afb529791 | 28 | 6 | 2017 | L4 | 2 |
| MBA-2017.06.28-L4-02 | 709e3e46c958 | 28 | 6 | 2017 | L4 | 2 |
| MBA-2017.06.28-L4-02 | 9a640edbc7fa | 28 | 6 | 2017 | L4 | 2 |
| MBA-2017.07.13-E1-01 | 3cb1b7b00de2 | 13 | 7 | 2017 | E1 | 1 |
| MBA-2017.07.13-E1-01 | d35e48294443 | 13 | 7 | 2017 | E1 | 1 |
| MBA-2017.07.13-E1-01 | f1906ce0686e | 13 | 7 | 2017 | E1 | 1 |
| MBA-2017.07.13-E1-02 | 095948237c31 | 13 | 7 | 2017 | E1 | 2 |
| MBA-2017.07.13-E1-02 | 6582a2cf3bdf | 13 | 7 | 2017 | E1 | 2 |
| MBA-2017.07.13-E1-02 | af7b640a84c3 | 13 | 7 | 2017 | E1 | 2 |
| MBA-2017.07.13-L5-01 | 14a1b4b54353 | 13 | 7 | 2017 | L5 | 1 |
| MBA-2017.07.13-L5-01 | 5e5dc5dd4ddd | 13 | 7 | 2017 | L5 | 1 |
| MBA-2017.07.13-L5-01 | ad224de7b7a9 | 13 | 7 | 2017 | L5 | 1 |
| MBA-2017.07.13-L5-02 | 2dc767694460 | 13 | 7 | 2017 | L5 | 2 |
| MBA-2017.07.13-L5-02 | 58f37cb5e156 | 13 | 7 | 2017 | L5 | 2 |
| MBA-2017.07.13-L5-02 | 8da271b216c9 | 13 | 7 | 2017 | L5 | 2 |
| MBA-2017.07.17-L4-01 | 135cf96525e2 | 17 | 7 | 2017 | L4 | 1 |
| MBA-2017.07.17-L4-01 | 2cbaf3dc09e3 | 17 | 7 | 2017 | L4 | 1 |
| MBA-2017.07.17-L4-01 | beb1e34f69c0 | 17 | 7 | 2017 | L4 | 1 |
| MBA-2017.07.17-L4-02 | 1d38ddca84b4 | 17 | 7 | 2017 | L4 | 2 |
| MBA-2017.07.17-L4-02 | c277a82520ad | 17 | 7 | 2017 | L4 | 2 |
| MBA-2017.07.17-L4-02 | cb1496f6bf69 | 17 | 7 | 2017 | L4 | 2 |
| MBA-2017.08.08-L4-01 | 34d00bf8deda | 8 | 8 | 2017 | L4 | 1 |
| MBA-2017.08.08-L4-01 | 38c1876c23a5 | 8 | 8 | 2017 | L4 | 1 |
| MBA-2017.08.08-L4-01 | 3d7ecb3569f3 | 8 | 8 | 2017 | L4 | 1 |

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|---|---|---|---|---|---|---|
| MBA-2017.08.08-L4-02 | 595b66336f5f | 8 | 8 | 2017 | L4 | 2 |
| MBA-2017.08.08-L4-02 | 8ac6a3510fba | 8 | 8 | 2017 | L4 | 2 |
| MBA-2017.08.08-L4-02 | e880e77d68af | 8 | 8 | 2017 | L4 | 2 |
| MBA-2017.08.24-E1-01 | 594a672e8198 | 24 | 8 | 2017 | E1 | 1 |
| MBA-2017.08.24-E1-01 | a39c20816fc0 | 24 | 8 | 2017 | E1 | 1 |
| MBA-2017.08.24-E1-01 | c7dbd6d16bf2 | 24 | 8 | 2017 | E1 | 1 |
| MBA-2017.08.24-E1-02 | 5899736c48ec | 24 | 8 | 2017 | E1 | 2 |
| MBA-2017.08.24-E1-02 | b9b5a3209d1c | 24 | 8 | 2017 | E1 | 2 |
| MBA-2017.08.24-E1-02 | c96e116e2121 | 24 | 8 | 2017 | E1 | 2 |
| MBA-2017.08.24-L5-01 | 187b2d8e7cb0 | 24 | 8 | 2017 | L5 | 1 |
| MBA-2017.08.24-L5-01 | 53f5c8474689 | 24 | 8 | 2017 | L5 | 1 |
| MBA-2017.08.24-L5-01 | ba728af55ee7 | 24 | 8 | 2017 | L5 | 1 |
| MBA-2017.08.24-L5-02 | 2.22445E+11 | 24 | 8 | 2017 | L5 | 2 |
| MBA-2017.08.24-L5-02 | 444b6f5011e2 | 24 | 8 | 2017 | L5 | 2 |
| MBA-2017.08.24-L5-02 | 9c837d5d62bf | 24 | 8 | 2017 | L5 | 2 |
| MBA-2017.08.30-L4-01 | 814a32ca16dc | 30 | 8 | 2017 | L4 | 1 |
| MBA-2017.08.30-L4-01 | 94a680e575a7 | 30 | 8 | 2017 | L4 | 1 |
| MBA-2017.08.30-L4-01 | e87679ac7388 | 30 | 8 | 2017 | L4 | 1 |
| MBA-2017.08.30-L4-02 | 25febdb27e82 | 30 | 8 | 2017 | L4 | 2 |
| MBA-2017.08.30-L4-02 | 4dcb4a1bef96 | 30 | 8 | 2017 | L4 | 2 |
| MBA-2017.08.30-L4-02 | f80a2d679931 | 30 | 8 | 2017 | L4 | 2 |
| MBA-2017.09.26-E1-01 | 074436b966b1 | 26 | 9 | 2017 | E1 | 1 |
| MBA-2017.09.26-E1-01 | 257fa2a15e96 | 26 | 9 | 2017 | E1 | 1 |
| MBA-2017.09.26-E1-01 | 494d366cff7f | 26 | 9 | 2017 | E1 | 1 |
| MBA-2017.09.26-E1-02 | 6b88fe3aeb6a | 26 | 9 | 2017 | E1 | 2 |
| MBA-2017.09.26-E1-02 | 9d09459ed5f4 | 26 | 9 | 2017 | E1 | 2 |
| MBA-2017.09.26-E1-02 | d6959d26f0c3 | 26 | 9 | 2017 | E1 | 2 |
| MBA-2017.09.26-L5-01 | 0f07f6e0a443 | 26 | 9 | 2017 | L5 | 1 |
| MBA-2017.09.26-L5-01 | 17bf6168cdc8 | 26 | 9 | 2017 | L5 | 1 |
| MBA-2017.09.26-L5-01 | f4e267ff7977 | 26 | 9 | 2017 | L5 | 1 |
| MBA-2017.09.26-L5-02 | 43440922c605 | 26 | 9 | 2017 | L5 | 2 |
| MBA-2017.09.26-L5-02 | 6eed3907650b | 26 | 9 | 2017 | L5 | 2 |
| MBA-2017.09.26-L5-02 | bacef6ba05aa | 26 | 9 | 2017 | L5 | 2 |
| MBA-2017.10.31-E1-01 | 320b4512c318 | 31 | 10 | 2017 | E1 | 1 |
| MBA-2017.10.31-E1-01 | 58c187999051 | 31 | 10 | 2017 | E1 | 1 |
| MBA-2017.10.31-E1-01 | e983ae6e3816 | 31 | 10 | 2017 | E1 | 1 |
| MBA-2017.10.31-E1-02 | c1f5ca41f365 | 31 | 10 | 2017 | E1 | 2 |
| MBA-2017.10.31-E1-02 | df90038ef041 | 31 | 10 | 2017 | E1 | 2 |
| MBA-2017.10.31-E1-02 | e50ced061475 | 31 | 10 | 2017 | E1 | 2 |
| MBA-2017.10.31-L5-01 | 13c7bd00af3b | 31 | 10 | 2017 | L5 | 1 |
| MBA-2017.10.31-L5-01 | 521667f47bc8 | 31 | 10 | 2017 | L5 | 1 |
| MBA-2017.10.31-L5-01 | 8320b5d42aa8 | 31 | 10 | 2017 | L5 | 1 |
| MBA-2017.10.31-L5-02 | 5eafcdbdb4dd | 31 | 10 | 2017 | L5 | 2 |
| MBA-2017.10.31-L5-02 | c393bb094a09 | 31 | 10 | 2017 | L5 | 2 |
| MBA-2017.10.31-L5-02 | ebe7f574b16e | 31 | 10 | 2017 | L5 | 2 |

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|---|---|---|---|---|---|---|
| MBA-2017.11.15-L4-01 | 335db24247fa | 15 | 11 | 2017 | L4 | 1 |
| MBA-2017.11.15-L4-01 | 842f3718a2d0 | 15 | 11 | 2017 | L4 | 1 |
| MBA-2017.11.15-L4-01 | fee03aab8790 | 15 | 11 | 2017 | L4 | 1 |
| MBA-2017.11.15-L4-02 | 02cd8b3f60e6 | 15 | 11 | 2017 | L4 | 2 |
| MBA-2017.11.15-L4-02 | 23b55e598540 | 15 | 11 | 2017 | L4 | 2 |
| MBA-2017.11.15-L4-02 | a2ebe5ca9cfe | 15 | 11 | 2017 | L4 | 2 |
| MBA-2017.11.16-E1-01 | 464ad117d041 | 16 | 11 | 2017 | E1 | 1 |
| MBA-2017.11.16-E1-01 | bad80291c6ea | 16 | 11 | 2017 | E1 | 1 |
| MBA-2017.11.16-E1-01 | c4ea688a82d6 | 16 | 11 | 2017 | E1 | 1 |
| MBA-2017.11.16-E1-02 | 76a4571dca65 | 16 | 11 | 2017 | E1 | 2 |
| MBA-2017.11.16-E1-02 | 79ed6a1ae077 | 16 | 11 | 2017 | E1 | 2 |
| MBA-2017.11.16-E1-02 | c54faa79ad66 | 16 | 11 | 2017 | E1 | 2 |
| MBA-2017.11.16-L5-01 | 672fc9cca204 | 16 | 11 | 2017 | L5 | 1 |
| MBA-2017.11.16-L5-01 | 747c63dc6373 | 16 | 11 | 2017 | L5 | 1 |
| MBA-2017.11.16-L5-01 | c2f3a44ccc50 | 16 | 11 | 2017 | L5 | 1 |
| MBA-2017.11.16-L5-02 | 1978aeb0ea20 | 16 | 11 | 2017 | L5 | 2 |
| MBA-2017.11.16-L5-02 | 1d98d5cca356 | 16 | 11 | 2017 | L5 | 2 |
| MBA-2017.11.16-L5-02 | b5aa48666725 | 16 | 11 | 2017 | L5 | 2 |
| MBA-2018.02.06-E1-01 | 0b2804995719 | 6 | 2 | 2018 | E1 | 1 |
| MBA-2018.02.06-E1-01 | 8d87a46471bd | 6 | 2 | 2018 | E1 | 1 |
| MBA-2018.02.06-E1-01 | bf5e37b49bd6 | 6 | 2 | 2018 | E1 | 1 |
| MBA-2018.02.06-E1-02 | 1c8e981a0df1 | 6 | 2 | 2018 | E1 | 2 |
| MBA-2018.02.06-E1-02 | 2039c3256623 | 6 | 2 | 2018 | E1 | 2 |
| MBA-2018.02.06-E1-02 | b2e2c990349e | 6 | 2 | 2018 | E1 | 2 |
| MBA-2018.02.06-L5-01 | 171efd5f5b08 | 6 | 2 | 2018 | L5 | 1 |
| MBA-2018.02.06-L5-01 | 3f577eaeecd8 | 6 | 2 | 2018 | L5 | 1 |
| MBA-2018.02.06-L5-01 | f97d6ba29b2f | 6 | 2 | 2018 | L5 | 1 |
| MBA-2018.02.06-L5-02 | 349bde58afe0 | 6 | 2 | 2018 | L5 | 2 |
| MBA-2018.02.06-L5-02 | 8ac282b4af47 | 6 | 2 | 2018 | L5 | 2 |
| MBA-2018.02.06-L5-02 | 8c13384a545e | 6 | 2 | 2018 | L5 | 2 |
| MBA-2018.03.21-E1-01 | 65d2732868d8 | 21 | 3 | 2018 | E1 | 1 |
| MBA-2018.03.21-E1-01 | 88c5051954a5 | 21 | 3 | 2018 | E1 | 1 |
| MBA-2018.03.21-E1-01 | 92424cf4f7df | 21 | 3 | 2018 | E1 | 1 |
| MBA-2018.03.21-E1-02 | 2b9c8cc5923d | 21 | 3 | 2018 | E1 | 2 |
| MBA-2018.03.21-E1-02 | c854746fe16e | 21 | 3 | 2018 | E1 | 2 |
| MBA-2018.03.21-E1-02 | e6d876c5558b | 21 | 3 | 2018 | E1 | 2 |
| MBA-2018.03.21-L5-01 | 8b394246f1c6 | 21 | 3 | 2018 | L5 | 1 |
| MBA-2018.03.21-L5-01 | c438bfa80a70 | 21 | 3 | 2018 | L5 | 1 |
| MBA-2018.03.21-L5-01 | f669734d4c01 | 21 | 3 | 2018 | L5 | 1 |
| MBA-2018.03.21-L5-02 | 00c47aec34c5 | 21 | 3 | 2018 | L5 | 2 |
| MBA-2018.03.21-L5-02 | 61c18b47ad7b | 21 | 3 | 2018 | L5 | 2 |
| MBA-2018.03.21-L5-02 | d99ca0c1e650 | 21 | 3 | 2018 | L5 | 2 |
| MBA-2018.04.20-E1-01 | 9edbe5c8dbc8 | 20 | 4 | 2018 | E1 | 1 |
| MBA-2018.04.20-E1-01 | d1c9e86c7322 | 20 | 4 | 2018 | E1 | 1 |
| MBA-2018.04.20-E1-01 | e22eb7f0e7cb | 20 | 4 | 2018 | E1 | 1 |

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|---|---|---|---|---|---|---|
| MBA-2018.04.20-E1-02 | 5bbca3d07f04 | 20 | 4 | 2018 | E1 | 2 |
| MBA-2018.04.20-E1-02 | cf1c98110c8c | 20 | 4 | 2018 | E1 | 2 |
| MBA-2018.04.20-E1-02 | efa47e0d6d25 | 20 | 4 | 2018 | E1 | 2 |
| MBA-2018.04.20-L5-01 | 03c3358d6531 | 20 | 4 | 2018 | L5 | 1 |
| MBA-2018.04.20-L5-01 | d62de314115a | 20 | 4 | 2018 | L5 | 1 |
| MBA-2018.04.20-L5-01 | d6af75cb7a11 | 20 | 4 | 2018 | L5 | 1 |
| MBA-2018.04.20-L5-02 | 101e8f1f268e | 20 | 4 | 2018 | L5 | 2 |
| MBA-2018.04.20-L5-02 | 46333114ee0d | 20 | 4 | 2018 | L5 | 2 |
| MBA-2018.04.20-L5-02 | b5c8448a5714 | 20 | 4 | 2018 | L5 | 2 |

**Supplementary Information Table S 2.2.** Primer efficiencies by fish species, and total reads recovered of all fish species.

| class | order | family | genus | species | efficiency | totalReads |
|---|---|---|---|---|---|---|
| Elasmobranchii | Carcharhiniformes | Carcharhinidae | Carcharhinus | Carcharhinus brevipinna | NA | 1164 |
| Elasmobranchii | Carcharhiniformes | Scyliorhinidae | Scyliorhinus | Scyliorhinus canicula | 0.927 | 5109711 |
| Elasmobranchii | Carcharhiniformes | Scyliorhinidae | Scyliorhinus | Scyliorhinus stellaris | 0.927 | 627547 |
| Elasmobranchii | Carcharhiniformes | Triakidae | Galeorhinus | Galeorhinus galeus | 0.886 | 8808 |
| Elasmobranchii | Carcharhiniformes | Triakidae | Mustelus | Mustelus asterias | 0.927 | 3023966 |
| Elasmobranchii | Lamniformes | Alopiidae | Alopias | Alopias vulpinus | 0.927 | 1344 |
| Elasmobranchii | Lamniformes | Lamnidae | Lamna | Lamna nasus | 0.927 | 112405 |
| Elasmobranchii | Rajiformes | Rajidae | Leucoraja | Leucoraja naevus | 0.927 | 92391 |
| Elasmobranchii | Rajiformes | Rajidae | Raja | Raja brachyura | 0.927 | 8657938 |
| Elasmobranchii | Rajiformes | Rajidae | Raja | Raja clavata | 0.927 | 1852230 |
| Elasmobranchii | Rajiformes | Rajidae | Raja | Raja microocellata | 0.927 | 2269319 |
| Elasmobranchii | Rajiformes | Rajidae | Raja | Raja montagui | 0.927 | 15907891 |
| Elasmobranchii | Rajiformes | Rajidae | Raja | Raja radula | NA | 39403 |
| Elasmobranchii | Rajiformes | Rajidae | Raja | Raja undulata | 0.927 | 34254 |
| Elasmobranchii | Squaliformes | Squalidae | Squalus | Squalus acanthias | 0.927 | 922507 |
| Actinopterygii | Beloniformes | Belonidae | Belone | Belone belone | 0.048 | 754359 |
| Actinopterygii | Clupeiformes | Clupeidae | Clupea | Clupea harengus | 0.049 | 77464 |
| Actinopterygii | Clupeiformes | Clupeidae | Sardina | Sardina pilchardus | 0.049 | 2226182 |
| Actinopterygii | Clupeiformes | Clupeidae | Sprattus | Sprattus sprattus | 0.048 | 8482070 |
| Actinopterygii | Clupeiformes | Engraulidae | Engraulis | Engraulis encrasicolus | 0.048 | 21592 |
| Actinopterygii | Gadiformes | Gadidae | Gadus | Gadus morhua | 0.048 | 22547 |
| Actinopterygii | Gadiformes | Gadidae | Melanogrammus | Melanogrammus aeglefinus | 0.048 | 84 |
| Actinopterygii | Gadiformes | Gadidae | Merlangius | Merlangius merlangus | 0.048 | 313162 |
| Actinopterygii | Gadiformes | Gadidae | Pollachius | Pollachius pollachius | 0.048 | 14799 |
| Actinopterygii | Gadiformes | Gadidae | Raniceps | Raniceps raninus | NA | 4 |

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|---|---|---|---|---|---|---|
| Actinopterygii | Gadiformes | Gadidae | Trisopterus | Trisopterus luscus | 0.048 | 113125 |
| Actinopterygii | Gadiformes | Gadidae | Trisopterus | Trisopterus minutus | 0.048 | 392184 |
| Actinopterygii | Gadiformes | Lotidae | Ciliata | Ciliata septentrionalis | 0.048 | 546 |
| Actinopterygii | Gadiformes | Lotidae | Molva | Molva molva | NA | 1542 |
| Actinopterygii | Gadiformes | Merlucciidae | Merluccius | Merluccius merluccius | 0.035 | 43 |
| Actinopterygii | Gobiesociformes | Gobiesocidae | Diplecogaster | Diplecogaster bimaculata | 0.049 | 685 |
| Actinopterygii | Lophiiformes | Lophiidae | Lophius | Lophius piscatorius | 0.015 | 67 |
| Actinopterygii | Mugiliformes | Mugilidae | Chelon | Chelon labrosus | 0.048 | 1 |
| Actinopterygii | Perciformes | Ammodytidae | Ammodytes | Ammodytes marinus | 0.048 | 6882 |
| Actinopterygii | Perciformes | Ammodytidae | Ammodytes | Ammodytes tobianus | 0.048 | 1348839 |
| Actinopterygii | Perciformes | Callionymidae | Callionymus | Callionymus lyra | NA | 3892 |
| Actinopterygii | Perciformes | Callionymidae | Callionymus | Callionymus maculatus | NA | 677 |
| Actinopterygii | Perciformes | Carangidae | Trachurus | Trachurus trachurus | 0.048 | 17809 |
| Actinopterygii | Perciformes | Cepolidae | Cepola | Cepola macrophthalma | 0.048 | 12838 |
| Actinopterygii | Perciformes | Gobiidae | Aphia | Aphia minuta | 0.048 | 634 |
| Actinopterygii | Perciformes | Gobiidae | Crystallogobius | Crystallogobius linearis | 0.016 | 6 |
| Actinopterygii | Perciformes | Gobiidae | Gobius | Gobius niger | 0.018 | 13 |
| Actinopterygii | Perciformes | Gobiidae | Gobius | Gobius paganellus | 0.048 | 3 |
| Actinopterygii | Perciformes | Gobiidae | Pomatoschistus | Pomatoschistus minutus | 0.048 | 747 |
| Actinopterygii | Perciformes | Labridae | Centrolabrus | Centrolabrus exoletus | 0.132 | 1355 |
| Actinopterygii | Perciformes | Labridae | Ctenolabrus | Ctenolabrus rupestris | 0.132 | 1195 |
| Actinopterygii | Perciformes | Labridae | Labrus | Labrus bergylta | 0.132 | 2820 |
| Actinopterygii | Perciformes | Labridae | Labrus | Labrus mixtus | 0.132 | 10694 |
| Actinopterygii | Perciformes | Moronidae | Dicentrarchus | Dicentrarchus labrax | 0.132 | 16875 |
| Actinopterygii | Perciformes | Mullidae | Mullus | Mullus surmuletus | 0.048 | 5785 |
| Actinopterygii | Perciformes | Scombridae | Scomber | Scomber scombrus | 0.048 | 70017 |
| Actinopterygii | Perciformes | Scombridae | Thunnus | Thunnus thynnus | 0.048 | 52 |
| Actinopterygii | Pleuronectiformes | Bothidae | Arnoglossus | Arnoglossus laterna | 0.024 | 102 |
| Actinopterygii | Pleuronectiformes | Pleuronectidae | Glyptocephalus | Glyptocephalus cynoglossus | 0.048 | 75 |

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|---|---|---|---|---|---|---|
| Actinopterygii | Pleuronectiformes | Pleuronectidae | Hippoglossoides | Hippoglossoides platessoides | 0.048 | 5 |
| Actinopterygii | Pleuronectiformes | Pleuronectidae | Limanda | Limanda limanda | 0.048 | 18095 |
| Actinopterygii | Pleuronectiformes | Pleuronectidae | Microstomus | Microstomus kitt | 0.048 | 17776 |
| Actinopterygii | Pleuronectiformes | Pleuronectidae | Platichthys | Platichthys flesus | 0.048 | 1367 |
| Actinopterygii | Pleuronectiformes | Pleuronectidae | Pleuronectes | Pleuronectes platessa | 0.048 | 111505 |
| Actinopterygii | Pleuronectiformes | Scophthalmidae | Phrynorhombus | Phrynorhombus norvegicus | 0.048 | 12029 |
| Actinopterygii | Pleuronectiformes | Scophthalmidae | Scophthalmus | Scophthalmus maximus | 0.048 | 801740 |
| Actinopterygii | Pleuronectiformes | Scophthalmidae | Scophthalmus | Scophthalmus rhombus | 0.048 | 497247 |
| Actinopterygii | Pleuronectiformes | Scophthalmidae | Zeugopterus | Zeugopterus punctatus | 0.048 | 7563 |
| Actinopterygii | Pleuronectiformes | Soleidae | Buglossidium | Buglossidium luteum | 0.048 | 4321 |
| Actinopterygii | Pleuronectiformes | Soleidae | Microchirus | Microchirus variegatus | 0.048 | 37165 |
| Actinopterygii | Pleuronectiformes | Soleidae | Solea | Solea solea | 0.048 | 2897 |
| Actinopterygii | Salmoniformes | Salmonidae | Oncorhynchus | Oncorhynchus mykiss | 0.055 | 4 |
| Actinopterygii | Salmoniformes | Salmonidae | Salmo | Salmo trutta | 0.048 | 237 |
| Actinopterygii | Scorpaeniformes | Cottidae | Micrenophrys | Micrenophrys lilljeborgii | 0.048 | 5 |
| Actinopterygii | Scorpaeniformes | Cottidae | Taurulus | Taurulus bubalis | 0.048 | 126 |
| Actinopterygii | Scorpaeniformes | Triglidae | Chelidonichthys | Chelidonichthys cuculus | 0.048 | 204401 |
| Actinopterygii | Scorpaeniformes | Triglidae | Chelidonichthys | Chelidonichthys lastoviza | 0.048 | 454 |
| Actinopterygii | Syngnathiformes | Syngnathidae | Syngnathus | Syngnathus acus | 0.048 | 552 |
| Actinopterygii | Zeiformes | Zeidae | Zeus | Zeus faber | 0.048 | 2513 |

**Supplementary Information Table S 2.3.** Summary of reads from extraction, field, PCR, tag blank and positive control samples by library.

| Libs | Control type | Event ID | Hash | Species name | Number reads | Note |
|---|---|---|---|---|---|---|
| lib1 | ExtractionBlank | ExtractionBlank | 7701bc7ea7ea | Raja radula* | 149 | |
| lib1 | ExtractionBlank | ExtractionBlank | 7701bc7ea7ea | Raja clavata | 1 | |
| lib1 | ExtractionBlank | ExtractionBlank | c99d9bb4ebbe | Sardina pilchardus | 60 | |
| lib1 | ExtractionBlank | ExtractionBlank | db229e7b11ad | NA | 0 | |
| lib1 | FieldBlank | MBA-2017.09.26-L5-02 | 3977b260b259 | Mustelus asterias | 1 | |
| lib1 | FieldBlank | MBA-2017.11.15-L4-02 | c472a5e5f947 | NA | 0 | |
| lib1 | FieldBlank | MBA-2018.04.20-E1-01 | 2256549ae625 | Sardina pilchardus | 2,444 | |
| lib1 | FieldBlank | MBA-2018.04.20-E1-01 | 2256549ae625 | Scyliorhinus canicula | 1,511 | |
| lib1 | FieldBlank | MBA-2018.04.20-E1-01 | 2256549ae625 | Belone belone | 872 | |
| lib1 | FieldBlank | MBA-2018.04.20-E1-01 | 2256549ae625 | Raja radula* | 437 | |
| lib1 | FieldBlank | MBA-2018.04.20-E1-01 | 2256549ae625 | Mustelus asterias | 25 | |
| lib1 | FieldBlank | MBA-2018.04.20-E1-01 | 2256549ae625 | Raja clavata | 1 | |
| lib1 | PCRBlank | PCRBlank | 16cdc3762063 | NA | 0 | |
| lib1 | PCRBlank | PCRBlank | 1a2e5951c582 | NA | 0 | |
| lib1 | PCRBlank | PCRBlank | 408f2d9fa8c5 | NA | 0 | |
| lib1 | PCRBlank | PCRBlank | b10d4b29c31b | Squalus acanthias | 2 | |
| lib1 | PCRBlank | PCRBlank | c04879523fad | NA | 0 | |
| lib1 | PCRBlank | PCRBlank | e91304a71b3b | NA | 0 | |
| lib1 | PCRBlank | PCRBlank | e9e09c3c7939 | NA | 0 | |
| lib1 | PositiveControl | PositiveControl | 0bdb3bf5809d | Scyliorhinus canicula† | 27,635 | Genomic DNA added per reaction = 0.0409 ng Carcharhinus brevipinna, 0.456 ng Raja radula |
| lib1 | PositiveControl | PositiveControl | 0bdb3bf5809d | Sprattus sprattus† | 15,958 | Genomic DNA added per reaction = 0.0409 ng Carcharhinus brevipinna, 0.456 ng Raja radula |
| lib1 | PositiveControl | PositiveControl | 0bdb3bf5809d | Sardina pilchardus† | 7,122 | Genomic DNA added per reaction = 0.0409 ng Carcharhinus brevipinna, 0.456 ng Raja radula |
| lib1 | PositiveControl | PositiveControl | 0bdb3bf5809d | Raja clavata† | 3,329 | Genomic DNA added per reaction = 0.0409 ng Carcharhinus brevipinna, 0.456 ng Raja radula |

| | | | | | | |
|---|---|---|---|---|---|---|
| lib1 | PositiveControl | PositiveControl | 0bdb3bf5809d | Mustelus asterias† | 3,222 | Genomic DNA added per reaction = 0.0409 ng Carcharhinus brevipinna, 0.456 ng Raja radula |
| lib1 | PositiveControl | PositiveControl | 0bdb3bf5809d | Raja radula* | 2,616 | Genomic DNA added per reaction = 0.0409 ng Carcharhinus brevipinna, 0.456 ng Raja radula |
| lib1 | PositiveControl | PositiveControl | 0bdb3bf5809d | Scyliorhinus stellaris† | 2,377 | Genomic DNA added per reaction = 0.0409 ng Carcharhinus brevipinna, 0.456 ng Raja radula |
| lib1 | PositiveControl | PositiveControl | 0bdb3bf5809d | Belone belone† | 2,014 | Genomic DNA added per reaction = 0.0409 ng Carcharhinus brevipinna, 0.456 ng Raja radula |
| lib1 | PositiveControl | PositiveControl | 0bdb3bf5809d | Carcharhinus brevipinna* | 1,787 | Genomic DNA added per reaction = 0.0409 ng Carcharhinus brevipinna, 0.456 ng Raja radula |
| lib1 | PositiveControl | PositiveControl | 0bdb3bf5809d | Raja microocellata† | 38 | Genomic DNA added per reaction = 0.0409 ng Carcharhinus brevipinna, 0.456 ng Raja radula |
| lib1 | PositiveControl | PositiveControl | 0bdb3bf5809d | Scomber scombrus† | 8 | Genomic DNA added per reaction = 0.0409 ng Carcharhinus brevipinna, 0.456 ng Raja radula |
| lib1 | PositiveControl | PositiveControl | 0bdb3bf5809d | Raja brachyura† | 1 | Genomic DNA added per reaction = 0.0409 ng Carcharhinus brevipinna, 0.456 ng Raja radula |
| lib2 | ExtractionBlank | ExtractionBlank | 1123e670d7de | NA | 0 | |
| lib2 | ExtractionBlank | ExtractionBlank | 5606c6d484d0 | NA | 0 | |
| lib2 | ExtractionBlank | ExtractionBlank | 57b80762f8f6 | NA | 0 | |
| lib2 | ExtractionBlank | ExtractionBlank | c6d4a5e5aa21 | NA | 0 | |
| lib2 | FieldBlank | MBA-2017.05.18-L4-01 | 9055f6c06942 | Raja clavata | 18 | |
| lib2 | FieldBlank | MBA-2017.05.18-L4-01 | 9055f6c06942 | Scyliorhinus canicula | 7 | |
| lib2 | FieldBlank | MBA-2017.07.17-L4-01 | 51fb639c4506 | Raja brachyura | 14 | |
| lib2 | FieldBlank | MBA-2017.07.17-L4-01 | 51fb639c4506 | Mustelus asterias | 7 | |
| lib2 | FieldBlank | MBA-2017.07.17-L4-01 | 51fb639c4506 | Raja montagui | 4 | |
| lib2 | FieldBlank | MBA-2017.07.17-L4-01 | 51fb639c4506 | Scyliorhinus canicula | 2 | |
| lib2 | FieldBlank | MBA-2017.08.30-L4-01 | 317555029694 | Scyliorhinus canicula | 4 | |
| lib2 | PCRBlank | PCRBlank | 1aa683edf8f7 | NA | 0 | |
| lib2 | PCRBlank | PCRBlank | 2170a363b1cd | NA | 0 | |
| lib2 | PCRBlank | PCRBlank | 7d6cb36b692c | NA | 0 | |
| lib2 | PCRBlank | PCRBlank | 9d41bfc15262 | Raja brachyura | 1 | |
| lib2 | PCRBlank | PCRBlank | e1e17fde4daa | NA | 0 | |
| lib2 | PCRBlank | PCRBlank | f8119a26c0db | Sardina pilchardus | 4 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| lib2 | PCRBlank | PCRBlank | f8119a26c0db | Scyliorhinus canicula | 3 | |
| lib2 | PCRBlank | PCRBlank | f85fadd0a769 | Raja radula* | 3 | |
| lib2 | PCRBlank | PCRBlank | f85fadd0a769 | Carcharhinus brevipinna* | 1 | |
| lib2 | PositiveControl | PositiveControl | d693a1eb360a | Raja radula* | 704,870 | Genomic DNA added per reaction = 0.409 ng Carcharhinus brevipinna, 4.56 ng Raja radula |
| lib2 | PositiveControl | PositiveControl | d693a1eb360a | Raja clavata† | 269,317 | Genomic DNA added per reaction = 0.409 ng Carcharhinus brevipinna, 4.56 ng Raja radula |
| lib2 | PositiveControl | PositiveControl | d693a1eb360a | Carcharhinus brevipinna* | 240,191 | Genomic DNA added per reaction = 0.409 ng Carcharhinus brevipinna, 4.56 ng Raja radula |
| lib2 | PositiveControl | PositiveControl | d693a1eb360a | Squalus acanthias† | 157,700 | Genomic DNA added per reaction = 0.409 ng Carcharhinus brevipinna, 4.56 ng Raja radula |
| lib2 | PositiveControl | PositiveControl | d693a1eb360a | Raja montagui† | 156,620 | Genomic DNA added per reaction = 0.409 ng Carcharhinus brevipinna, 4.56 ng Raja radula |
| lib2 | PositiveControl | PositiveControl | d693a1eb360a | Raja brachyura† | 475 | Genomic DNA added per reaction = 0.409 ng Carcharhinus brevipinna, 4.56 ng Raja radula |
| lib2 | PositiveControl | PositiveControl | d693a1eb360a | Raja microocellata† | 19 | Genomic DNA added per reaction = 0.409 ng Carcharhinus brevipinna, 4.56 ng Raja radula |
| lib2 | TagBlank | TagBlank | c89960ee43fa | NA | 0 | |
| lib2 | TagBlank | TagBlank | faf40dc7707a | Trisopterus minutus | 1 | |
| lib3 | ExtractionBlank | ExtractionBlank | 62162fc93266 | Raja clavata | 1 | |
| lib3 | ExtractionBlank | ExtractionBlank | 62162fc93266 | Scyliorhinus canicula | 1 | |
| lib3 | FieldBlank | MBA-2017.06.13-E1-01 | 38bef2b38e82 | Dicentrarchus labrax | 15 | |
| lib3 | FieldBlank | MBA-2017.06.13-E1-01 | 38bef2b38e82 | Sardina pilchardus | 4 | |
| lib3 | FieldBlank | MBA-2017.06.13-E1-01 | 38bef2b38e82 | Raja clavata | 2 | |
| lib3 | PCRBlank | PCRBlank | 16cfacc6a696 | NA | 0 | |
| lib3 | PCRBlank | PCRBlank | 64f0d2efc61d | Pleuronectes platessa | 826 | |
| lib3 | PCRBlank | PCRBlank | 64f0d2efc61d | Trachurus trachurus | 621 | |
| lib3 | PCRBlank | PCRBlank | 64f0d2efc61d | Platichthys flesus | 2 | |
| lib3 | PCRBlank | PCRBlank | 9b3f9e24d6c9 | NA | 0 | |
| lib3 | PCRBlank | PCRBlank | e886e420a0c3 | Raja radula* | 1 | |
| lib3 | PositiveControl | PositiveControl | c3dd6f2bef6a | Raja radula* | 1,947,865 | Genomic DNA added per reaction = 0.818 ng Carcharhinus brevipinna, 9.12 ng Raja radula |
| lib3 | PositiveControl | PositiveControl | c3dd6f2bef6a | Carcharhinus brevipinna* | 831,771 | Genomic DNA added per reaction = 0.818 ng Carcharhinus brevipinna, 9.12 ng Raja radula |

| | | | | | | |
|---|---|---|---|---|---|---|
| lib3 | PositiveControl | PositiveControl | c3dd6f2bef6a | Raja brachyura† | 2,213 | Genomic DNA added per reaction = 0.818 ng Carcharhinus brevipinna, 9.12 ng Raja radula |
| lib3 | PositiveControl | PositiveControl | c3dd6f2bef6a | Raja clavata† | 1,862 | Genomic DNA added per reaction = 0.818 ng Carcharhinus brevipinna, 9.12 ng Raja radula |
| lib3 | PositiveControl | PositiveControl | c3dd6f2bef6a | Raja montagui† | 143 | Genomic DNA added per reaction = 0.818 ng Carcharhinus brevipinna, 9.12 ng Raja radula |
| lib3 | PositiveControl | PositiveControl | c3dd6f2bef6a | Raja microocellata† | 6 | Genomic DNA added per reaction = 0.818 ng Carcharhinus brevipinna, 9.12 ng Raja radula |
| lib3 | PositiveControl | PositiveControl | c3dd6f2bef6a | Scyliorhinus canicula† | 2 | Genomic DNA added per reaction = 0.818 ng Carcharhinus brevipinna, 9.12 ng Raja radula |
| lib3 | PositiveControl | PositiveControl | c3dd6f2bef6a | Mustelus asterias† | 1 | Genomic DNA added per reaction = 0.818 ng Carcharhinus brevipinna, 9.12 ng Raja radula |
| lib3 | TagBlank | TagBlank | 0dca194f547f | NA | 0 | |
| lib3 | TagBlank | TagBlank | 12214cc0d5f1 | Raja clavata | 4 | |
| lib3 | TagBlank | TagBlank | 12214cc0d5f1 | Sprattus sprattus | 3 | |
| lib3 | TagBlank | TagBlank | 16bc66099b5a | NA | 0 | |
| lib3 | TagBlank | TagBlank | 16f0f57bf2d5 | NA | 0 | |
| lib3 | TagBlank | TagBlank | 1b14b0e39f5f | Raja montagui | 32 | |
| lib3 | TagBlank | TagBlank | 376f0beef7b6 | NA | 0 | |
| lib3 | TagBlank | TagBlank | 3e08cf574eae | NA | 0 | |
| lib3 | TagBlank | TagBlank | 3f24193b0c65 | NA | 0 | |
| lib3 | TagBlank | TagBlank | 49b79788c7ba | Scyliorhinus canicula | 1 | |
| lib3 | TagBlank | TagBlank | 4c793f9bb637 | NA | 0 | |
| lib3 | TagBlank | TagBlank | 4f5acda611c7 | Raja montagui | 27 | |
| lib3 | TagBlank | TagBlank | 4f5acda611c7 | Scyliorhinus canicula | 24 | |
| lib3 | TagBlank | TagBlank | 4f5acda611c7 | Mustelus asterias | 3 | |
| lib3 | TagBlank | TagBlank | 4f5acda611c7 | Merlangius merlangus | 1 | |
| lib3 | TagBlank | TagBlank | 4f5acda611c7 | Pleuronectes platessa | 1 | |
| lib3 | TagBlank | TagBlank | 4f5acda611c7 | Scomber scombrus | 1 | |
| lib3 | TagBlank | TagBlank | 4f5acda611c7 | Sprattus sprattus | 1 | |
| lib3 | TagBlank | TagBlank | 4f5acda611c7 | Trisopterus minutus | 1 | |
| lib3 | TagBlank | TagBlank | 5ab91744b7e5 | NA | 0 | |

| | | | | | |
|---|---|---|---|---|---|
| lib3 | TagBlank | TagBlank | 6b1e757fa096 | Raja brachyura | 53 |
| lib3 | TagBlank | TagBlank | 6b1e757fa096 | Raja montagui | 17 |
| lib3 | TagBlank | TagBlank | 6b1e757fa096 | Scyliorhinus canicula | 13 |
| lib3 | TagBlank | TagBlank | 6b1e757fa096 | Raja microocellata | 5 |
| lib3 | TagBlank | TagBlank | 6b1e757fa096 | Mustelus asterias | 1 |
| lib3 | TagBlank | TagBlank | 6b1e757fa096 | Pleuronectes platessa | 1 |
| lib3 | TagBlank | TagBlank | 76b3c2c85ecf | Raja clavata | 1 |
| lib3 | TagBlank | TagBlank | 7a95307cdc57 | NA | 0 |
| lib3 | TagBlank | TagBlank | 877ed4a80da1 | NA | 0 |
| lib3 | TagBlank | TagBlank | 8a2d5aea0d01 | NA | 0 |
| lib3 | TagBlank | TagBlank | 8a5cdb19e7c1 | NA | 0 |
| lib3 | TagBlank | TagBlank | 8dd21c4c5672 | NA | 0 |
| lib3 | TagBlank | TagBlank | 99ab85556ec8 | Raja montagui | 52 |
| lib3 | TagBlank | TagBlank | 99ab85556ec8 | Microstomus kitt | 1 |
| lib3 | TagBlank | TagBlank | 9e7ef7ba02e6 | NA | 0 |
| lib3 | TagBlank | TagBlank | a64a0d182f61 | NA | 0 |
| lib3 | TagBlank | TagBlank | a7da8760cb26 | NA | 0 |
| lib3 | TagBlank | TagBlank | ad7322f946b6 | NA | 0 |
| lib3 | TagBlank | TagBlank | ae639c678e9d | Raja radula* | 60 |
| lib3 | TagBlank | TagBlank | ae639c678e9d | Carcharhinus brevipinna* | 36 |
| lib3 | TagBlank | TagBlank | ae639c678e9d | Raja clavata | 20 |
| lib3 | TagBlank | TagBlank | ae639c678e9d | Raja montagui | 18 |
| lib3 | TagBlank | TagBlank | ae639c678e9d | Squalus acanthias | 18 |
| lib3 | TagBlank | TagBlank | ae639c678e9d | Raja microocellata | 1 |
| lib3 | TagBlank | TagBlank | b93b50ea775a | Raja clavata | 3 |
| lib3 | TagBlank | TagBlank | b93b50ea775a | Buglossidium luteum | 1 |
| lib3 | TagBlank | TagBlank | b93b50ea775a | Scyliorhinus canicula | 1 |
| lib3 | TagBlank | TagBlank | bd400d4b16d2 | NA | 0 |
| lib3 | TagBlank | TagBlank | be1212a0d0df | Raja clavata | 2 |

| | | | | | |
|---|---|---|---|---|---|
| lib3 | TagBlank | TagBlank | c233cf219fa3 | NA | 0 |
| lib3 | TagBlank | TagBlank | c2ba5c0c26cb | NA | 0 |
| lib3 | TagBlank | TagBlank | c3a404f8a5a2 | Raja microocellata | 5 |
| lib3 | TagBlank | TagBlank | cc017195baf6 | Raja brachyura | 1 |
| lib3 | TagBlank | TagBlank | cfe8a2ccd462 | Raja brachyura | 1 |
| lib3 | TagBlank | TagBlank | d0f8e148b4ab | Raja montagui | 13 |
| lib3 | TagBlank | TagBlank | d0f8e148b4ab | Scophthalmus maximus | 4 |
| lib3 | TagBlank | TagBlank | d0f8e148b4ab | Scyliorhinus canicula | 4 |
| lib3 | TagBlank | TagBlank | d0f8e148b4ab | Sardina pilchardus | 1 |
| lib3 | TagBlank | TagBlank | d3e623ebd186 | NA | 0 |
| lib3 | TagBlank | TagBlank | dd6d5d1d7663 | Raja brachyura | 25 |
| lib3 | TagBlank | TagBlank | dd6d5d1d7663 | Raja montagui | 6 |
| lib3 | TagBlank | TagBlank | dd6d5d1d7663 | Scyliorhinus canicula | 4 |
| lib3 | TagBlank | TagBlank | dd6d5d1d7663 | Phrynorhombus norvegicus | 1 |
| lib3 | TagBlank | TagBlank | dd6d5d1d7663 | Sprattus sprattus | 1 |
| lib3 | TagBlank | TagBlank | e0ef8ef20fa7 | NA | 0 |
| lib3 | TagBlank | TagBlank | e64f3d5a1017 | Raja montagui | 3 |
| lib3 | TagBlank | TagBlank | e64f3d5a1017 | Mustelus asterias | 1 |
| lib3 | TagBlank | TagBlank | e64f3d5a1017 | Scyliorhinus canicula | 1 |
| lib3 | TagBlank | TagBlank | fc265d2ac3fa | NA | 0 |
| lib3 | TagBlank | TagBlank | fc45f3b6b9fd | NA | 0 |
| lib3 | TagBlank | TagBlank | fdf6dcb7f56a | NA | 0 |

**Note:** * = positive control species; † = contamination, most plausibly due to contamination during field collection and handling of the tissue.

**Supplementary Information Table S 2.4** eDNA metabarcode data filtering. Number of reads remaining at each bioinformatic step for each library. Taxonomy assigned reads are reads assigned to species level using the curated UK fishes reference library (Collins et al., 2021), and after exclusion of contaminant reads.

| Filtering step | Library 1 | Library 2 | Library 3 |
|---|---|---|---|
| Total passing filter | 32094650 | 55505076 | 73583927 |
| Detect primers | 28181730 | 47352242 | 63313910 |
| Demultiplex | 22342450 | 37984530 | 49758019 |
| Trim primers | 22336438 | 37975395 | 49750091 |
| Quality filter | 22116406 | 37598668 | 49196087 |
| Merge | 18874453 | 14001903 | 36120517 |
| Remove chimaeras | 18314787 | 13735134 | 35396061 |
| Homology search | 18306751 | 13717693 | 35261367 |
| Taxonomy assigned | 18060683 | 10938063 | 29686177 |

**Supplementary Information Table S 2.5.** Number of reads assigned to each elasmobranch species in each field sample.

| Sample | localityID | Depth (1 = surface, 2=bottom) | Month | Year | Alopias vulpinus | Galeorhinus galeus | Lamna nasus | Leucoraja naevus | Mustelus asterias |
|---|---|---|---|---|---|---|---|---|---|
| MBA-2017.02.08-L4-01 | L4 | 1 | 2 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.08-L4-01 | L4 | 1 | 2 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.08-L4-01 | L4 | 1 | 2 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.08-L4-02 | L4 | 2 | 2 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.08-L4-02 | L4 | 2 | 2 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.08-L4-02 | L4 | 2 | 2 | 2017 | 0 | 0 | 0 | 0 | 188 |
| MBA-2017.02.16-E1-01 | E1 | 1 | 2 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.16-E1-01 | E1 | 1 | 2 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.16-E1-01 | E1 | 1 | 2 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.16-E1-02 | E1 | 2 | 2 | 2017 | 0 | 0 | 0 | 0 | 11 |
| MBA-2017.02.16-E1-02 | E1 | 2 | 2 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.16-E1-02 | E1 | 2 | 2 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.16-L5-01 | L5 | 1 | 2 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.16-L5-01 | L5 | 1 | 2 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.16-L5-02 | L5 | 2 | 2 | 2017 | 0 | 0 | 0 | 0 | 9147 |
| MBA-2017.02.16-L5-02 | L5 | 2 | 2 | 2017 | 0 | 0 | 0 | 0 | 1 |
| MBA-2017.02.16-L5-02 | L5 | 2 | 2 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.03.09-L4-01 | L4 | 1 | 3 | 2017 | 0 | 0 | 0 | 0 | 33397 |
| MBA-2017.03.09-L4-01 | L4 | 1 | 3 | 2017 | 0 | 0 | 0 | 0 | 67 |
| MBA-2017.03.09-L4-01 | L4 | 1 | 3 | 2017 | 0 | 0 | 0 | 92389 | 222611 |
| MBA-2017.03.09-L4-02 | L4 | 2 | 3 | 2017 | 0 | 0 | 0 | 0 | 452223 |
| MBA-2017.03.09-L4-02 | L4 | 2 | 3 | 2017 | 0 | 0 | 0 | 0 | 13 |
| MBA-2017.03.09-L4-02 | L4 | 2 | 3 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.03.15-L4-01 | L4 | 1 | 3 | 2017 | 0 | 0 | 0 | 0 | 331748 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| MBA-2017.03.15-L4-01 | L4 | 1 | 3 | 2017 | 0 | 0 | 0 | 0 | 401367 |
| MBA-2017.03.15-L4-01 | L4 | 1 | 3 | 2017 | 0 | 0 | 0 | 0 | 164874 |
| MBA-2017.03.15-L4-02 | L4 | 2 | 3 | 2017 | 0 | 0 | 0 | 0 | 77062 |
| MBA-2017.03.15-L4-02 | L4 | 2 | 3 | 2017 | 0 | 0 | 0 | 0 | 248891 |
| MBA-2017.03.15-L4-02 | L4 | 2 | 3 | 2017 | 0 | 0 | 0 | 0 | 36537 |
| MBA-2017.04.05-L4-01 | L4 | 1 | 4 | 2017 | 0 | 0 | 0 | 0 | 4735 |
| MBA-2017.04.05-L4-01 | L4 | 1 | 4 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.04.05-L4-01 | L4 | 1 | 4 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.04.05-L4-02 | L4 | 2 | 4 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.04.05-L4-02 | L4 | 2 | 4 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.04.05-L4-02 | L4 | 2 | 4 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.04.10-E1-01 | E1 | 1 | 4 | 2017 | 0 | 0 | 0 | 0 | 17 |
| MBA-2017.04.10-E1-01 | E1 | 1 | 4 | 2017 | 0 | 0 | 0 | 0 | 8 |
| MBA-2017.04.10-E1-01 | E1 | 1 | 4 | 2017 | 0 | 0 | 0 | 0 | 28 |
| MBA-2017.04.10-E1-02 | E1 | 2 | 4 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.04.10-E1-02 | E1 | 2 | 4 | 2017 | 0 | 0 | 0 | 0 | 53 |
| MBA-2017.04.10-E1-02 | E1 | 2 | 4 | 2017 | 0 | 0 | 0 | 0 | 89 |
| MBA-2017.04.10-L5-01 | L5 | 1 | 4 | 2017 | 0 | 0 | 0 | 0 | 245692 |
| MBA-2017.04.10-L5-01 | L5 | 1 | 4 | 2017 | 0 | 0 | 0 | 0 | 14 |
| MBA-2017.04.10-L5-01 | L5 | 1 | 4 | 2017 | 0 | 0 | 0 | 0 | 41 |
| MBA-2017.04.10-L5-02 | L5 | 2 | 4 | 2017 | 0 | 0 | 0 | 0 | 22 |
| MBA-2017.04.10-L5-02 | L5 | 2 | 4 | 2017 | 0 | 0 | 0 | 0 | 57 |
| MBA-2017.04.10-L5-02 | L5 | 2 | 4 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.05.18-L4-01 | L4 | 1 | 5 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.05.18-L4-01 | L4 | 1 | 5 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.05.18-L4-01 | L4 | 1 | 5 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.05.18-L4-02 | L4 | 2 | 5 | 2017 | 0 | 0 | 0 | 0 | 16534 |
| MBA-2017.05.18-L4-02 | L4 | 2 | 5 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.05.18-L4-02 | L4 | 2 | 5 | 2017 | 0 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|
| MBA-2017.05.31-E1-01 | E1 | 1 | 5 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.05.31-E1-01 | E1 | 1 | 5 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.05.31-E1-01 | E1 | 1 | 5 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.05.31-E1-02 | E1 | 2 | 5 | 2017 | 0 | 0 | 0 | 0 | 5 |
| MBA-2017.05.31-E1-02 | E1 | 2 | 5 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.05.31-E1-02 | E1 | 2 | 5 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.05.31-L5-01 | L5 | 1 | 5 | 2017 | 0 | 0 | 0 | 0 | 612 |
| MBA-2017.05.31-L5-01 | L5 | 1 | 5 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.05.31-L5-01 | L5 | 1 | 5 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.05.31-L5-02 | L5 | 2 | 5 | 2017 | 0 | 1 | 0 | 0 | 0 |
| MBA-2017.05.31-L5-02 | L5 | 2 | 5 | 2017 | 0 | 0 | 0 | 0 | 7 |
| MBA-2017.05.31-L5-02 | L5 | 2 | 5 | 2017 | 0 | 0 | 0 | 0 | 4 |
| MBA-2017.06.13-E1-01 | E1 | 1 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-E1-01 | E1 | 1 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-E1-01 | E1 | 1 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-E1-02 | E1 | 2 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-E1-02 | E1 | 2 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-E1-02 | E1 | 2 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-L5-01 | L5 | 1 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-L5-01 | L5 | 1 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-L5-01 | L5 | 1 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-L5-02 | L5 | 2 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-L5-02 | L5 | 2 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-L5-02 | L5 | 2 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.15-L4-01 | L4 | 1 | 6 | 2017 | 0 | 0 | 0 | 0 | 14842 |
| MBA-2017.06.15-L4-01 | L4 | 1 | 6 | 2017 | 0 | 0 | 0 | 0 | 76178 |
| MBA-2017.06.15-L4-01 | L4 | 1 | 6 | 2017 | 0 | 0 | 0 | 0 | 2 |
| MBA-2017.06.15-L4-02 | L4 | 2 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.15-L4-02 | L4 | 2 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|
| MBA-2017.06.15-L4-02 | L4 | 2 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.28-L4-01 | L4 | 1 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.28-L4-01 | L4 | 1 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.28-L4-01 | L4 | 1 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.28-L4-02 | L4 | 2 | 6 | 2017 | 0 | 0 | 0 | 0 | 13531 |
| MBA-2017.06.28-L4-02 | L4 | 2 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.28-L4-02 | L4 | 2 | 6 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-E1-01 | E1 | 1 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-E1-01 | E1 | 1 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-E1-01 | E1 | 1 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-E1-02 | E1 | 2 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-E1-02 | E1 | 2 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-E1-02 | E1 | 2 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-L5-01 | L5 | 1 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-L5-01 | L5 | 1 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-L5-01 | L5 | 1 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-L5-02 | L5 | 2 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-L5-02 | L5 | 2 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-L5-02 | L5 | 2 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.17-L4-01 | L4 | 1 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.17-L4-01 | L4 | 1 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.17-L4-01 | L4 | 1 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.17-L4-02 | L4 | 2 | 7 | 2017 | 0 | 0 | 4 | 0 | 4 |
| MBA-2017.07.17-L4-02 | L4 | 2 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.17-L4-02 | L4 | 2 | 7 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.08-L4-01 | L4 | 1 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.08-L4-01 | L4 | 1 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.08-L4-01 | L4 | 1 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.08-L4-02 | L4 | 2 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|
| MBA-2017.08.08-L4-02 | L4 | 2 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.08-L4-02 | L4 | 2 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-E1-01 | E1 | 1 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-E1-01 | E1 | 1 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-E1-01 | E1 | 1 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-E1-02 | E1 | 2 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-E1-02 | E1 | 2 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-E1-02 | E1 | 2 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-L5-01 | L5 | 1 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-L5-01 | L5 | 1 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-L5-01 | L5 | 1 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-L5-02 | L5 | 2 | 8 | 2017 | 0 | 0 | 0 | 0 | 1 |
| MBA-2017.08.24-L5-02 | L5 | 2 | 8 | 2017 | 0 | 0 | 23 | 0 | 0 |
| MBA-2017.08.24-L5-02 | L5 | 2 | 8 | 2017 | 0 | 0 | 0 | 0 | 2 |
| MBA-2017.08.30-L4-01 | L4 | 1 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.30-L4-01 | L4 | 1 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.30-L4-01 | L4 | 1 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.30-L4-02 | L4 | 2 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.30-L4-02 | L4 | 2 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.30-L4-02 | L4 | 2 | 8 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-E1-01 | E1 | 1 | 9 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-E1-01 | E1 | 1 | 9 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-E1-01 | E1 | 1 | 9 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-E1-02 | E1 | 2 | 9 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-E1-02 | E1 | 2 | 9 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-E1-02 | E1 | 2 | 9 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-L5-01 | L5 | 1 | 9 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-L5-01 | L5 | 1 | 9 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-L5-01 | L5 | 1 | 9 | 2017 | 0 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|
| MBA-2017.09.26-L5-02 | L5 | 2 | 9 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-L5-02 | L5 | 2 | 9 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-L5-02 | L5 | 2 | 9 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-E1-01 | E1 | 1 | 10 | 2017 | 0 | 0 | 76811 | 0 | 0 |
| MBA-2017.10.31-E1-01 | E1 | 1 | 10 | 2017 | 0 | 0 | 31770 | 0 | 6 |
| MBA-2017.10.31-E1-01 | E1 | 1 | 10 | 2017 | 0 | 0 | 0 | 2 | 2 |
| MBA-2017.10.31-E1-02 | E1 | 2 | 10 | 2017 | 0 | 0 | 0 | 0 | 24661 |
| MBA-2017.10.31-E1-02 | E1 | 2 | 10 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-E1-02 | E1 | 2 | 10 | 2017 | 0 | 0 | 3797 | 0 | 0 |
| MBA-2017.10.31-L5-01 | L5 | 1 | 10 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-L5-01 | L5 | 1 | 10 | 2017 | 1344 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-L5-01 | L5 | 1 | 10 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-L5-02 | L5 | 2 | 10 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-L5-02 | L5 | 2 | 10 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-L5-02 | L5 | 2 | 10 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.15-L4-01 | L4 | 1 | 11 | 2017 | 0 | 0 | 0 | 0 | 1119 |
| MBA-2017.11.15-L4-01 | L4 | 1 | 11 | 2017 | 0 | 0 | 0 | 0 | 42975 |
| MBA-2017.11.15-L4-01 | L4 | 1 | 11 | 2017 | 0 | 4403 | 0 | 0 | 0 |
| MBA-2017.11.15-L4-02 | L4 | 2 | 11 | 2017 | 0 | 0 | 0 | 0 | 384909 |
| MBA-2017.11.15-L4-02 | L4 | 2 | 11 | 2017 | 0 | 0 | 0 | 0 | 61458 |
| MBA-2017.11.15-L4-02 | L4 | 2 | 11 | 2017 | 0 | 0 | 0 | 0 | 128741 |
| MBA-2017.11.16-E1-01 | E1 | 1 | 11 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-E1-01 | E1 | 1 | 11 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-E1-01 | E1 | 1 | 11 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-E1-02 | E1 | 2 | 11 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-E1-02 | E1 | 2 | 11 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-E1-02 | E1 | 2 | 11 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-L5-01 | L5 | 1 | 11 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-L5-01 | L5 | 1 | 11 | 2017 | 0 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|
| MBA-2017.11.16-L5-01 | L5 | 1 | 11 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-L5-02 | L5 | 2 | 11 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-L5-02 | L5 | 2 | 11 | 2017 | 0 | 0 | 0 | 0 | 29346 |
| MBA-2017.11.16-L5-02 | L5 | 2 | 11 | 2017 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.02.06-E1-01 | E1 | 1 | 2 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.02.06-E1-01 | E1 | 1 | 2 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.02.06-E1-01 | E1 | 1 | 2 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.02.06-E1-02 | E1 | 2 | 2 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.02.06-E1-02 | E1 | 2 | 2 | 2018 | 0 | 0 | 0 | 0 | 2 |
| MBA-2018.02.06-E1-02 | E1 | 2 | 2 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.02.06-L5-01 | L5 | 1 | 2 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.02.06-L5-01 | L5 | 1 | 2 | 2018 | 0 | 0 | 0 | 0 | 5 |
| MBA-2018.02.06-L5-01 | L5 | 1 | 2 | 2018 | 0 | 0 | 0 | 0 | 2 |
| MBA-2018.02.06-L5-02 | L5 | 2 | 2 | 2018 | 0 | 0 | 0 | 0 | 25 |
| MBA-2018.02.06-L5-02 | L5 | 2 | 2 | 2018 | 0 | 0 | 0 | 0 | 2 |
| MBA-2018.02.06-L5-02 | L5 | 2 | 2 | 2018 | 0 | 0 | 0 | 0 | 3 |
| MBA-2018.03.21-E1-01 | E1 | 1 | 3 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-E1-01 | E1 | 1 | 3 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-E1-01 | E1 | 1 | 3 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-E1-02 | E1 | 2 | 3 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-E1-02 | E1 | 2 | 3 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-E1-02 | E1 | 2 | 3 | 2018 | 0 | 0 | 0 | 0 | 2 |
| MBA-2018.03.21-L5-01 | L5 | 1 | 3 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-L5-01 | L5 | 1 | 3 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-L5-01 | L5 | 1 | 3 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-L5-02 | L5 | 2 | 3 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-L5-02 | L5 | 2 | 3 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-L5-02 | L5 | 2 | 3 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-E1-01 | E1 | 1 | 4 | 2018 | 0 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|
| MBA-2018.04.20-E1-01 | E1 | 1 | 4 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-E1-01 | E1 | 1 | 4 | 2018 | 0 | 0 | 0 | 0 | 93 |
| MBA-2018.04.20-E1-02 | E1 | 2 | 4 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-E1-02 | E1 | 2 | 4 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-E1-02 | E1 | 2 | 4 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-L5-01 | L5 | 1 | 4 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-L5-01 | L5 | 1 | 4 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-L5-01 | L5 | 1 | 4 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-L5-02 | L5 | 2 | 4 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-L5-02 | L5 | 2 | 4 | 2018 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-L5-02 | L5 | 2 | 4 | 2018 | 0 | 0 | 0 | 0 | 0 |

| **Sample** | **Raja brachyura** | **Raja clavata** | **Raja microocellata** | **Raja montagui** | **Raja_ ndulata** | **Scyliorhinus canicula** | **Scyliorhinus stellaris** | **Squalus acanthias** |
|---|---|---|---|---|---|---|---|---|
| MBA-2017.02.08-L4-01 | 329 | 3 | 142451 | 82609 | 0 | 0 | 0 | 0 |
| MBA-2017.02.08-L4-01 | 202 | 0 | 3278 | 84875 | 0 | 0 | 0 | 0 |
| MBA-2017.02.08-L4-01 | 0 | 0 | 1270 | 8 | 0 | 0 | 0 | 0 |
| MBA-2017.02.08-L4-02 | 2 | 197 | 10278 | 398 | 0 | 0 | 0 | 0 |
| MBA-2017.02.08-L4-02 | 21 | 0 | 20207 | 16037 | 0 | 0 | 0 | 0 |
| MBA-2017.02.08-L4-02 | 55 | 9531 | 70752 | 26697 | 0 | 83 | 0 | 0 |
| MBA-2017.02.16-E1-01 | 12 | 6 | 15790 | 252 | 0 | 59 | 0 | 0 |
| MBA-2017.02.16-E1-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.16-E1-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.16-E1-02 | 0 | 610 | 0 | 557 | 0 | 1286 | 0 | 0 |
| MBA-2017.02.16-E1-02 | 2 | 0 | 232 | 33 | 0 | 4 | 0 | 0 |
| MBA-2017.02.16-E1-02 | 0 | 2 | 369 | 4 | 0 | 0 | 0 | 0 |
| MBA-2017.02.16-L5-01 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| MBA-2017.02.16-L5-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|
| MBA-2017.02.16-L5-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.02.16-L5-02 | 0 | 2 | 0 | 0 | 0 | 4 | 0 | 0 |
| MBA-2017.02.16-L5-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.03.09-L4-01 | 3223 | 27 | 2001 | 245512 | 0 | 197486 | 1462 | 0 |
| MBA-2017.03.09-L4-01 | 2019 | 2 | 93207 | 1045703 | 0 | 213096 | 0 | 1 |
| MBA-2017.03.09-L4-01 | 114899 | 18 | 539679 | 917439 | 0 | 645282 | 193981 | 0 |
| MBA-2017.03.09-L4-02 | 2598 | 12 | 229845 | 1161985 | 0 | 54513 | 29820 | 0 |
| MBA-2017.03.09-L4-02 | 17 | 2 | 278064 | 61290 | 0 | 173746 | 5863 | 0 |
| MBA-2017.03.09-L4-02 | 411 | 4 | 270223 | 88037 | 0 | 16283 | 0 | 0 |
| MBA-2017.03.15-L4-01 | 1230276 | 3087 | 47571 | 1522881 | 33880 | 281072 | 0 | 2 |
| MBA-2017.03.15-L4-01 | 1296481 | 32732 | 69139 | 1078273 | 1 | 336379 | 0 | 0 |
| MBA-2017.03.15-L4-01 | 772339 | 23673 | 56848 | 1133534 | 2 | 314795 | 0 | 0 |
| MBA-2017.03.15-L4-02 | 258074 | 17906 | 28093 | 189847 | 0 | 108701 | 0 | 0 |
| MBA-2017.03.15-L4-02 | 238725 | 75772 | 15644 | 331297 | 0 | 353483 | 27573 | 0 |
| MBA-2017.03.15-L4-02 | 297805 | 13350 | 39504 | 229410 | 0 | 64627 | 0 | 0 |
| MBA-2017.04.05-L4-01 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.04.05-L4-01 | 23 | 9 | 2 | 7034 | 0 | 6 | 0 | 0 |
| MBA-2017.04.05-L4-01 | 1616 | 4 | 202 | 624542 | 0 | 0 | 0 | 0 |
| MBA-2017.04.05-L4-02 | 0 | 1 | 0 | 0 | 0 | 4 | 0 | 0 |
| MBA-2017.04.05-L4-02 | 7776 | 6 | 234 | 593406 | 0 | 1 | 0 | 0 |
| MBA-2017.04.05-L4-02 | 176 | 49 | 70518 | 1230 | 0 | 0 | 0 | 0 |
| MBA-2017.04.10-E1-01 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 15 |
| MBA-2017.04.10-E1-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.04.10-E1-01 | 1 | 0 | 0 | 7 | 0 | 1 | 0 | 26 |
| MBA-2017.04.10-E1-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.04.10-E1-02 | 238 | 1 | 6 | 304 | 7 | 65 | 0 | 1 |
| MBA-2017.04.10-E1-02 | 0 | 0 | 22 | 1 | 0 | 0 | 0 | 107 |
| MBA-2017.04.10-L5-01 | 1136 | 11 | 148 | 324767 | 0 | 72 | 247 | 0 |
| MBA-2017.04.10-L5-01 | 59 | 4 | 8 | 49 | 0 | 17 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|
| MBA-2017.04.10-L5-01 | 3219 | 5 | 13 | 278 | 0 | 10272 | 0 | 0 |
| MBA-2017.04.10-L5-02 | 42 | 3 | 8 | 331 | 0 | 242 | 0 | 0 |
| MBA-2017.04.10-L5-02 | 59 | 15 | 3 | 77 | 0 | 74 | 11 | 0 |
| MBA-2017.04.10-L5-02 | 284 | 58 | 17760 | 126888 | 0 | 37466 | 0 | 0 |
| MBA-2017.05.18-L4-01 | 20 | 12 | 0 | 2 | 0 | 26 | 16604 | 0 |
| MBA-2017.05.18-L4-01 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.05.18-L4-01 | 29 | 0 | 4 | 12053 | 0 | 5805 | 0 | 0 |
| MBA-2017.05.18-L4-02 | 8 | 0 | 1 | 5919 | 0 | 451 | 0 | 0 |
| MBA-2017.05.18-L4-02 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| MBA-2017.05.18-L4-02 | 9 | 0 | 0 | 8 | 0 | 0 | 74 | 0 |
| MBA-2017.05.31-E1-01 | 0 | 0 | 0 | 135 | 0 | 12 | 0 | 0 |
| MBA-2017.05.31-E1-01 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| MBA-2017.05.31-E1-01 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 |
| MBA-2017.05.31-E1-02 | 1 | 9 | 2 | 46 | 0 | 0 | 0 | 0 |
| MBA-2017.05.31-E1-02 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| MBA-2017.05.31-E1-02 | 0 | 0 | 0 | 1 | 0 | 88 | 0 | 0 |
| MBA-2017.05.31-L5-01 | 51312 | 0 | 25 | 2093 | 0 | 43145 | 0 | 0 |
| MBA-2017.05.31-L5-01 | 12 | 0 | 0 | 3428 | 0 | 1712 | 0 | 0 |
| MBA-2017.05.31-L5-01 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 |
| MBA-2017.05.31-L5-02 | 582 | 0 | 50 | 2343 | 0 | 1489 | 0 | 0 |
| MBA-2017.05.31-L5-02 | 74 | 12 | 4 | 662 | 0 | 14 | 12 | 9 |
| MBA-2017.05.31-L5-02 | 0 | 0 | 0 | 24 | 0 | 23 | 0 | 0 |
| MBA-2017.06.13-E1-01 | 4 | 55562 | 44 | 2 | 0 | 172168 | 0 | 0 |
| MBA-2017.06.13-E1-01 | 183 | 2 | 19 | 58100 | 0 | 79693 | 0 | 0 |
| MBA-2017.06.13-E1-01 | 0 | 1 | 0 | 0 | 0 | 5 | 0 | 0 |
| MBA-2017.06.13-E1-02 | 0 | 2 | 0 | 0 | 0 | 3 | 0 | 3 |
| MBA-2017.06.13-E1-02 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-E1-02 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 |
| MBA-2017.06.13-L5-01 | 0 | 0 | 0 | 0 | 0 | 542544 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MBA-2017.06.13-L5-01 | 3 | 0 | 0 | 0 | 0 | 224 | 0 | 0 |
| MBA-2017.06.13-L5-01 | 8 | 0 | 0 | 2 | 0 | 7309 | 0 | 0 |
| MBA-2017.06.13-L5-02 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-L5-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.06.13-L5-02 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 |
| MBA-2017.06.15-L4-01 | 599514 | 23 | 41352 | 161860 | 0 | 130450 | 2 | 0 |
| MBA-2017.06.15-L4-01 | 1109729 | 39848 | 68413 | 722087 | 2 | 143725 | 0 | 0 |
| MBA-2017.06.15-L4-01 | 17 | 1 | 1 | 5 | 0 | 2 | 0 | 0 |
| MBA-2017.06.15-L4-02 | 343434 | 1 | 837 | 84625 | 1 | 41145 | 1 | 0 |
| MBA-2017.06.15-L4-02 | 18858 | 79 | 1 | 3434 | 0 | 1619 | 0 | 0 |
| MBA-2017.06.15-L4-02 | 4555 | 0 | 85 | 3953 | 0 | 2369 | 0 | 0 |
| MBA-2017.06.28-L4-01 | 0 | 19 | 0 | 0 | 0 | 66884 | 0 | 2 |
| MBA-2017.06.28-L4-01 | 0 | 32072 | 1 | 1 | 0 | 0 | 0 | 0 |
| MBA-2017.06.28-L4-01 | 19 | 36793 | 0 | 15 | 0 | 0 | 0 | 0 |
| MBA-2017.06.28-L4-02 | 47398 | 0 | 0 | 783 | 0 | 22 | 0 | 0 |
| MBA-2017.06.28-L4-02 | 0 | 0 | 0 | 0 | 0 | 175467 | 0 | 0 |
| MBA-2017.06.28-L4-02 | 101427 | 0 | 13 | 15305 | 0 | 135 | 0 | 0 |
| MBA-2017.07.13-E1-01 | 0 | 0 | 0 | 0 | 0 | 57 | 0 | 0 |
| MBA-2017.07.13-E1-01 | 0 | 0 | 0 | 0 | 0 | 1062 | 0 | 0 |
| MBA-2017.07.13-E1-01 | 44 | 18631 | 2 | 5692 | 0 | 17529 | 0 | 0 |
| MBA-2017.07.13-E1-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-E1-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-E1-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-L5-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-L5-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-L5-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-L5-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-L5-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.07.13-L5-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MBA-2017.07.17-L4-01 | 0 | 0 | 0 | 0 | 0 | 36200 | 0 | 0 |
| MBA-2017.07.17-L4-01 | 49423 | 14 | 2 | 817 | 0 | 0 | 0 | 0 |
| MBA-2017.07.17-L4-01 | 0 | 8 | 0 | 0 | 0 | 29704 | 0 | 0 |
| MBA-2017.07.17-L4-02 | 538 | 61 | 75 | 171147 | 0 | 2 | 306650 | 0 |
| MBA-2017.07.17-L4-02 | 15674 | 10 | 0 | 223 | 0 | 9 | 0 | 0 |
| MBA-2017.07.17-L4-02 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.08-L4-01 | 422 | 0 | 0 | 17 | 0 | 3 | 0 | 0 |
| MBA-2017.08.08-L4-01 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| MBA-2017.08.08-L4-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.08-L4-02 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| MBA-2017.08.08-L4-02 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 |
| MBA-2017.08.08-L4-02 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-E1-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-E1-01 | 370 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-E1-01 | 0 | 0 | 0 | 0 | 0 | 40 | 0 | 0 |
| MBA-2017.08.24-E1-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-E1-02 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| MBA-2017.08.24-E1-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-L5-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-L5-01 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-L5-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.08.24-L5-02 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| MBA-2017.08.24-L5-02 | 196 | 23 | 0 | 13 | 0 | 3 | 0 | 0 |
| MBA-2017.08.24-L5-02 | 47909 | 51 | 8 | 668 | 0 | 6 | 1 | 0 |
| MBA-2017.08.30-L4-01 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 |
| MBA-2017.08.30-L4-01 | 0 | 0 | 0 | 0 | 0 | 22415 | 0 | 0 |
| MBA-2017.08.30-L4-01 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| MBA-2017.08.30-L4-02 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| MBA-2017.08.30-L4-02 | 0 | 0 | 0 | 0 | 0 | 49 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MBA-2017.08.30-L4-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-E1-01 | 3 | 39093 | 0 | 0 | 0 | 2363 | 0 | 0 |
| MBA-2017.09.26-E1-01 | 204 | 0 | 0 | 348 | 0 | 2180 | 0 | 1085 |
| MBA-2017.09.26-E1-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-E1-02 | 14 | 52620 | 1 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-E1-02 | 4 | 37689 | 0 | 1 | 0 | 2267 | 0 | 0 |
| MBA-2017.09.26-E1-02 | 0 | 5682 | 0 | 3 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-L5-01 | 8 | 91557 | 1 | 0 | 0 | 30615 | 0 | 0 |
| MBA-2017.09.26-L5-01 | 8375 | 1226 | 1553 | 29 | 0 | 20735 | 0 | 0 |
| MBA-2017.09.26-L5-01 | 0 | 8812 | 0 | 0 | 0 | 2 | 0 | 0 |
| MBA-2017.09.26-L5-02 | 0 | 530 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-L5-02 | 13 | 73518 | 1 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.09.26-L5-02 | 4 | 48798 | 0 | 884 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-E1-01 | 991701 | 111802 | 7 | 401 | 0 | 3 | 0 | 0 |
| MBA-2017.10.31-E1-01 | 107233 | 294599 | 4 | 2185 | 0 | 1 | 2 | 0 |
| MBA-2017.10.31-E1-01 | 421692 | 191858 | 9 | 16582 | 0 | 4 | 1 | 0 |
| MBA-2017.10.31-E1-02 | 56570 | 7960 | 7 | 22653 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-E1-02 | 26546 | 101408 | 2 | 13 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-E1-02 | 1205 | 26631 | 0 | 2 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-L5-01 | 5 | 29152 | 0 | 0 | 0 | 11003 | 0 | 0 |
| MBA-2017.10.31-L5-01 | 2221 | 1309 | 0 | 1 | 0 | 147 | 0 | 0 |
| MBA-2017.10.31-L5-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-L5-02 | 21 | 95009 | 1 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-L5-02 | 3 | 31719 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.10.31-L5-02 | 34848 | 15286 | 40 | 65913 | 0 | 0 | 0 | 0 |
| MBA-2017.11.15-L4-01 | 5 | 0 | 0 | 714 | 0 | 0 | 0 | 2529 |
| MBA-2017.11.15-L4-01 | 46 | 721 | 0 | 217 | 0 | 0 | 0 | 92 |
| MBA-2017.11.15-L4-01 | 81 | 0 | 3 | 13875 | 0 | 15541 | 0 | 0 |
| MBA-2017.11.15-L4-02 | 13 | 23 | 87046 | 1196 | 0 | 0 | 0 | 516873 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MBA-2017.11.15-L4-02 | 211 | 0 | 12 | 25142 | 0 | 31422 | 0 | 76310 |
| MBA-2017.11.15-L4-02 | 14245 | 0 | 60 | 63808 | 0 | 0 | 0 | 136231 |
| MBA-2017.11.16-E1-01 | 142 | 0 | 11 | 26476 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-E1-01 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-E1-01 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-E1-02 | 1 | 4637 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-E1-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-E1-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-L5-01 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-L5-01 | 3 | 21574 | 0 | 183 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-L5-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-L5-02 | 1115 | 229 | 6481 | 114130 | 0 | 0 | 0 | 0 |
| MBA-2017.11.16-L5-02 | 2335 | 64725 | 28590 | 75714 | 0 | 86291 | 45243 | 44663 |
| MBA-2017.11.16-L5-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.02.06-E1-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.02.06-E1-01 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 |
| MBA-2018.02.06-E1-01 | 0 | 0 | 0 | 2 | 0 | 65 | 0 | 0 |
| MBA-2018.02.06-E1-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.02.06-E1-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.02.06-E1-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.02.06-L5-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.02.06-L5-01 | 230 | 748 | 50 | 121427 | 0 | 43 | 0 | 5 |
| MBA-2018.02.06-L5-01 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 |
| MBA-2018.02.06-L5-02 | 15 | 127739 | 128 | 42 | 0 | 3 | 0 | 0 |
| MBA-2018.02.06-L5-02 | 353072 | 0 | 4 | 7957 | 0 | 3 | 0 | 0 |
| MBA-2018.02.06-L5-02 | 12 | 0 | 0 | 11 | 0 | 9 | 0 | 144552 |
| MBA-2018.03.21-E1-01 | 1182 | 1 | 168 | 380851 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-E1-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-E1-01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MBA-2018.03.21-E1-02 | 910 | 1 | 137 | 415781 | 0 | 177598 | 0 | 0 |
| MBA-2018.03.21-E1-02 | 407 | 0 | 123 | 191510 | 0 | 56466 | 0 | 0 |
| MBA-2018.03.21-E1-02 | 1229 | 0 | 195 | 448830 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-L5-01 | 1030 | 1 | 170 | 485061 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-L5-01 | 1735 | 0 | 262 | 727847 | 0 | 0 | 0 | 0 |
| MBA-2018.03.21-L5-01 | 237 | 0 | 56 | 146652 | 0 | 313191 | 0 | 0 |
| MBA-2018.03.21-L5-02 | 4032 | 1 | 639 | 1352346 | 0 | 2427 | 0 | 0 |
| MBA-2018.03.21-L5-02 | 2 | 4125 | 9213 | 658 | 361 | 0 | 0 | 0 |
| MBA-2018.03.21-L5-02 | 0 | 0 | 0 | 101 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-E1-01 | 2 | 0 | 0 | 0 | 0 | 136 | 0 | 0 |
| MBA-2018.04.20-E1-01 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| MBA-2018.04.20-E1-01 | 644 | 728 | 0 | 347 | 0 | 639 | 0 | 0 |
| MBA-2018.04.20-E1-02 | 426 | 355 | 36 | 46165 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-E1-02 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-E1-02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-L5-01 | 3 | 0 | 0 | 710 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-L5-01 | 1 | 0 | 0 | 0 | 0 | 23790 | 0 | 0 |
| MBA-2018.04.20-L5-01 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| MBA-2018.04.20-L5-02 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| MBA-2018.04.20-L5-02 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| MBA-2018.04.20-L5-02 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |

**Supplementary Information Table S 2.6** Results of the Indicator species analyses (IndVal) analyses.

***Wisconsin Transformed***

| **Depth** | | Surface | Bottom | Index | Stat | P-value |
|---|---|---|---|---|---|---|
| | Alopias_vulpinus | 1.00000 | 0.00000 | 1.00000 | 0.10721 | 1.00000 |
| | Galeorhinus_galeus | 1.00000 | 0.00000 | 1.00000 | 0.10459 | 1.00000 |
| | Lamna_nasus | 0.00000 | 1.00000 | 2.00000 | 0.13759 | 0.74280 |
| | Leucoraja_naevus | 1.00000 | 0.00000 | 1.00000 | 0.15162 | 0.49860 |
| | Mustelus_asterias | 0.00000 | 1.00000 | 2.00000 | 0.43839 | 0.45040 |
| | Raja_brachyura | 0.00000 | 1.00000 | 2.00000 | 0.60318 | 0.55810 |
| | Raja_clavata | 0.00000 | 1.00000 | 2.00000 | 0.58872 | 0.14460 |
| | Raja_microocellata | 0.00000 | 1.00000 | 2.00000 | 0.58648 | 0.14690 |
| | Raja_montagui | 1.00000 | 0.00000 | 1.00000 | 0.61758 | 0.74910 |
| | Raja_undulata | 0.00000 | 1.00000 | 2.00000 | 0.15310 | 0.78380 |
| | Scyliorhinus_canicula | 1.00000 | 0.00000 | 1.00000 | 0.63585 | 0.05190 |
| | Scyliorhinus_stellaris | 0.00000 | 1.00000 | 2.00000 | 0.27729 | 0.40230 |
| | Squalus_acanthias | 0.00000 | 1.00000 | 2.00000 | 0.25254 | 0.72400 |

| **Stations** | | E1 | L4 | L5 | Index | Stat | P-value |
|---|---|---|---|---|---|---|---|
| | Alopias_vulpinus | 0.00000 | 0.00000 | 1.00000 | 3.00000 | 0.13363 | 0.63300 |
| | Galeorhinus_galeus | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.12194 | 1.00000 |
| | Lamna_nasus | 1.00000 | 0.00000 | 0.00000 | 1.00000 | 0.19211 | 0.25550 |
| | Leucoraja_naevus | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.12499 | 1.00000 |
| | Mustelus_asterias | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.41851 | 0.20110 |
| | Raja_brachyura | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.59636 | 0.06970 |
| | Raja_clavata | 0.00000 | 0.00000 | 1.00000 | 3.00000 | 0.45576 | 0.76260 |
| | **Raja_microocellata** | **0.00000** | **1.00000** | **0.00000** | **2.00000** | **0.60200** | **0.01090** |
| | Raja_montagui | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.54217 | 0.49350 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Raja_undulata | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.15820 | 0.69830 |
| | Scyliorhinus_canicula | 0.00000 | 0.00000 | 1.00000 | 3.00000 | 0.48508 | 0.72020 |
| | **Scyliorhinus_stellaris** | **0.00000** | **1.00000** | **0.00000** | **2.00000** | **0.37516** | **0.02070** |
| | Squalus_acanthias | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.21514 | 0.89000 |

***Hellinger Tranformed***

| **Depth** | | Surface | Bottom | Index | Stat | P-value | |
|---|---|---|---|---|---|---|---|
| | Alopias_vulpinus | 1.00000 | 0.00000 | 1.00000 | 0.10721 | 1.00000 | |
| | Galeorhinus_galeus | 1.00000 | 0.00000 | 1.00000 | 0.10505 | 1.00000 | |
| | Lamna_nasus | 0.00000 | 1.00000 | 2.00000 | 0.13801 | 0.68680 | |
| | Leucoraja_naevus | 1.00000 | 0.00000 | 1.00000 | 0.15162 | 0.50160 | |
| | Mustelus_asterias | 0.00000 | 1.00000 | 2.00000 | 0.44273 | 0.36560 | |
| | Raja_brachyura | 0.00000 | 1.00000 | 2.00000 | 0.59168 | 0.61440 | |
| | Raja_clavata | 0.00000 | 1.00000 | 2.00000 | 0.58534 | 0.13960 | |
| | Raja_microocellata | 0.00000 | 1.00000 | 2.00000 | 0.58520 | 0.09170 | |
| | Raja_montagui | 0.00000 | 1.00000 | 2.00000 | 0.61339 | 0.78500 | |
| | Raja_undulata | 0.00000 | 1.00000 | 2.00000 | 0.15764 | 0.79910 | |
| | Scyliorhinus_canicula | 1.00000 | 0.00000 | 1.00000 | 0.62487 | 0.06580 | |
| | Scyliorhinus_stellaris | 0.00000 | 1.00000 | 2.00000 | 0.27776 | 0.36450 | |
| | Squalus_acanthias | 0.00000 | 1.00000 | 2.00000 | 0.25095 | 0.74730 | |

| **Stations** | | E1 | L4 | L5 | Index | Stat | P-value |
|---|---|---|---|---|---|---|---|
| | Alopias_vulpinus | 0.00000 | 0.00000 | 1.00000 | 3.00000 | 0.13363 | 0.63290 |
| | Galeorhinus_galeus | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.12248 | 1.00000 |
| | Lamna_nasus | 1.00000 | 0.00000 | 0.00000 | 1.00000 | 0.20317 | 0.18810 |
| | Leucoraja_naevus | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.12440 | 1.00000 |
| | Mustelus_asterias | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.41970 | 0.15610 |
| | Raja_brachyura | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.54971 | 0.16820 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Raja_clavata | 0.00000 | 0.00000 | 1.00000 | 3.00000 | 0.45716 | 0.72990 |
| | Raja_microocellata | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.58859 | 0.00580 |
| | Raja_montagui | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.57769 | 0.06810 |
| | Raja_undulata | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.14879 | 0.82800 |
| | Scyliorhinus_canicula | 0.00000 | 0.00000 | 1.00000 | 3.00000 | 0.47408 | 0.84490 |
| | Scyliorhinus_stellaris | 0.00000 | 1.00000 | 0.00000 | 2.00000 | 0.36038 | 0.02190 |
| | Squalus_acanthias | 1.00000 | 0.00000 | 0.00000 | 1.00000 | 0.21127 | 0.91410 |

**Supplementary Information Table S 2.7.** GAM model summary details.

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> S_can_eDNA_gam <- gam(Scyliorhinus_canicula ~ s(month, k=5) + year + minimumDepthInMeters + localityID, family=nb, select=TRUE ,method="REML", data=Elasmo_Hell_zeros)

Family: Negative Binomial(194102.5)

Link function: log

Formula:

Scyliorhinus_canicula ~ s(month, k = 5) + year + minimumDepthInMeters + localityID

Parametric coefficients:

| | Estimate | Std. Error | z value | Pr(>\|z\|) |
|---|---|---|---|---|
| (Intercept) | -252.40704 | 868.09374 | -0.291 | 0.771 |
| year | 0.12450 | 0.43033 | 0.289 | 0.772 |
| minimumDepthInMeters2 | -0.39509 | 0.27590 | -1.432 | 0.152 |
| localityIDL4 | -0.02143 | 0.35329 | -0.061 | 0.952 |
| localityIDL5 | 0.07155 | 0.32885 | 0.218 | 0.828 |

Approximate significance of smooth terms:

| | edf | Ref.df | Chi.sq | p-value |
|---|---|---|---|---|
| s(month) | 1.858 | 4 | 9.02 | 0.00564 ** |

---

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) = 0.109 Deviance explained = 9.82%

-REML = 139.12 Scale est. = 1 n = 209

---

> M_ast_eDNA_gam <- gam(Mustelus_asterias~ s(month, k=5) + year + minimumDepthInMeters + localityID, family=nb, select=TRUE,method="REML", data=Elasmo_Hell_zeros)

Family: Negative Binomial(132457.282)

Link function: log

Formula:

Mustelus_asterias ~ s(month, k = 5) + year + minimumDepthInMeters + localityID

Parametric coefficients:

| | Estimate | Std. Error | z value | Pr(>\|z\|) |
|---|---|---|---|---|
| (Intercept) | 1.981e+03 | 1.708e+03 | 1.159 | 0.246 |
| year | -9.833e-01 | 8.469e-01 | -1.161 | 0.246 |
| minimumDepthInMeters2 | 1.837e-01 | 4.541e-01 | 0.404 | 0.686 |
| localityIDL4 | 4.375e-01 | 5.721e-01 | 0.765 | 0.444 |
| localityIDL5 | 4.225e-04 | 6.205e-01 | 0.001 | 0.999 |

Approximate significance of smooth terms:

```
       edf Ref.df Chi.sq p-value
s(month) 1.4      4  4.674  0.0337 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  0.0367   Deviance explained = 9.15%
-REML = 71.294  Scale est. = 1        n = 209
```

---

```
> R_bra_eDNA_gam <- gam(Raja_brachyura~ s(month, k=5) + year + minimumDepthInMeters + localityID, family=nb,
select=TRUE,method="REML", data=Elasmo_Hell_zeros)

Family: Negative Binomial(182385.756)
Link function: log

Formula:
Raja_brachyura ~ s(month, k = 5) + year + minimumDepthInMeters +  localityID

Parametric coefficients:
                    Estimate Std. Error z value Pr(>|z|)
(Intercept)         1828.39741 1441.84425   1.268    0.205
year                -0.90747    0.71480  -1.270    0.204
minimumDepthInMeters2   0.09094    0.34766   0.262    0.794
localityIDL4            0.41203    0.43277   0.952    0.341
localityIDL5            0.08725    0.46410   0.188    0.851

Approximate significance of smooth terms:
            edf Ref.df Chi.sq p-value
s(month) 7.867e-06      4      0   0.593

R-sq.(adj) =  0.0162   Deviance explained = 3.56%
-REML = 106.03  Scale est. = 1        n = 209
```

---

```
> R_cla_eDNA_gam <- gam(Raja_clavata~ s(month, k=5) + year + minimumDepthInMeters + localityID, family=nb,
select=TRUE,method="REML", data=Elasmo_Hell_zeros)

Family: Negative Binomial(198756.026)
Link function: log

Formula:
Raja_clavata ~ s(month, k = 5) + year + minimumDepthInMeters +  localityID

Parametric coefficients:
                    Estimate Std. Error z value Pr(>|z|)
(Intercept)         1266.76348 1576.80841   0.803    0.422
year                -0.62901    0.78170  -0.805    0.421
```

```
minimumDepthInMeters2   0.38245   0.35461   1.079   0.281
localityIDL4           -0.62644   0.49168  -1.274   0.203
localityIDL5            0.01115   0.38773   0.029   0.977

Approximate significance of smooth terms:
          edf Ref.df Chi.sq p-value
s(month) 1.507      4  6.627  0.0112 *

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  0.162   Deviance explained = 13.9%
-REML = 97.663  Scale est. = 1         n = 209
```

---

```
> R_mic_eDNA_gam <- gam(Raja_microocellata~ s(month, k=5) + year + minimumDepthInMeters + localityID, family=nb,
select=TRUE,method="REML", data=Elasmo_Hell_zeros)

Family: Negative Binomial(171275.704)
Link function: log

Formula:
Raja_microocellata ~ s(month, k = 5) + year + minimumDepthInMeters +  localityID

Parametric coefficients:
                    Estimate Std. Error z value Pr(>|z|)
(Intercept)         3627.6966  2169.4233   1.672   0.0945 .
year                -1.8002     1.0755  -1.674   0.0942 .
minimumDepthInMeters2   0.5141     0.4939   1.041   0.2979
localityIDL4            0.7551     0.6314   1.196   0.2317
localityIDL5            0.0623     0.7372   0.085   0.9327
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:
          edf Ref.df Chi.sq  p-value
s(month) 1.912      4  18.38 3.65e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  0.402   Deviance explained = 34.6%
-REML = 57.055  Scale est. = 1         n = 209
```

---

```
> R_mon_eDNA_gam <- gam(Raja_montagui~  s(month, k=5) + year + minimumDepthInMeters + localityID, family=nb,
select=TRUE,method="REML", data=Elasmo_Hell_zeros)

Family: Negative Binomial(211064.405)
```

Link function: log

Formula:
Raja_montagui ~ s(month, k = 5) + year + minimumDepthInMeters + localityID

Parametric coefficients:

| | Estimate | Std. Error | z value | Pr(>\|z\|) | |
|---|---|---|---|---|---|
| (Intercept) | -828.64478 | 725.46953 | -1.142 | 0.2534 | |
| year | 0.41007 | 0.35962 | 1.140 | 0.2542 | |
| minimumDepthInMeters2 | 0.00489 | 0.24496 | 0.020 | 0.9841 | |
| localityIDL4 | 0.62373 | 0.33101 | 1.884 | 0.0595 | . |
| localityIDL5 | 0.14167 | 0.31974 | 0.443 | 0.6577 | |

---
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:

| | edf | Ref.df | Chi.sq | p-value | |
|---|---|---|---|---|---|
| s(month) | 1.067 | 4 | 2.592 | 0.086 | . |

---
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) = 0.0841 Deviance explained = 6.82%
-REML = 158.04 Scale est. = 1 n = 209

---

# Chapter 3

# Utilising environmental DNA-based metabarcoding to quantify mesopelagic fish diversity in the Southern Ocean

An adapted version of this chapter is in preparation to be submitted to a peer-reviewed journal:

Liu, Z., Collins, R.A., Baillie, C., Freer, J.J., Saunders, R.A., Tarling, G.A., Mariani, S. & Genner, M.J. Utilising environmental DNA-based metabarcoding to quantify mesopelagic fish diversity in the Southern Ocean.

Author contributions: eDNA and RMT sampling: GAT, RAS and other colleagues from BAS. DNA extraction: RAC and JJF. PCR and Sequencing: CB and SM. Bioinformatics: ZL. Data analysis: ZL and MJG. Manuscript: ZL and MJG.

**Abstract**

Mesopelagic fish occupy key trophic positions in food webs of the Southern Ocean, but distributions and abundance can be challenging to quantify using survey trawls. Here we initially explored the capacity of environmental DNA metabarcoding to quantify the structure of mesopelagic fish assemblage of the Scotia Sea across a depth gradient from the surface to 850 m. We then compared the eDNA-based results to trawls undertaken at the same locations and depths. Overall, using eDNA-based methods we identified a gradient in community structure with increasing depth, albeit less prominent than that observed in the trawl survey data. We also found evidence that across species and sampling locations, fish species abundance in the net survey was decoupled from eDNA read abundance. In addition, we explored broader patterns of fish in surface-collected samples, identifying the presence of multiple epipelagic and demersal fish species. Finally, we mapped the distribution of ten marine mammal species encountered as “molecular bycatch”. Collectively, these results indicate that eDNA-based methods may be useful for ecological assessments of fish stocks alongside conventional survey approaches, and may also be valuable for mapping of distributions of pelagic marine mammal species in oceanic environments.

## 3.1 Introduction

In the Southern Ocean mesopelagic fish that occupy the twilight zone between 200 m to 1000 m are fundamental to food webs, linking the primary consumers including zooplankton and Antarctic krill, to top predators including fin whales, elephant seals and king penguins (Saunders et al. 2018; Dornan et al. 2022). Due to their high abundance, these fish are also a key component of the oceanic biological carbon pump, transporting large amounts of biomass during their diel vertical migration from the surface in darkness, to deeper waters during daylight hours (Saba et al. 2021; Dornan et al. 2022). Despite this ecosystem-level importance, there is still considerable uncertainty regarding key aspects of their biology, including their distributions and abundance over time, space and depth, and relevance to food webs of the region (Saunders et al. 2019). Much of our present understanding of the biology of these species comes from capture-based net survey methods, which can lead to underestimates of abundance due to net avoidance behaviour at sampled locations (Saunders et al. 2019). More recently, good insight into broader patterns of mesopelagic biomass has been gained using active acoustic methods (Dornan et al. 2022), but species composition information still needs to be groundtruthed with sampling methods that provide species-level abundance and biomass information (Dornan et al. 2019). A further constraint on our understanding is that surveys of the Southern Ocean ecosystem tend to be undertaken during the Austral summer from November to March (Woods et al. 2022), and our understanding of the distributions and abundance of species during other seasons is relatively limited.

Aquatic environmental DNA (eDNA) is emerging as an efficient, non-invasive, and potentially inexpensive method of capturing information on the distributions and abundance of specific species and can also provide information on the relative abundance of species within whole ecological communities (Taberlet et al. 2018). In general, two approaches are typically employed in eDNA-based analyses. First, to reliably provide information on the abundance of DNA of target species, it possible the employ quantitative PCR (qPCR) on eDNA-samples. This method requires the development of PCR primer and probe combinations that target specific species, and do not amplify other taxa in the environment (Langlois et al. 2021). Second, to explore the distributions and abundance of multiple species simultaneously, it possible to use eDNA metabarcoding. This method requires the use of PCR primers that can

target multiple species, and species level information is derived from sequencing amplified reads and assigning identities to those reads using curated sequence reference libraries (Taberlet et al. 2012). Metabarcoding data derived from aquatic eDNA samples are typically considered to be only semi-quantitative (Li et al. 2019; Blabolil et al. 2021; Russo et al. 2021; Stoeckle et al. 2021), partly because different DNA templates amplify with different efficiencies (primer bias; Collins et al. 2022), but also because there can be stochastic variation between PCR reactions, resulting in different copy numbers in PCR amplicons despite a homogenous DNA template (Beng & Corlett 2020; Griffin et al. 2020).

Despite these technical issues, there is increasing evidence from eDNA-based research on marine fish communities that eDNA-based metabarcoding can provide a good indication of the relative species abundance in the sampling area. Specifically, several studies have now shown significant positive associations between the abundance of species in the environment sampling using conventional methods (e.g. trawl surveys) and the relative abundance of species in eDNA (Collins et al. 2022). This relationship can, however, be affected by the various factors that influence the abundance of eDNA in the environment including whether a species is breeding, the activity levels of species (Ghosal et al. 2018; Murakami et al. 2021), the rate of eDNA decay (Collins et al. 2018; Murakami et al. 2019), and the presence of oceanographic features such as currents, fronts or stratification (Harrison et al. 2019). Therefore, the reliability of eDNA-based metabarcoding to reflect the relative abundance of species in the local communities is likely to vary considerably among marine habitats.

In principle eDNA-based methods may be particularly valuable to survey the biodiversity of habitats that are difficult to access, or species groups that are difficult to sample reliably using other means. In this study, we explore eDNA metabarcoding to study patterns of fish diversity across a depth gradient in the Southern Ocean. Although the high throughput DNA-based methods have been used to assess biodiversity of other species groups in the Southern Ocean (Lejzerowicz *et al.* 2014; Cowart *et al.* 2018; Flaviani *et al.* 2018; O'Rorke et al. 2022) to date, no studies have used eDNA-based methods to explore fish diversity of the region. In this study, we first explored the divergence of mesopelagic fish communities across depth gradients sampled using eDNA-based metabarcoding data and stratified net samples. We also evaluated the ability of eDNA-based metabarcoding to resolve the

diversity of surface samples across a larger latitudinal gradient. Finally, we explored spatial patterns of marine mammal "molecular bycatch" (*sensu* Mariani *et al.* 2021) in the metabarcoding dataset.

## 3.2 Methods

### *3.2.1 eDNA sampling – Community structure by depth and latitude*

We analysed a total of 127 environmental DNA samples collected during cruise JR16003 (British Antarctic Survey; December 2016 to January 2017) from 43 sampling stations. Samples were taken from multiple depths at seven of the stations (Table 3.1; Figure 3.1). using CTD water bottles (100 m, 300 m, 550-650 m and 850 m). Surface samples were collected from the same sampling locations using the ship's underway water supply. In addition, surface samples were collected from a further 11 surface locations during the same cruise (Table 3.1; Figure 3.1). At each depth or sampling location, typically three replicate water samples were collected and analysed.

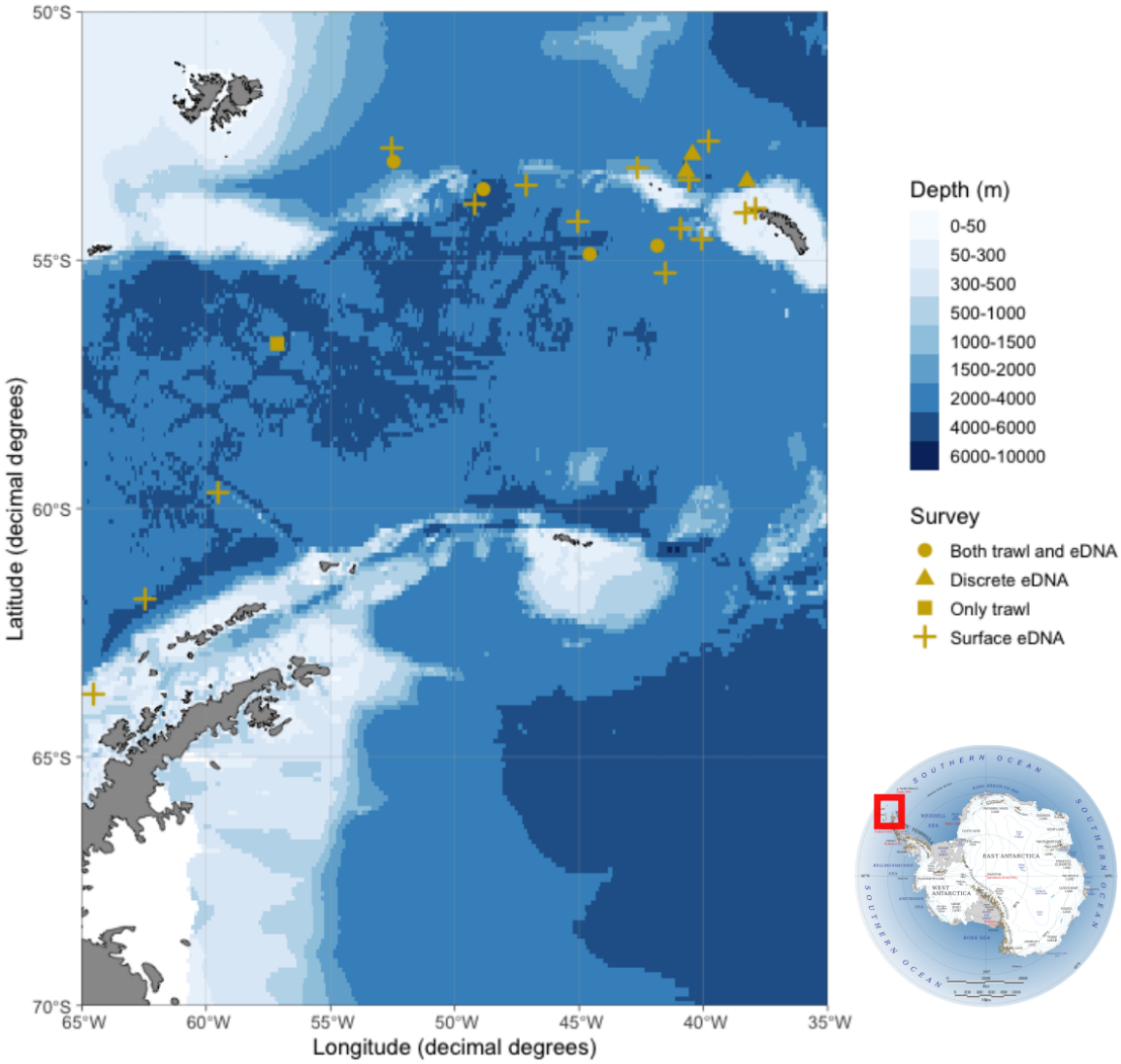



**Figure 3.1.** Sampling locations in the Southern Ocean. At locations indicated circles and triangles, eDNA sampling took place across the depth gradient using CTD bottles. Stratified trawling took place using RMT25 net. Surface eDNA sampling via the ship's underway water supply. Full sampling details are available in Table 3.1 and 3.2.

**Table 3.1.** Summary details of 43 eDNA sampling locations, including use of samples in analyses and total read counts of fish and mammals.

| In depth analyses - Code | In surface analyses | Event ID | Latitude | Longitude | Sampling depth | Replicates | Day | Month | Year | Total eDNA reads of fish | Total eDNA reads of mammals |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Yes – E73 | Yes | BAS-2016.12.27-Und-01 | -52.8079 | -40.1132 | 0 | 3 | 27 | 12 | 2016 | 1 | 1 |
| Yes – E73 | No | BAS-2016.12.27-E73-01 | -52.8079 | -40.1132 | 100 | 3 | 27 | 12 | 2016 | 44 | 4 |
| Yes – E73 | No | BAS-2016.12.27-E73-02 | -52.8079 | -40.1132 | 300 | 3 | 27 | 12 | 2016 | 1818 | 1 |
| Yes – E73 | No | BAS-2016.12.27-E73-03 | -52.8079 | -40.1132 | 550 | 3 | 27 | 12 | 2016 | 3 | 1056 |
| Yes – E73 | No | BAS-2016.12.27-E73-04 | -52.8079 | -40.1132 | 850 | 3 | 27 | 12 | 2016 | 4406 | 6 |
| Yes – E21 | Yes | BAS-2016.12.16-Und-01 | -52.8187 | -40.1364 | 0 | 3 | 16 | 12 | 2016 | 1137 | 3243 |
| Yes – E21 | No | BAS-2016.12.16-E21-01 | -52.8187 | -40.1364 | 100 | 3 | 16 | 12 | 2016 | 1491 | 48 |
| Yes – E21 | No | BAS-2016.12.16-E21-02 | -52.8187 | -40.1364 | 300 | 3 | 16 | 12 | 2016 | 112 | 2 |
| Yes – E21 | No | BAS-2016.12.16-E21-03 | -52.8187 | -40.1364 | 550 | 3 | 16 | 12 | 2016 | 2147 | 3 |
| Yes – E21 | No | BAS-2016.12.16-E21-04 | -52.8187 | -40.1364 | 650 | 3 | 16 | 12 | 2016 | 512 | 3 |
| Yes – E170 | Yes | BAS-2017.01.06-Und-01 | -53.2577 | -52.1684 | 0 | 3 | 6 | 1 | 2017 | 5837 | 2 |
| Yes – E170 | No | BAS-2017.01.06-E170-01 | -53.2956 | -52.1878 | 100 | 3 | 6 | 1 | 2017 | 3074 | 1 |
| Yes – E170 | No | BAS-2017.01.06-E170-02 | -53.2956 | -52.1878 | 300 | 3 | 6 | 1 | 2017 | 14 | 1 |
| Yes – E170 | No | BAS-2017.01.06-E170-03 | -53.2956 | -52.1878 | 550 | 3 | 6 | 1 | 2017 | 2 | 0 |
| Yes – E170 | No | BAS-2017.01.06-E170-04 | -53.2956 | -52.1878 | 850 | 3 | 6 | 1 | 2017 | 5241 | 1 |

**Table 3.1.** continued

| In depth analyses - Code | In surface analyses | Event ID | Latitude | Longitude | Sampling depth | Replicates | Day | Month | Year | Total eDNA reads of fish | Total eDNA reads of mammals |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Yes – E50 | Yes | BAS-2016.12.22-Und-01 | -53.7154 | -37.9642 | 0 | 3 | 22 | 12 | 2016 | 9777 | 4912 |
| Yes – E50 | No | BAS-2016.12.22-E50-01 | -53.7154 | -37.9642 | 100 | 3 | 22 | 12 | 2016 | 27594 | 4486 |
| Yes – E150 | Yes | BAS-2017.01.05-Und-01 | -53.9049 | -49.274 | 0 | 3 | 5 | 1 | 2017 | 1629 | 1572 |
| Yes – E150 | No | BAS-2017.01.05-E150-01 | -53.9049 | -49.274 | 100 | 3 | 5 | 1 | 2017 | 3423 | 2 |
| Yes – E150 | No | BAS-2017.01.05-E150-02 | -53.9049 | -49.274 | 300 | 3 | 5 | 1 | 2017 | 12796 | 5 |
| Yes – E150 | No | BAS-2017.01.05-E150-03 | -53.9049 | -49.274 | 550 | 3 | 5 | 1 | 2017 | 763 | 3 |
| Yes – E150 | No | BAS-2017.01.05-E150-04 | -53.9049 | -49.274 | 850 | 3 | 5 | 1 | 2017 | 1704 | 6 |
| Yes - E134 | Yes | BAS-2017.01.03-Und-01 | -54.538 | -45.0937 | 0 | 3 | 3 | 1 | 2017 | 724 | 6 |
| Yes - E134 | No | BAS-2017.01.03-E134-01 | -54.538 | -45.0937 | 100 | 3 | 3 | 1 | 2017 | 7946 | 4 |
| Yes - E134 | No | BAS-2017.01.03-E134-02 | -54.538 | -45.0937 | 300 | 3 | 3 | 1 | 2017 | 8465 | 36 |
| Yes - E134 | No | BAS-2017.01.03-E134-03 | -54.538 | -45.0937 | 550 | 3 | 3 | 1 | 2017 | 2485 | 3 |
| Yes - E134 | No | BAS-2017.01.03-E134-04 | -54.538 | -45.0937 | 850 | 3 | 3 | 1 | 2017 | 5177 | 5 |
| Yes – E94 | Yes | BAS-2016.12.30-Und-01 | -55.2443 | -41.2741 | 0 | 3 | 30 | 12 | 2016 | 4645 | 374 |
| Yes – E94 | No | BAS-2016.12.30-E94-01 | -55.2443 | -41.2741 | 100 | 2 | 30 | 12 | 2016 | 8829 | 1 |
| Yes – E94 | No | BAS-2016.12.30-E94-02 | -55.2443 | -41.2741 | 300 | 3 | 30 | 12 | 2016 | 1534 | 5 |
| Yes – E94 | No | BAS-2016.12.30-E94-03 | -55.2443 | -41.2741 | 550 | 3 | 30 | 12 | 2016 | 599 | 675 |
| Yes – E94 | No | BAS-2016.12.30-E94-04 | -55.2443 | -41.2741 | 850 | 3 | 30 | 12 | 2016 | 117 | 4 |

**Table 3.1.** continued

| Use in depth Analyses | Use in surface Analysis | Event ID | Latitude | Longitude | Sampling Depth | Replicates | Day | Month | Year | Total eDNA reads of fish | Total eDNA reads of mammals |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No | Yes | BAS-2016.12.14-Und-01 | -53.4828 | -47.1514 | 0 | 3 | 14 | 12 | 2016 | 4335 | 305 |
| No | Yes | BAS-2016.12.15-Und-01 | -53.4996 | -43.1409 | 0 | 2 | 15 | 12 | 2016 | 2819 | 1 |
| No | Yes | BAS-2016.12.23-Und-01 | -53.7574 | -37.6191 | 0 | 3 | 23 | 12 | 2016 | 50 | 7997 |
| No | Yes | BAS-2016.12.19-Und-01 | -53.7929 | -40.6673 | 0 | 3 | 19 | 12 | 2016 | 4 | 20 |
| No | Yes | BAS-2016.12.18-Und-01 | -54.4444 | -39.8757 | 0 | 3 | 18 | 12 | 2016 | 3794 | 8458 |
| No | Yes | BAS-2017.01.08-Und-01 | -59.5303 | -59.7675 | 0 | 3 | 8 | 1 | 2017 | 5615 | 3643 |
| No | Yes | BAS-2017.01.09-Und-01 | -62.0118 | -62.6962 | 0 | 3 | 9 | 1 | 2017 | 5415 | 2490 |
| No | Yes | BAS-2017.01.09-Und-02 | -63.1625 | -64.2067 | 0 | 3 | 9 | 1 | 2017 | 732 | 1 |
| No | Yes | BAS-2017.01.10-Und-01 | -65.4901 | -67.1774 | 0 | 3 | 10 | 1 | 2017 | 0 | 2 |
| No | Yes | BAS-2017.01.10-Und-02 | -66.4194 | -68.4399 | 0 | 3 | 10 | 1 | 2017 | 3 | 1 |
| No | Yes | BAS-2017.01.11-E175-01 | -67.5750 | -68.2441 | 5 | 3 | 11 | 1 | 2017 | 8163 | 168 |

Each water sample was prefiltered through a 250 µm nylon mesh, and then collected by bleach-cleaned 2 L Nalgene PE bottles. These bottles were kept in chilled containers until filtering. All samples were filtered through an 0.22 µm Sterivex-GP PES filter (SVGP01050; Merck Millipore, US), using a peristaltic pump at a rate of 150 mL/min (Masterflex, Cole Palmer). To remove residual water air was pumped through for a further 3 minutes. Field negative controls (n=14) were 2 L of MilliQ water and were processed the same as eDNA samples. Filter cartridges were then stored individually in sealed whirlpak bags at -20 °C before processing.

*3.2.2 eDNA extraction, PCR amplification and Illumina sequencing*

eDNA was extracted from the 0.22 µm Sterivex filters using DNeasy Blood & Tissue Kit (QIAGEN, Carlsbad, CA), following an optimised extraction protocol (Spens et al. 2017). Among three possible metabarcoding primers: MiFish-U/E primers (Miya et al., 2015), tele01 teleost fish primers (Valentini et al., 2016) and tele02 modified from (Taberlet et al. 2018), after comparing the PCR in *silico* test using crabs v0.1.2 (Jeunen et al., 2022; Supplementary Information Figure S3.1), we decided to use tele02 amplifying mitochondrial 12S gene. Primers were adapted with unique 8-mer sample-identifying barcode tags, identical on both the forward and reverse primer, and incorporating 2-4 random 5` bases to increase sequencing heterogeneity. Four PCR replicates were performed on each eDNA template, using the PCR methods described in Collins et al. (2022). Alongside the extracted eDNA samples and 14 field negatives (Table S4), we included 7 extraction negative controls, 5 negative no-template PCR controls and 9 blank controls (Supplementary Information Table S3.4). The eDNA extractions, pre-PCR preparations and post-PCR procedures were carried out in separate rooms.

The PCR product were pooled and purified using the MinElute PCR Purification Kit (Qiagen, Hilden, Germany) following manufacturer's protocol. Illumina sequencing adapters were attached to the amplicons using the Hyper Prep PCR-free (Kapa Biosystems Inc, Switzerland) following manufacturer's protocol. A total of two libraries using unique indexes were created. Libraries were then quantified using a NEBNext Library Quant kit (New England Biolabs, MA) and sequenced on an Illumina MiSeq using v2 (2 × 150 bp paired-end)

chemistry with a 10% phiX spike. Sequence data are available at the Sequence Read Archive BioProject PRJNA605313.

### *3.2.3 Bioinformatics*

Paired-end sequences were processed using obitools3 (Boyer et al. 2015). Raw sequences were aligned using the "alignpairedend" function. Alignment scores were calculated using "stats" function, and sequence pairs with alignment scores higher than 0.7 were retained. Sequences were then assigned to samples using the "ngsfilter" function, and dereplicated using the "uniq" function. Then these unique sequences were trimmed using the "annotate" function to remove tags. The "grep" function was then used to remove sequences with less than 10 replicates, and those shorter than 129 bp, which is the minimal length of the targeted metabarcode region (Taberlet et al. 2018). We then used the "clean" function to remove PCR/sequencing errors, setting the error rate at 0.05. The resulting data were exported, and identities assigned by comparing them to a customised reference database using BLAST (https://blast.ncbi.nlm.nih.gov).

The reference database was constructed and annotated using meta-fish-lib pipeline (Collins et al. 2021). Initially a species list of fish was constructed for the sampling region, 12S sequences were then downloaded from NCBI GenBank and the NCBI SRA archivers. In addition, 12S sequences were generated from tissue samples (*Gymnoscopelus braueri*, *Gymnoscopelus fraseri, Krefftichthys anderssoni and Electrona antarctica*) using the primers Aa22-PheF and Aa633-12SR as described in Collins et al. (2021). The final database also included all species in the NCBI RefSeq v206 reference library. Identities were confirmed by a close match to the reference sequence and previous records of the species being in the sampling region. The final species list was also evaluated based on negative controls and distributions of species in samples. If a species was found more than once across all eDNA samples, or a species was found in an eDNA sample but never in negative controls, it was considered as "detected".

### *3.2.4 Trawl surveys*

During cruise JR16003 trawl surveys took place at five stations using a Rectangular Midwater Trawl 25 net (RMT25, 5 mm mesh at cod end; Ref) at four stratified depth

intervals (20-200 m, 200-400 m, 400-700 m, 700-1000 m) (Table 2). All fish were identified to species level where possible. Average catch per unit effort (CPUE) was calculated as the average catch per cubic metre of water fished. One catch record assigned only to "Actinopterygii" was removed from downstream analysis.

**Table 3.2.** Trawl survey locations and depths.

| Event | Station | Depth (m) | Date | Start time | Trawl duration (minutes) | Event | Net | Latitude | Longitude | $m^3$ filtered | Corresponding eDNA sampling location |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 129 | APF2 | Stratified 20-200 | 03/01/2017 | 01:17:00 | 31 | 129 | 2 | -54.66 | -45.2 | 72484.9795 | E134 |
| 130 | APF2 | Stratified 200-400 | 03/01/2017 | 03:50:00 | 39 | 130 | 1 | -54.6 | -45.13 | 74653.5925 | E134 |
| 130 | APF2 | Stratified 400-700 | 03/01/2017 | 04:30:00 | 40 | 130 | 2 | -54.59 | -45.11 | 83836.8809 | E134 |
| 129 | APF2 | Stratified 700-1000 | 03/01/2017 | 00:35:00 | 41 | 129 | 1 | -54.68 | -45.22 | 87422.9671 | E134 |
| 146 | APF4 | Stratified 20-200 | 05/01/2017 | 00:25:00 | 42 | 146 | 2 | -53.95 | -49.19 | 68692.9357 | E150 |
| 147 | APF4 | Stratified 200-400 | 05/01/2017 | 02:48:00 | 39 | 147 | 1 | -53.96 | -49.24 | 60951.3508 | E150 |
| 147 | APF4 | Stratified 400-700 | 05/01/2017 | 03:28:00 | 39 | 147 | 2 | -53.94 | -49.25 | 74181.1014 | E150 |
| 146 | APF4 | Stratified 700-1000 | 04/01/2017 | 23:43:00 | 41 | 146 | 1 | -53.94 | -49.17 | 57619.6829 | E150 |
| 163 | APF6 | Stratified 20-200 | 06/01/2017 | 01:00:00 | 44 | 163 | 2 | -53.26 | -52.17 | 91687.5021 | E170 |
| 164 | APF6 | Stratified 200-400 | 06/01/2017 | 03:06:00 | 41 | 164 | 1 | -53.29 | -52.2 | 73018.0464 | E170 |
| 164 | APF6 | Stratified 400-700 | 06/01/2017 | 03:48:00 | 39 | 164 | 2 | -53.3 | -52.2 | 69456.1905 | E170 |
| 163 | APF6 | Stratified 700-1000 | 06/01/2017 | 00:20:00 | 40 | 163 | 1 | -53.25 | -52.16 | 78021.6059 | E170 |
| 113 | P2 | Stratified 20-200 | 01/01/2017 | 03:42:00 | 33 | 113 | 1 | -55.29 | -41.35 | 62768.6243 | E94 |
| 113 | P2 | Stratified 200-400 | 01/01/2017 | 04:16:00 | 27 | 113 | 2 | -55.3 | -41.36 | 54300.1301 | E94 |
| 171 | APF7 | Stratified 20-200 | 08/01/2017 | 01:32:00 | 41 | 171 | 1 | -56.71 | -56.85 | 99344.2808 | - |
| 171 | APF7 | Stratified 200-400 | 08/01/2017 | 02:14:00 | 9 | 171 | 2 | -56.73 | -56.86 | 26931.9922 | - |

*3.2.5 Data analysis - Community structure by depth*

To compare community structure among sampling zones in the trawl survey data, we $\log_{10}(x+1)$ transformed the CPUE data for each trawl. We then ordinated the community structure by generating a distance matrix based using vegdist function and the Bray Curtis index in the R package vegan 2.6-4 (Okansen et al. 2020), and then conducted a Principal Coordinate Analysis using the package ape 5.6-2 (Paradis and Schliep 2019). To test for differences between sampling depths and stations, we used a two-way Permanova with 10,000 permutations using the adonis2 function in vegan. This was followed by pairwise post-hoc tests using the pairwise.adonis 0.4 with 10,000 permutations (Martinez Arbizu, 2020).

We conducted two sets of analyses to compare the community structure among depth zones in the eDNA samples, the first using all species recovered in the eDNA, and the second focussed on species known to occupy deep water pelagic habitats [classified as "benthopelagic" in Fishbase (Froese & Pauly 2022)]. Both datasets were "Hellinger" transformed using the decostand function in vegan. Following this, Principal Coordinates Analysis, Permanova and pairwise post-hoc tests were conducted as described above for the trawl survey data.

*3.2.6 Data analysis - Testing for associations between read abundance and CPUE*

We tested for an association between read abundance in eDNA and mesopelagic fish abundance in trawls. We restricted this analysis to eDNA data only from those taxa known to occupy deep water pelagic habitats (classified as "benthopelagic" in Fishbase), and only to those sampling events that had a corresponding net catch. We then used a generalised linear mixed-effects model (GLMM) approach using glmmTMB 1.1.5 (Brooks et al. 2017), broadly following Collins et al. (2022). The model used the untransformed total number of reads assigned to a taxon in an eDNA sample as the response variable, while the $\log_{10}$ transformed total number of fish reads from a sample was used as an offset. Due to the large number of zero data points, we used a negative binomial family distribution with a zero-inflated component. The fixed factor was scaled CPUE data for the taxon in the corresponding trawl. We used "sampling event" and "species" as random factors.

We explored the associations between the total number of eDNA reads of each taxon group to their total CPUE in the net samples and their total frequency of occurrence in net samples. For these analyses we only considered eDNA sampling events that had a corresponding net catch, and included taxa known to occupy deep water pelagic habitats and were represented in the eDNA reference library.

*3.2.7 Data analysis - Species distributions in surface eDNA*

To evaluate the potential of surface eDNA to provide information on species distributions we tested for the presence of fish community structure between northeasterly sites (north of 55°S and east of 58°W) and the southeasterly sites in the vicinity of the Antarctic Peninsula (south of 52°S and west of 58°W. As described above for analysis of community structure using eDNA data, data were "Hellinger" transformed enabling Principal Coordinates Analysis for ordination and Permanova for statistical testing. We also plotted the $\log_{10}(x+1)$ read counts of the ten most abundant fish taxa, and all ten marine mammal taxa recovered in eDNA metabarcoding data. Maps were generated using ggOceans 1.3.7 (Vihtakari 2022).

**3.3 Results**

*3.3.1 eDNA sequencing and negative controls*

A total of 31,721,988 raw sequencing reads were generated for two libraries (Supplementary Information Table S3.1). After filtering, error removal, taxonomy assignment, removal of non-fish species there were a total of 154,976 reads assigned to native fish taxa across 127 samples (Table 3.1). In addition, a total of 39,557 reads assigned to native marine mammal taxa were recorded from the 127 samples, as "molecular bycatch" (Mariani et al. 2021). In total across the 127 samples, we detected a total of 29 fish taxa (24 identified at the species level) and 10 marine mammal taxa (8 identified at the species level) (Supplementary Information Table S3.2). Of 35 negative controls, 34 yielded less than 10 contamination reads each, with the exception of one field blank that yielded a total of 1721 reads (Supplementary Information Table S3.4).

*3.3.2 Fish community structure by depth in net catches*

In total the 19 taxa were sampled by the RMT across 16 sampling events. The net samples were dominated by myctophid species, comprising on average 74.43% of the CPUE (95% confidence intervals 60.52 to 88.35%). Overall, there were significant difference in fish community structure across the four depth zones (Table 3.3; Figure 3.2). In *post-hoc* comparisons, there were significant differences between all depth strata, except for the two deepest strata (400-700 m vs 700-100 m; Table 3.4; Figure 2). Myctophidae were broadly distributed across depth zones, except for *G. braueri* that declined in abundance with increasing depth. The bathypelagic taxa including the deep-sea smelts (Bathylagidae), pearleyes (*B. elongata* and *L. macropinna*) and bristlemouths (*Cyclothone* sp.) were primarily found in the samples from greater than 400 m depth. (Figure 3.3).

*3.3.3 Fish community structure by depth in eDNA samples*

Across 85 eDNA samples for the depth-focused analyses there were a total of 28 species recovered. These eDNA reads were dominated by a group of 14 meso-bathypelagic taxa, which comprised 66.11% of the reads of all fishes. These meso-bathypelagic taxa included myctophids (*E. antarctica, G. fraseri, G. nicholsi, K. anderssoni*), longfish ice devil (*A. mitopteryx*; a pelagic icefish) and deep-sea smelts (*Bathylagidae sp*.). Other abundant taxa in the eDNA reads were marbled rockcod (*N. rossii*; a demersal icefish), longtail southern cod (*P. ramsayi*; a benthopelagic icefish) and a representative of the genus *Lampris* (opahs, pelagic lampriforms) identified only to genus level.

Using the full dataset of all 28 recovered taxa in the eDNA there was a highly significant, but gradual shift in community structure across the depth zones (Table 3.3, Figure 3.2). In *post-hoc* comparisons significant differences were present between the surface samples and all deeper water samples, and between the samples collected at 100 m and samples collected from the more extreme depth (550-650 m, 850 m). Samples collected from 300 m, 550-650 m and 850 m contain more species homogenous fish communities (Table 3.4, Figure 3.2). Focusing on the 14 meso-bathypelagic taxa, a similar significant pattern of community structuring was present to that of the full set of 28 recovered taxa, albeit less prominent than in the full set of 28 recovered taxa (Table 3.3, Figure 3.2). In *post-hoc* comparisons significant differences were only present between the surface and 100, surface and 850, and

between the 100 m and 550-650 m sample set (Table 3.4, Figure 3.2). Exploring the changes in the proportion of species within reads across sampling depths (Figure 3.3) revealed relative increases in some species with increasing depth such as the myctophids *E. antarctica* and *P. bolini,* and deep-sea smelts (Bathylagidae). There were also declines in the proportions of reads of some species including the myctophid *G. fraseri* and the pelagic icefish *A. mitopteryx.* Notably some species were exclusively found in samples from deep waters including the myctophid *L. achirus*. There were also examples of deep water or demersal taxa being found in surface waters, including Patagonian toothfish (*D. eleginoides*), black icefish (*C. aceratus*), Antarctic dragonfish (*P. charcoti*) and barbeled plunderfish (*Pogonophyne* sp.).

**Table 3.3.** Global statistical differences in fish community structure between depths and stations, using RMT net sampling and eDNA metabarcoding, using Permanova.

| Dataset | Factor | Df | SS | $r^2$ | *F* | *P* |
|---|---|---|---|---|---|---|
| Meso-bathypelagic fishes (RMT) | Site | 4 | 1.1714 | 0.28274 | 1.7552 | 0.035 |
| | Depth | 3 | 1.6369 | 0.39509 | 3.2702 | < 0.001 |
| | Residual | 8 | 1.3348 | 0.32218 | | |
| | Total | 15 | 4.1431 | 1 | | |
| All fishes (eDNA) | Depth | 4 | 2.758 | 0.08578 | 1.9557 | 0.003 |
| | Station | 6 | 4.009 | 0.12467 | 1.8948 | 0.001 |
| | Residual | 72 | 25.387 | 0.78955 | | |
| | Total | 82 | 32.154 | 1 | | |
| Meso-bathypelagic fishes (eDNA) | Depth | 4 | 2.5281 | 0.08862 | 1.8978 | 0.009 |
| | Station | 6 | 3.6871 | 0.12924 | 1.8452 | 0.005 |
| | Residual | 67 | 22.3136 | 0.78214 | | |
| | Total | 77 | 28.5288 | 1 | | |

**Table 3.4.** Pairwise tests of differences in community structure by depth using RMT net sampling and eDNA metabarcoding, using Permanova.

| Dataset | Depth 1 | Depth 2 | SS | $r^2$ | *F* | *P* |
|---|---|---|---|---|---|---|
| Meso-bathypelagic fishes (RMT) | 20-200 m | 200-400 m | 0.534 | 0.266 | 2.897 | **0.034** |
| | 20-200 m | 400-700 m | 0.694 | 0.394 | 3.906 | **0.019** |
| | 20-200 m | 700-1000 m | 0.811 | 0.392 | 3.868 | **0.019** |
| | 200-400 m | 400-700 m | 0.557 | 0.341 | 3.101 | **0.018** |
| | 200-400 m | 700-1000 m | 0.804 | 0.388 | 3.802 | **0.017** |
| | 700-1000 m | 400-700 m | 0.112 | 0.115 | 0.522 | 0.700 |
| All fishes (eDNA) | 0 m | 100 m | 0.951 | 0.056 | 2.133 | **0.005** |
| | 0 m | 300 m | 0.703 | 0.049 | 1.588 | **0.050** |
| | 0 m | 550-650 m | 0.944 | 0.062 | 2.189 | **0.011** |
| | 0 m | 850 m | 0.942 | 0.069 | 2.163 | **0.004** |
| | 100 m | 300 m | 0.477 | 0.030 | 1.025 | 0.429 |
| | 100 m | 550-650 m | 1.009 | 0.060 | 2.227 | **0.001** |
| | 10 0m | 850 m | 0.710 | 0.047 | 1.544 | **0.040** |
| | 300 m | 550-650 m | 0.627 | 0.044 | 1.392 | 0.128 |
| | 300 m | 850 m | 0.318 | 0.026 | 0.693 | 0.863 |
| | 550-650 m | 850 m | 0.660 | 0.050 | 1.488 | 0.081 |
| Meso-bathypelagic fishes (eDNA) | 0 m | 100 m | 0.734 | 0.047 | 1.617 | **0.033** |
| | 0 m | 300 m | 0.584 | 0.041 | 1.279 | 0.150 |
| | 0 m | 550-650 m | 0.663 | 0.043 | 1.477 | 0.086 |
| | 0 m | 850 m | 0.726 | 0.054 | 1.602 | **0.022** |
| | 100 m | 300 m | 0.454 | 0.032 | 0.968 | 0.500 |
| | 100 m | 550-650 m | 0.848 | 0.054 | 1.842 | **0.009** |
| | 100 m | 850 m | 0.583 | 0.044 | 1.249 | 0.160 |
| | 300 m | 550-650 m | 0.667 | 0.047 | 1.440 | 0.063 |
| | 300 m | 850 m | 0.304 | 0.026 | 0.644 | 0.968 |
| | 550-650 m | 850 m | 0.640 | 0.049 | 1.389 | 0.087 |

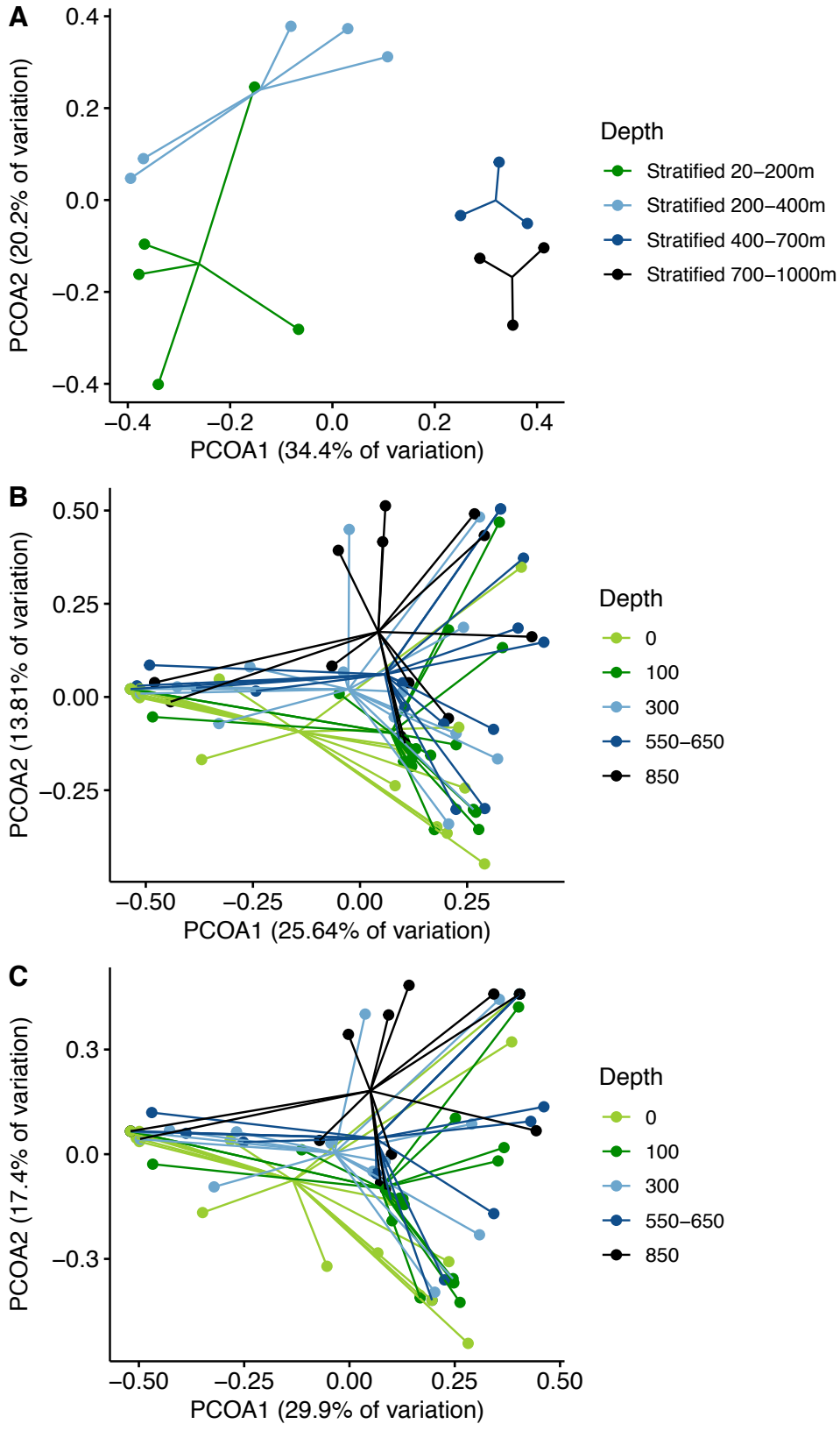



**Figure 3.2.** Comparisons of fish community structure between depths and stations (Principal Coordinate Analysis), using a) RMT net sampling, b) eDNA metabarcoding (all recovered fish) and c) eDNA metabarcoding (only meso-bathypelagic fishes).

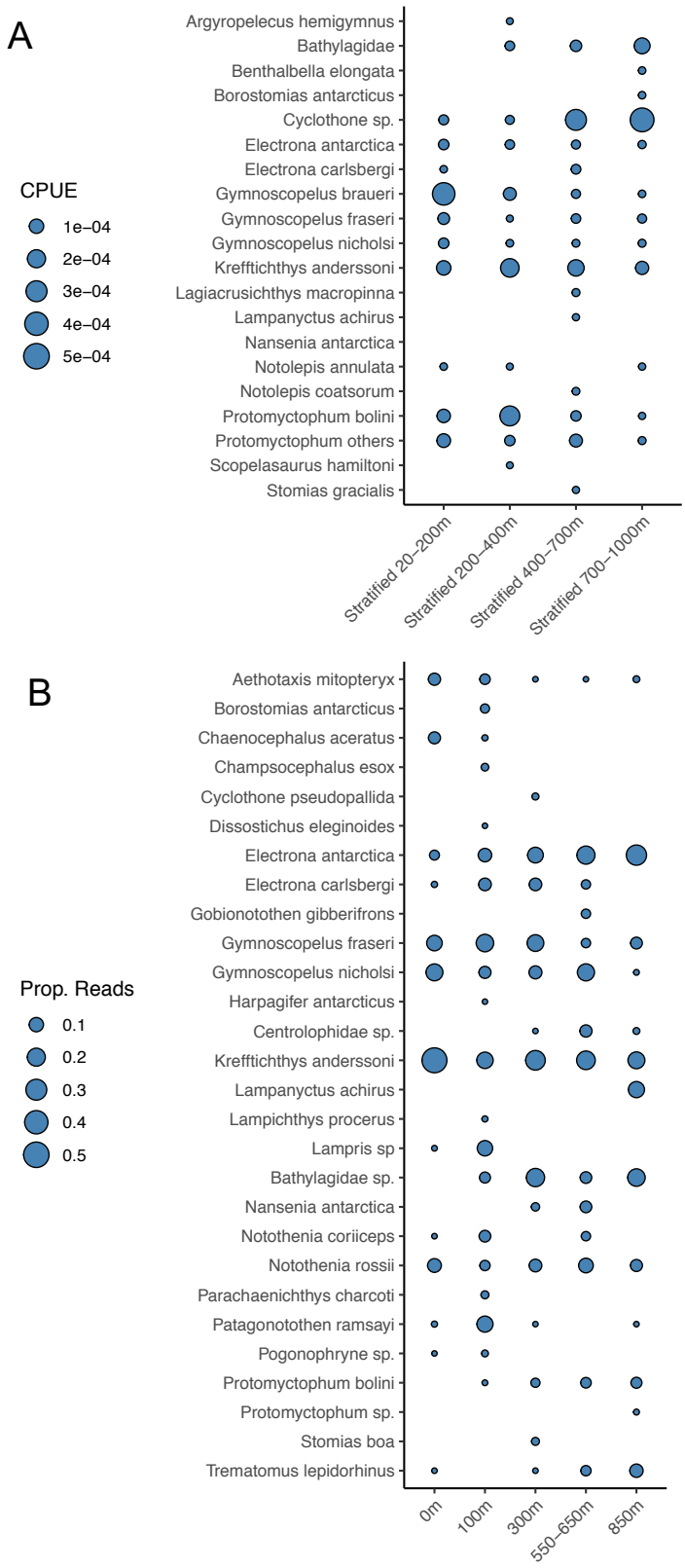



**Figure 3.3.** a) Mean catch per unit effort of each taxon in the RMT catches at each depth across all sampling stations. b) Mean proportion of reads in samples at each depth across sampling stations.

*3.3.4 Associations between species abundance in net samples and eDNA reads.*

Samples were conducted at four stations and across four depth zones with both eDNA methods and trawling (Tables 3.1 and 3.2). Across a total of 33 eDNA samples that contained reads, we found no evidence that CPUE predicted an increased eDNA read count for the 14 meso-bathypelagic taxa present in the eDNA dataset (CPUE estimate = 0.2124. $z$ = 0.633, $P$ = 0.527)

We compared the total number of reads across all eDNA samples that were linked to net sampling events for the 16 meso-bathypelagic taxa that were present in either the eDNA or the net survey data, and for which a reference eDNA sequence was available. We found a non-significant positive association between CPUE in net surveys and eDNA reads ($F_{1,14}$ = 0.8823, $r^2$ = 0.059, $P$ = 0.365), and a non-significant positive association between frequency of occurrence of species in net surveys and eDNA reads ($F_{1,14}$ = 2.10, $r^2$ = 0.131, $P$ = 0.169).

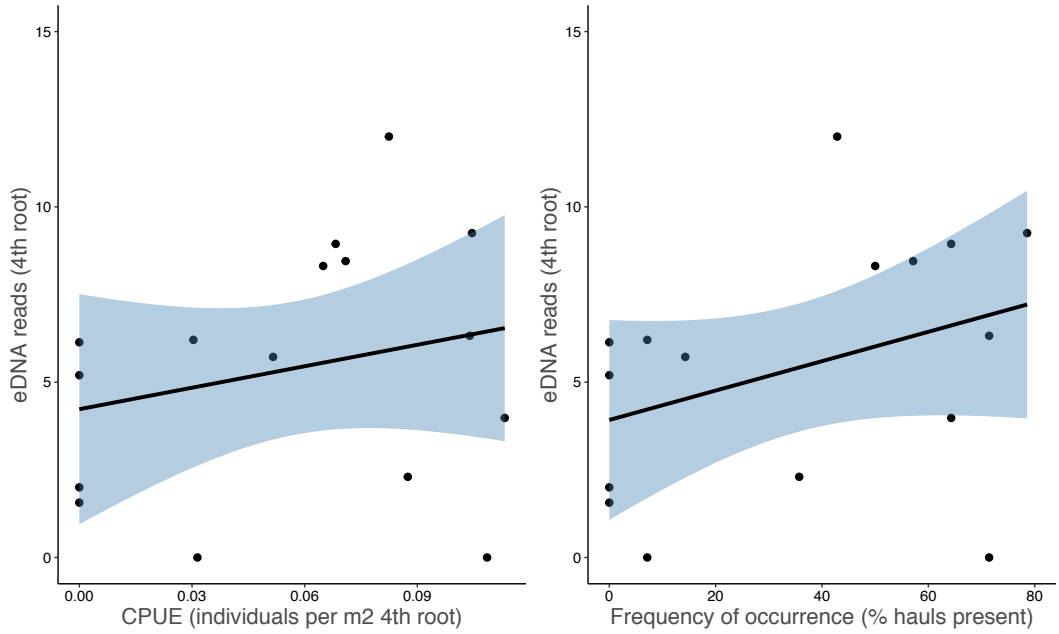



**Figure 3.4.** Associations between eDNA read abundance and a) mean catch per unit effort of each taxon in the RMT catches at each depth across sampling stations. b) frequency of occurrence of each taxon in the RMT catches at each depth across sampling stations. Analyses only include events where eDNA samples (n = 44) and net samples (n = 14) were taken.

*3.3.5 Spatial patterns of species recorded in surface reads*

In total we observed 18 fish taxa in the eDNA samples from the 53 surface samples from 18 sampling stations. We found no clear association between community composition between the more northeasterly sites (north of 55°S and east of 58°W) and the southeasterly sites in the vicinity of the Antarctic Peninsula (south of 52°S and west of 58°W) (Permanova $F_{1,40}$ = 1.371, $P$ = 0.226). Plotting the distribution of the ten most common species (Figure 3.6) showed that some deep-water species including Patagonian toothfish (*D. eleginoides*), black icefish (*C. aceratus*) were on only recorded in the more northerly samples, open water mesopelagic myctophids including *E. antarctica*, *E. carlsbergi* and *G. nicholsi* and *K. anderssoni* were absent from samples in the shelf seas of the Antarctic peninsula. By contrast the Antarctic spiny plunderfish *H. antacticus* was only found in a sample proximate to the Antarctic peninsula.

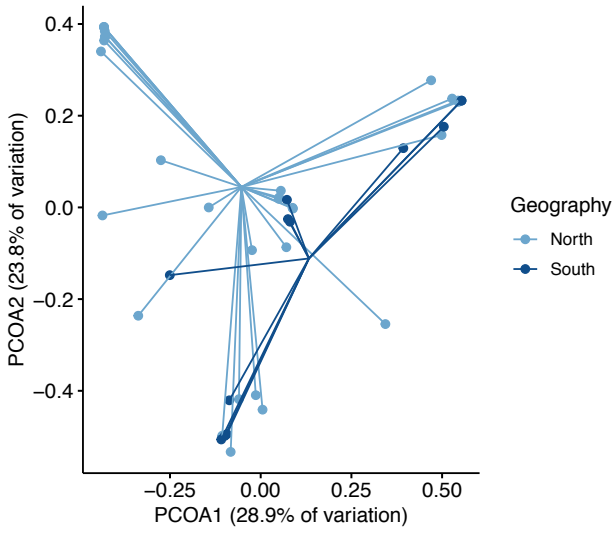



**Figure 3.5.** Comparison of fish community structure in surface samples between stations in the northeasterly and southwesterly sampling location, using Principal Coordinate Analysis.

*3.3.6 Marine mammal "molecular bycatch"*

We assigned species-level identities to eight marine mammal species. Reads of Antarctic fur seal (*A. gazella*) had a broad distribution but were most abundant in the vicinity of South Georgia. Reads of southern elephant seal (*M. leonine*) and crabeater seal (*L. carcinophaga*) were exclusively in the northern sector of the sampling region, while reads of Weddell seal

were only found proximate to the Antarctic Peninsula. Southern right whale (*E. australis*) and humpback whale (*M. novaengliae*) were only found in the northerly samples. Reads from the fin whale (*B. physalus*) and Antarctic minke whale (*B. bonaerensis*) had broader distributions. Reads assigned to the bottlenose whales (*Hyperoodon* sp.) which can most plausibly be assigned to the southern bottlenose whale (*H. planifrons*) were found only in a single more northerly sample. Similarly reads assigned to the white-sided dolphins (*Lagenorhynchus* sp.) were also restricted to a single more northerly sample. These reads most plausibly belong to Peale's dolphin (*L. australis*), hourglass dolphin (*L. cruciger*) or dusky dolphin (*L. obscurus*).

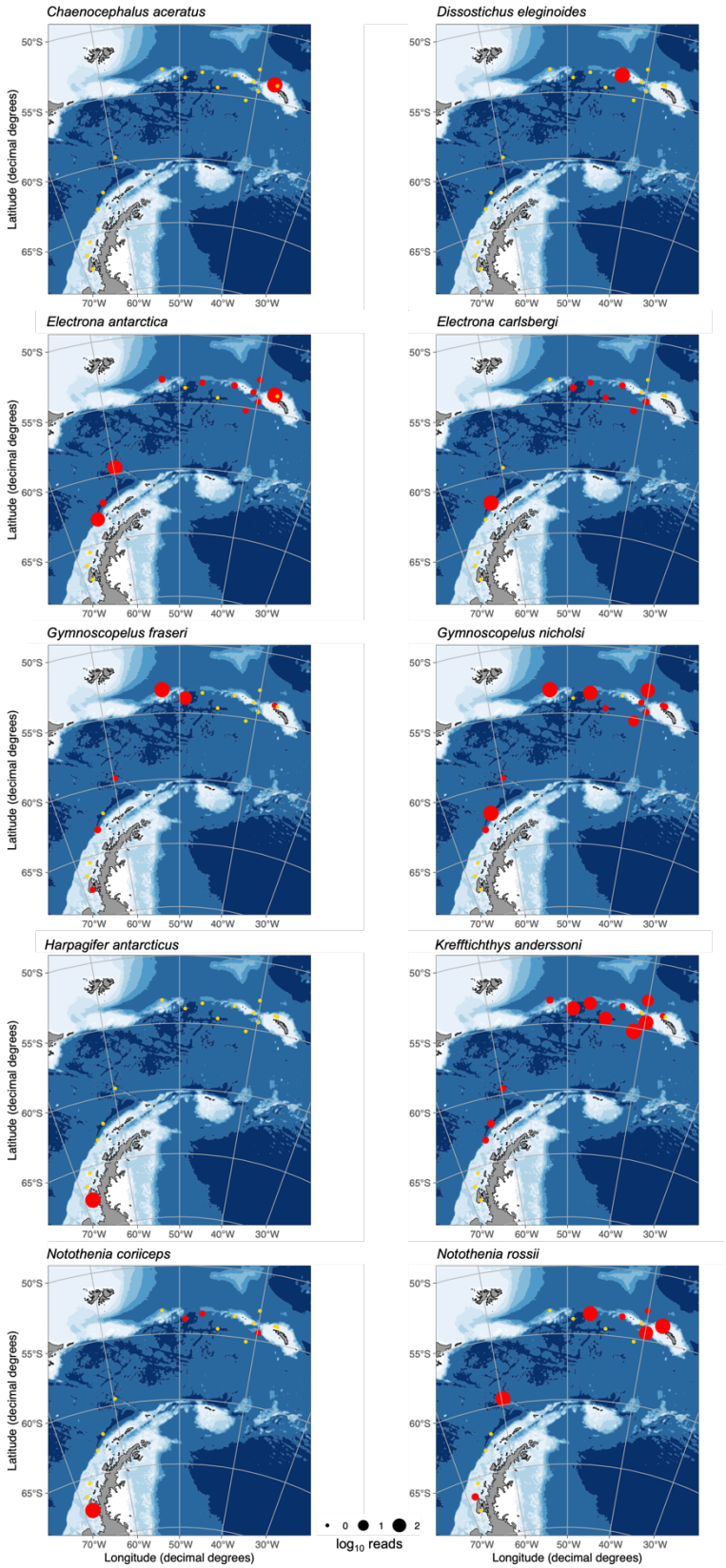



**Figure 3.6.** Average eDNA metabarcoding read abundance in surface collected samples, for the ten most abundant fish species. Red circles indicate presence, size is $\log_{10}(x+1)$ transformed abundance. Gold circles indicate absence.

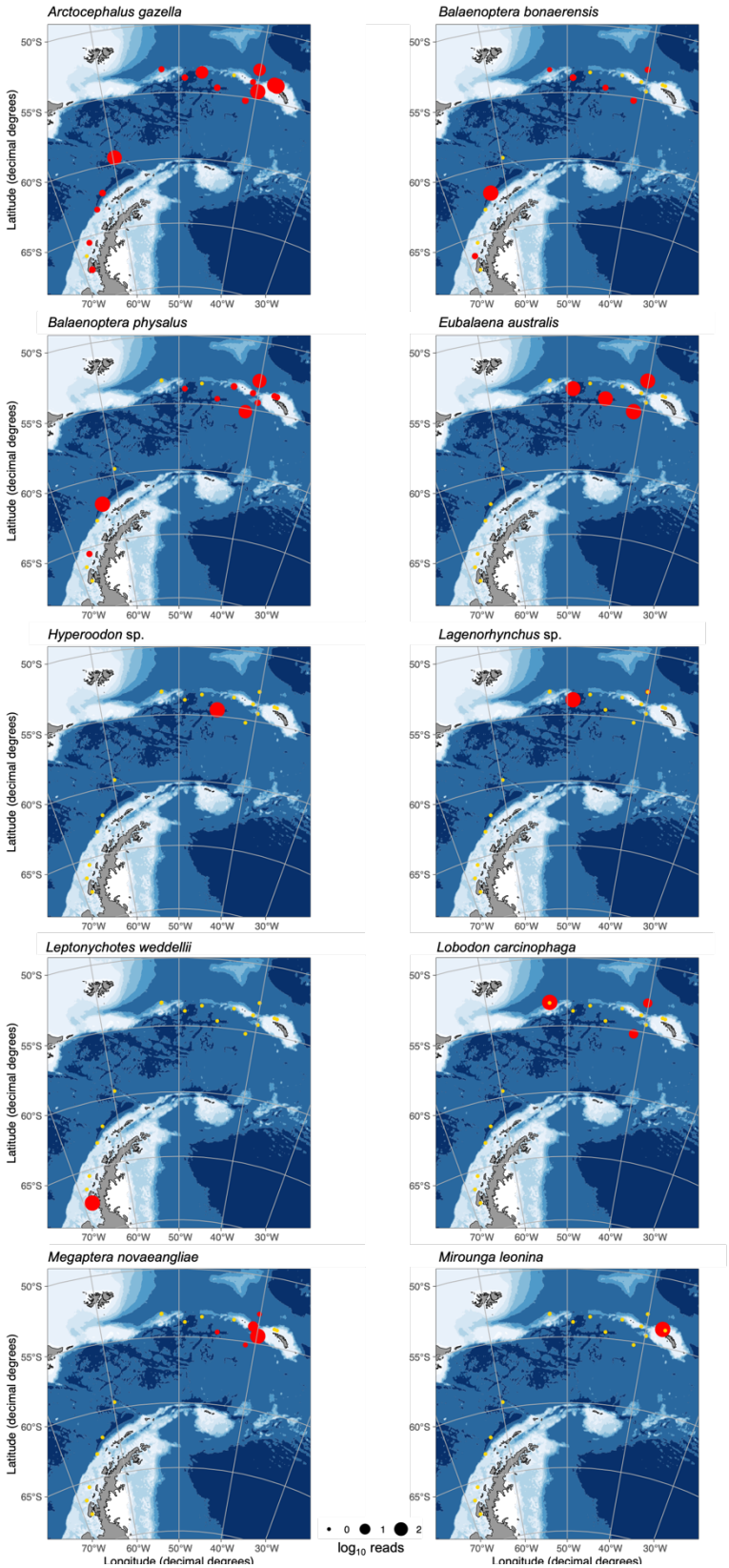



**Figure 3.7.** Average eDNA metabarcoding read abundance in all samples, for ten marine mammal taxa observed as "molecular bycatch". Red circles indicate presence, size is $\log_{10}(x+1)$ transformed abundance. Gold circles indicate absence.

## 3.4 Discussion

This study demonstrates eDNA metabarcoding has the capability to provide information on the distributions of fish and mammal species in oceanic environments, and can be used to map their distributions over large geographic scales of hundreds of km, and over depth gradients. While the study has provided biological insight into the distribution of the DNA of species, it has also revealed multiple issues that need to be considered in designing and implementing further research using eDNA-based methods for studying the marine fish and mammal distributions.

### *3.4.1 Inferring mesopelagic fish abundance from eDNA data*

Our study demonstrated environmental DNA of key mesopelagic species is present in the samples taken from the Southern Ocean. We also found evidence of structuring in the assemblage over depth using eDNA samples, a pattern broadly expected given depth-based structuring of mesopelagic and bathypelagic species of the region that was also present in the RMT data. However, there was no clear segregation of the species by depth zones as we see in net capture data (Collins et al. 2012); eDNA samples were considerably more homogeneous in species composition. We also found no evidence of a link between the abundance of fish captured in RMT trawl nets and eDNA abundance. In part this decoupling of fish abundance from eDNA may be linked to mixing and transport of eDNA in the water column, although recent modelling work has suggested these effects may be relatively modest (Allan et al. 2021). The pattern may instead be driven by the diurnal vertical migrations of many mesopelagic fishes that may lead to eDNA being deposited across the depths over daily cycles (Collins et al. 2012). Although eDNA degrades at an exponential rate, it has a half-life of 1-2 days in temperate conditions (Collins et al. 2018), which may be longer in the colder conditions of the Southern Ocean, given evidence of greater persistence times in cooler waters (McCartin et al. 2022).

The distributions of eDNA in the environment may also be linked to contributions from eggs and larval fish in surface waters. Our surveys took place during December-January, a period of the year when larval fish, including pelagic myctophids and demersal notothenioids, are reliably present in the Southern Ocean (Loeb et al. 1993; Saunders et al. 2017). Breeding seasonality has been found to influence the number of eDNA reads in samples in temperate

marine environments, with a greater number of reads in during the breeding season (Collins et al. 2022). Thus, generation of eDNA from eggs and larvae may break down expected positive associations between the abundance of larger fishes caught in nets (adults and subadults) and the abundance of eDNA sampled from the local environment. A contribution of eggs and larvae to eDNA is also a plausible explanation for the presence of reads belonging to species that are exclusively demersal as adults in eDNA samples collected from surface waters. Such species include, for example, the Antarctic spiny plunderfish (*H. antarcticus*) and black icefish (*C. aceratus*). In principle, further research could quantify the contributions of the ichthyoplankton to aquatic eDNA signatures by comparing the metabarcode read compositions of homogenised ichthyoplankton net samples to those of aquatic DNA collected using the methods in this study.

### *3.4.2 Spatial records of fish abundance.*

Our spatial records of fish taxa comprehensively matched known biogeographic ranges of the species. The typically sub-Antarctic Patagonian toothfish (*D. eleginoides*) were recorded in eDNA samples from the northern sector of the Scotia Sea, where it supports a fishery (Shust and Kozlov, 2016). Similarly reads from the blackfin icefish were recorded around South Georgia, where it is bycatch in commercial fisheries for krill and mackerel icefish (*Champsocephalus gunnari*) (Reid et al. 2007). The myctophids *E. antarctica*, *E. carlsbergi*, *G. fraseri*, *G. nicholsi* and *K. anderssoni* all had a broad distribution in eDNA samples across oceanic waters, consistent with expectations from distributions and ecological niche models (Freer et al. 2019). Reads from the marbled rockcod (*N. rossii*) and black rockcod (*N. coriiceps*) are compatible with their known broad distributions across the sampled region (Calì et al. 2017). Collectively, these results suggest that occurrence records provided by eDNA metabarcoding can be reliable indicators of the presence of the species.

### *3.4.3 Requirements for reference library*

Where comprehensive reference libraries are available eDNA metabarcoding can be highly successful at describing species richness of aquatic habitats (Closek et al. 2019; Fraija-Fernández et al. 2020). Incomplete reference databases are however a significant limitation on the capability of eDNA metabarcoding studies (Collins et al. 2021; Lim & Thompson, 2021). We compiled a list of 353 fish species that have been recorded in the Southern Ocean

and could plausibly be present in eDNA reads. However, only 22.63% of these had appropriate sequences of our target 12S region of the mitochondrial genome available for inclusion in our analyses, including from NCBI Genbank and SRA databases (Supplementary Information Table S3.5). Hence, our analyses are likely to have considerably underestimated the diversity of species present in the metabarcoding reads.

### *3.4.4 Marine mammal distributions*

Our study generated metabarcoding reads that we were able to reliably assign to marine mammal species, despite targeting teleost fish. Thus, our results are comparable with those of other fish-focused studies that have been able to generate "bycatch" data on the occurrences of non-target vertebrate groups including mammals and birds (Mariani et al. 2021; Ritter et al., 2022). Where reads could reliably be assigned to species, our results typically fit within known biogeographic ranges. The distribution of reads assigned to Antarctic minke whale (*B. bonaerensis*) and fin whale (*B. physalus*) are reflective of known occurrences in recent years (Lee et al. 2017; Herr et al. 2022), while the distributions of reads assigned to southern right whale (*E. australis*) and humpback whale (*M. novaengliae*) are in relatively close proximity to recent observations of these species around South Georgia (Jackson et al. 2020). Our observation of reads from Antarctic fur seal (*A. gazella*) match the distribution of breeding colonies on South Georgia during the Austal summer breeding season (e.g. Hooker et al. 2015), while reads present around the Antarctic Peninsula match the distribution of non-breeding individuals are during the same period of the year (Casaux et al. 2003). Our observations of reads assigned to the southern elephant seal (*M. leonina*) at South Georgia and the Weddell seal (*L. weddellii*) on the Antarctic Peninsula are compatible with known ranges (Boyd et al. 1996; Larue et al. 2021). Only reads assigned to crabeater seals contrast with expectations; they suggest habitat use in the northern sectors of our sampling range. Although this krill feeding species is known to be pelagic, it is typically associated with pack ice of the Antarctic continental waters (Hückstädt et al. 2020). Thus, these reads may suggest more extensive use of open pelagic waters than is typically associated with the species.

### *3.4.5 The usage and concerns of eDNA bycatch*

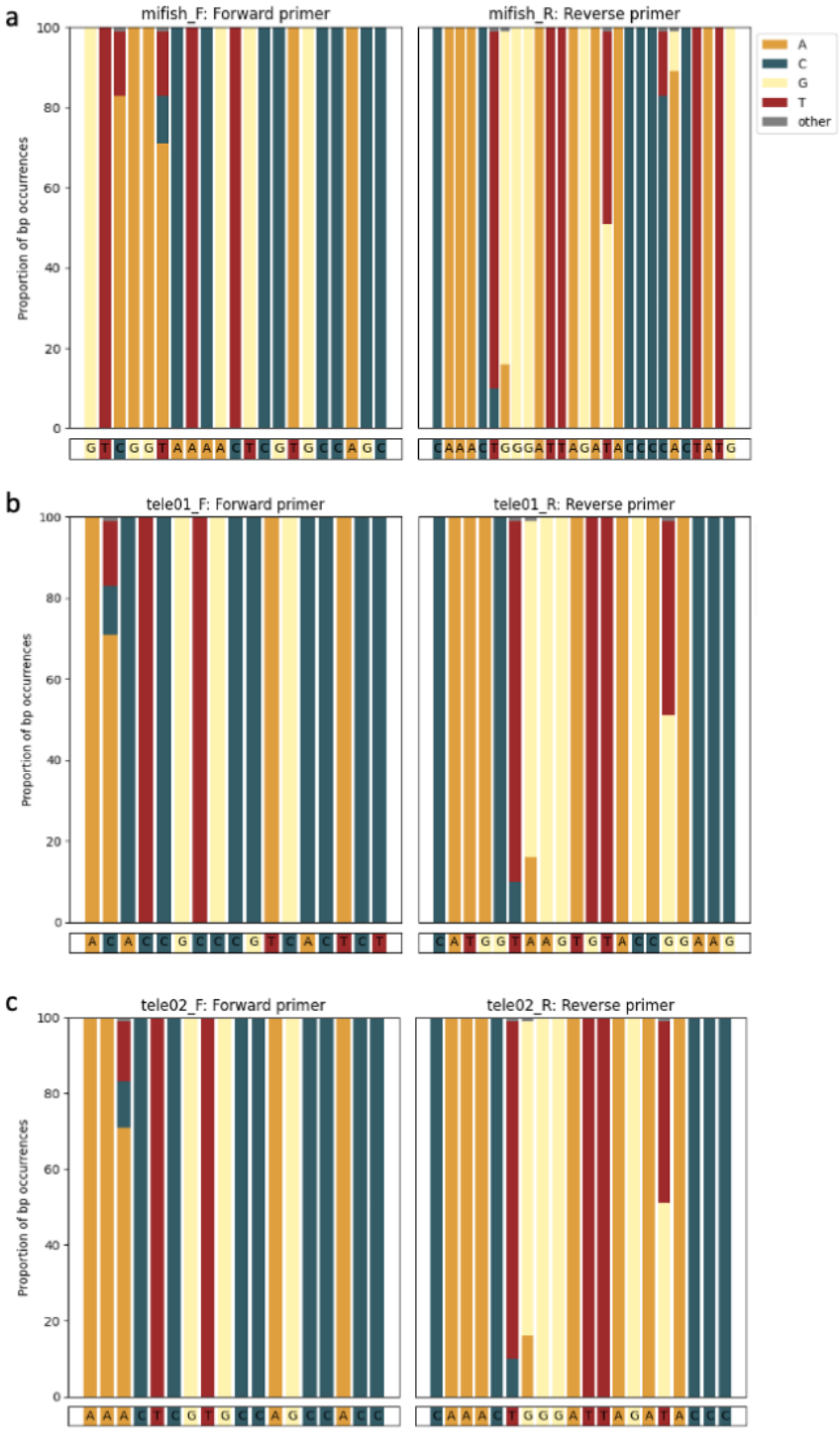
A literature search using Web of Science (9th July 2023) and the search terms "environmental DNA", "Antarctica" and "metabarcoding" revealed a total of 61 papers, with evidence of a growing use of these methods for understanding the biodiversity of the region (Supplementary Information Figure S3.2). Our eDNA "bycatch" data provided information on the distribution of several highly mobile marine mammals in the Southern Ocean, a region where spatial distributions of marine megafauna are not comprehensively understood (Griffiths et al., 2010). Since metabarcoding methods can successfully amplify non-target species, the presence of such bycatch reads is likely to be commonplace in eDNA metabarcoding studies. This has been clearly shown in previous studies where metabarcoding using teleost fish primers have provided insight into the spatial and temporal distribution of multiple bird and mammal species (e.g. Mariani et al., 2021). Hence, researchers may be able to investigate eDNA metabarcoding data more broadly to obtain biodiversity information for non-target species groups, including from those previous studies that focused on Antarctic habitats.

There is potential for eDNA bycatch data to be misused. Researchers pointed out the concern over ethics and privacy risks of eDNA-derived data of human origin. A study focused on identifying herpes virus infections that cause tumours in sea turtles extracted DNA from sand where sea turtles nest, then they found that human genetic bycatch (HGB) recovered from eDNA samples was intact enough for recognizing X and Y chromosomes. Such environmental samples collected from water and air are all capable of providing high-quality human eDNA which contains information on sex, ancestry and vulnerability to disease (Whitmore et al., 2023). A commentary published in Science discussed this issue and reported that at least one researcher is considering withdrawing raw sequencing data from public databases to avoid potential violation of privacy (Vogel, 2023). If researchers are unable to share eDNA samples and data, this would constrain future research. Therefore, implementation of protocols that allow sharing of eDNA samples and data, while preventing misuse of human-derived data, may be necessary as the field of research develops.

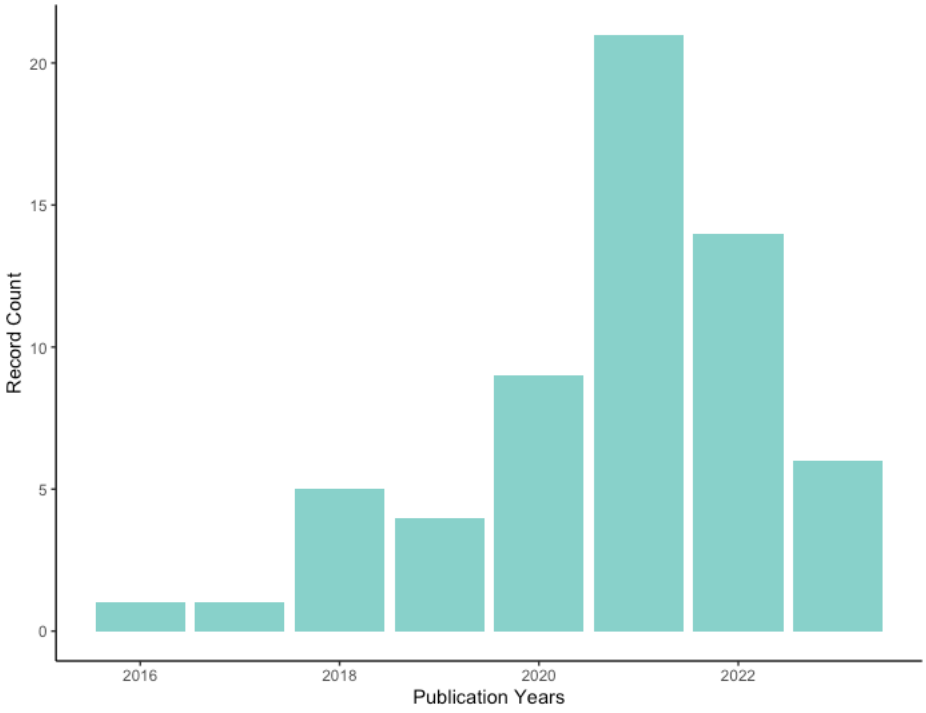
### *3.4.6 Concluding remarks.*

This study has demonstrated the ability of eDNA to detect mesopelagic fish diversity across space and provide insight into the geographic distribution of mammals. Our analyses would have likely yielded more information if a greater volume of water had been filtered and if samples had been sequenced at higher coverage. Moreover, it seems likely that with the additional use of primers that specifically target marine mammals a greater coverage of their diversity could be achieved. Such future research using eDNA will benefit considerably from the development of comprehensive reference libraries of target eDNA sequences. This needs to be a priority if the potential of eDNA-based methods for monitoring and management of vertebrate species in the Southern Ocean is to be realised.

**Supporting Information**




**Supplementary Information Figure S 3.1.** Mismatches between primers and targeted fish species in the Southern Ocean.




**Supplementary Information Figure S 3.2.** All published environmental DNA research conducted in Antarctica. Created using a Web of Science search of publications with the *topic*: "environmental DNA" *and* " Antarctica" *and* "metabarcoding", the number of publications was counted in each publication year since the first paper was published in 2016 till the date 9$^{th}$ July 2023.

**Supplementary Information Table S 3.1.** Numbers of reads remaining after filtering and assignment steps

| Filtering Step | Library 1 | Library 2 | Total Number |
|---|---|---|---|
| Paired-end merge | 6,230,305 | 9,630,694 | 15,860,999 |
| High Alignment Score | 4,315,828 | 7,371,439 | 11,687,267 |
| Demultiplex | 3,443,171 | 6,091,183 | 9,534,354 |
| Dereplicate | 3,443,171 | 6,091,183 | 9,534,354 |
| Trim primers | 3,443,171 | 6,091,183 | 9,534,354 |
| Remove outsize and low replicates | 2,955,049 | 5,419,485 | 8,374,534 |
| Remove PCR/sequencing errors | 2,176,430 | 3,992,451 | 6,168,881 |
| Taxonomy assigned | 146,502 | 945,459 | 1,091,961 |
| Reads retained from focal samples | 146,502 | 50,552 | 197,054 |

**Supplementary Information Table S 3.2.** List of species in environmental DNA reads

| Taxon | Common name | Family | Total reads in 127 samples | Notes |
|---|---|---|---|---|
| Fishes | | | | |
| *Pogonophryne* sp. | Plunderfish | Artedidraconidae | 570 | |
| *Borostomias antarcticus* | Snaggletooth | Stomiidae | 758 | |
| *Stomias boa* | Boa dragonfish | Stomiidae | 730 | |
| *Parachaenichthys charcoti* | Charcot's dragonfish | Bathydraconidae | 1612 | |
| Bathylagidae | Deep-sea smelts | Bathylagidae | 20778 | Originally assigned to *Lipolagus ochotensis* replaced with Bathylagidae. |
| Centrolophidae | Medusafish | Centrolophidae | 2146 | Originally assigned to *Hyperoglyphe* replaced with Centrolophidae |
| *Chaenocephalus aceratus* | Blackfin icefish | Channichthyidae | 1789 | |
| *Champsocephalus esox* | Pike icefish | Channichthyidae | 1283 | |
| *Cyclothone pseudopallida* | Slender bristlemouth | Gonostomatidae | 251 | |
| *Harpagifer antarcticus* | Antarctic spiny plunderfish | Harpagiferidae | 1977 | |
| Lampridae | Moonfish | Lampridae | 8834 | Originally assigned to *Lampris guttatus* replaced with Lampridae |
| Microstomatidae | Pencil smelts | Microstomatidae | 1419 | Originally assigned to *Nansenia ardesiaca* replaced with Microstomatidae |
| *Electrona antarctica* | Antarctica lanternfish | Myctophidae | 17906 | |
| *Electrona carlsbergi* | Electron subantarctic lanternfish | Myctophidae | 3824 | |
| *Gymnoscopelus fraseri* | Fraser’s lanternfish | Myctophidae | 8454 | |
| *Gymnoscopelus nicholsi* | Nichol's lanternfish | Myctophidae | 14896 | |
| *Krefftichthys anderssoni* | Rhombic lanternfish | Myctophidae | 17114 | |
| *Lampanyctus achirus* | Lantern fish | Myctophidae | 1593 | |
| *Lampichthys procerus* | Blackhead lanternfish | Myctophidae | 16 | |
| *Protomyctophum bolini* | Bolin’s lanternfish | Myctophidae | 3359 | |
| *Protomyctophum* sp. | Lanternfishes | Myctophidae | 28 | |
| *Aethotaxis mitopteryx* | Longfin icedevil | Nototheniidae | 5759 | |
| *Dissostichus eleginoides* | Patagonian toothfish | Nototheniidae | 2816 | |
| *Dissostichus mawsoni* | Antarctic toothfish | Nototheniidae | 0 | |
| *Gobionotothen gibberifrons* | Humped rockcod | Nototheniidae | 1 | |
| *Notothenia coriiceps* | Black rockcod | Nototheniidae | 6187 | |
| *Notothenia rossii* | Marbled rockcod | Nototheniidae | 15263 | |
| *Patagonotothen ramsayi* | Longtail southern cod | Nototheniidae | 18034 | |

| | | | |
|---|---|---|---|
| *Trematomus lepidorhinus* | Slender scalyhead | Nototheniidae | 20 |
| *Amblyraja georgiana* | Antarctic starry skate | Rajidae | 48 |
| Mammals | | | |
| *Lagenorhynchus* sp. | White-sided dolphins | Delphinidae | 1572 |
| *Arctocephalus gazella* | Antarctic fur seal | Otariidae | 30318 |
| *Leptonychotes weddellii* | Weddell seal | Phocidae | 166 |
| *Lobodon carcinophaga* | Crabeater seal | Phocidae | 3 |
| *Mirounga leonina* | Southern elephant seal | Phocidae | 394 |
| *Hyperoodon* sp. | bottlenose whales | Ziphiidae | 35 |
| *Eubalaena australis* | Southern right whale | Balaenidae | 4 |
| *Balaenoptera bonaerensis* | Antarctic minke whale | Balaenopteridae | 1071 |
| *Balaenoptera physalus* | Fin whale | Balaenopteridae | 4620 |
| *Megaptera novaeangliae* | Humpback whale | Balaenopteridae | 1406 |

**Supplementary Information Table S 3.3.** List of species in trawl survey data.

| Scientific Name | Common name | Family | Average CPUE |
|---|---|---|---|
| *Electrona antarctica* | Antarctic lanternfish | Myctophidae | 0.00035745 |
| *Electrona carlsbergi* | Electron subantarctic lanternfish | Myctophidae | 0.0000276 |
| *Gymnoscopelus fraseri* | Fraser's lanternfish | Myctophidae | 0.0003986 |
| *Gymnoscopelus nicholsi* | Nichol's lanternfish | Myctophidae | 0.00024867 |
| *Krefftichthys anderssoni* | Rhombic lanternfish | Myctophidae | 0.00228906 |
| *Lampanyctus achirus* | Lantern fish | Myctophidae | 0.00001498 |
| *Protomyctophum bolini* | Bolin's lanternfish | Myctophidae | 0.0000786 |
| *Protomyctophum* sp. | Lanternfish | Myctophidae | 0.00022538 |
| *Borostomias antarcticus* | Snaggletooth | Stomiidae | 0.0000174 |
| *Stomias boa* | Boa dragonfish | Stomiidae | 0.00004678 |
| *Bathylagus* sp. | Smelt | Bathylagidae | 0.0000215 |
| *Bathylagus tenuis* | -- | Bathylagidae | 0.00066063 |
| *Cyclothone signata* | Showy bristlemouth | Gonostomatidae | 0.0000492 |
| *Cyclothone* sp. | Bristlemouth | Gonostomatidae | 0.00232302 |
| *Cynomacrurus piriei* | Dogtooth grenadier | Macrouridae | 0.00000308 |
| *Gymnoscopelus braueri* | Brauer's lanternfish | Myctophidae | 0.00224986 |
| *Gymnoscopelus piabilis* | Southern blacktip lanternfish | Myctophidae | 0.000056 |
| *Lampanyctus macdonaldi* | Rakery beaconlamp | Myctophidae | 0.0000521 |
| *Notothenia neglecta* | Yellowbelly rockcod | Nototheniidae | 0.00000308 |
| *Argyropelecus hemigymnus* | Half-naked hatchetfish | Sternoptychidae | 0.0000137 |
| *Paradiplospinus gracilis* | Slender escolar | Gempylidae | 0.00000308 |
| *Nansenia antarctica* | -- | Microstomatidae | 0.00000308 |
| *Protomyctophum andriashevi* | Andriashev's lanternfish | Myctophidae | 0.00000308 |
| *Protomyctophum parallelum* | Parallel lanternfish | Myctophidae | 0.00184672 |
| *Protomyctophum tenisoni* | Tenison's lanternfish | Myctophidae | 0.00052533 |
| *Scopelosaurus hamiltoni* | Smallscale waryfish | Notosudidae | 0.0000138 |
| *Notolepis annulata* | Ringed barracudina | Paralepididae | 0.00006168 |
| *Notolepis coatsi* | Antarctic jonasfish | Paralepididae | 0.0000218 |
| *Benthalbella elongata* | -- | Scopelarchidae | 0.0000174 |
| *Benthalbella macropinna* | -- | Scopelarchidae | 0.0000255 |

**Supplementary Information Table S 3.4.** Reads assigned in negative controls.

| SampleID | Sample Number | EventID | Sample Type | Description | Taxon | Reads |
|---|---|---|---|---|---|---|
| lib1_A12 | Smpl185 | BLANK | BLANK | Empty well/barcode | *Krefftichthys anderssoni* | 1 |
| lib1_A12 | Smpl185 | BLANK | BLANK | Empty well/barcode | *Lagenorhynchus sp.* | 1 |
| lib1_E12 | Smpl189 | BLANK | BLANK | Empty well/barcode | *Notothenia rossii* | 1 |
| lib1_F12 | Smpl190 | BLANK | BLANK | Empty well/barcode | *Notothenia rossii* | 3 |
| lib1_G12 | Smpl191 | BLANK | BLANK | Empty well/barcode | *Notothenia rossii* | 4 |
| lib1_A02 | Smpl105 | BAS-2017.01.10-Und-01 | NEG | Field Negative | *Arctocephalus australis* | 2 |
| lib1_A02 | Smpl105 | BAS-2017.01.10-Und-01 | NEG | Field Negative | *Electrona antarctica* | 1 |
| lib1_A02 | Smpl105 | BAS-2017.01.10-Und-01 | NEG | Field Negative | *Krefftichthys anderssoni* | 1 |
| lib1_A02 | Smpl105 | BAS-2017.01.10-Und-01 | NEG | Field Negative | *Patagonotothen ramsayi* | 2 |
| lib1_A08 | Smpl153 | EXT_07 | EXT BLANK | Extraction negative | *Arctocephalus gazella* | 2 |
| lib1_A08 | Smpl153 | EXT_07 | EXT BLANK | Extraction negative | *Krefftichthys anderssoni* | 1 |
| lib1_A08 | Smpl153 | EXT_07 | EXT BLANK | Extraction negative | *Notothenia coriiceps* | 1 |
| lib1_A12 | Smpl185 | BLANK | BLANK | Empty well/barcode | *Krefftichthys anderssoni* | 1 |
| lib1_A12 | Smpl185 | BLANK | BLANK | Empty well/barcode | *Lagenorhynchus sp.* | 1 |
| lib1_B11 | Smpl178 | EXT_06 | EXT BLANK | Extraction negative | *Aethotaxis mitopteryx* | 2 |
| lib1_B11 | Smpl178 | EXT_06 | EXT BLANK | Extraction negative | *Krefftichthys anderssoni* | 1 |
| lib1_B11 | Smpl178 | EXT_06 | EXT BLANK | Extraction negative | *Notothenia rossii* | 2 |
| lib1_C11 | Smpl179 | PCR-BLANK-05 | PCR BLANK | PCR Negative | *Notothenia rossii* | 5 |
| lib1_D04 | Smpl124 | BAS-2017.01.09-Und-02 | NEG | Field Negative | *Arctocephalus gazella* | 2 |
| lib1_D04 | Smpl124 | BAS-2017.01.09-Und-02 | NEG | Field Negative | *Balaenoptera bonaerensis* | 1 |
| lib1_D04 | Smpl124 | BAS-2017.01.09-Und-02 | NEG | Field Negative | *Krefftichthys anderssoni* | 1 |
| lib1_D04 | Smpl124 | BAS-2017.01.09-Und-02 | NEG | Field Negative | *Patagonotothen ramsayi* | 1 |
| lib1_D10 | Smpl172 | BAS-2016.12.30-E94-02 | NEG | Field Negative | *Gymnoscopelus fraseri* | 1 |
| lib1_D10 | Smpl172 | BAS-2016.12.30-E94-02 | NEG | Field Negative | *Gymnoscopelus nicholsi* | 2 |
| lib1_D10 | Smpl172 | BAS-2016.12.30-E94-02 | NEG | Field Negative | *Krefftichthys anderssoni* | 1 |
| lib1_D10 | Smpl172 | BAS-2016.12.30-E94-02 | NEG | Field Negative | *Lampridae* | 1 |
| lib1_E08 | Smpl157 | BAS-2017.01.09-Und-01 | NEG | Field Negative | *Aethotaxis mitopteryx* | 1 |
| lib1_E08 | Smpl157 | BAS-2017.01.09-Und-01 | NEG | Field Negative | *Krefftichthys anderssoni* | 1 |
| lib1_E12 | Smpl189 | BLANK | BLANK | Empty well/barcode | *Notothenia rossii* | 1 |
| lib1_F03 | Smpl118 | EXT_03 | EXT BLANK | Extraction negative | *Arctocephalus gazella* | 1 |
| lib1_F05 | Smpl134 | EXT_04 | EXT BLANK | Extraction negative | *Arctocephalus gazella* | 1 |
| lib1_F12 | Smpl190 | BLANK | BLANK | Empty well/barcode | *Krefftichthys anderssoni* | 1 |
| lib1_F12 | Smpl190 | BLANK | BLANK | Empty well/barcode | *Notothenia rossii* | 3 |
| lib1_G12 | Smpl191 | BLANK | BLANK | Empty well/barcode | *Notothenia rossii* | 4 |
| lib1_H06 | Smpl144 | BAS-2017.01.03-E134-02 | NEG | Field Negative | *Notothenia rossii* | 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| lib1_H09 | Smpl168 | BAS-2016.12.16-E21-04 | NEG | Field Negative | *Arctocephalus gazella* | 1 |
| lib1_H12 | Smpl192 | BLANK | BLANK | Empty well/barcode | *Krefftichthys anderssoni* | 1 |
| lib2_A05 | Smpl033 | BAS-2016.12.27-E73-02 | NEG | Field Negative | *Balaenoptera bonaerensis* | 1 |
| lib2_A05 | Smpl033 | BAS-2016.12.27-E73-02 | NEG | Field Negative | *Electrona antarctica* | 2 |
| lib2_A05 | Smpl033 | BAS-2016.12.27-E73-02 | NEG | Field Negative | *Gymnoscopelus fraseri* | 1 |
| lib2_C02 | Smpl011 | BAS-2017.01.06-Und-01 | NEG | Field Negative | *Arctocephalus gazella* | 1 |
| lib2_C02 | Smpl011 | BAS-2017.01.06-Und-01 | NEG | Field Negative | *Bathylagidae* | 1 |
| lib2_C02 | Smpl011 | BAS-2017.01.06-Und-01 | NEG | Field Negative | *Gymnoscopelus fraseri* | 1 |
| lib2_D10 | Smpl076 | PCR-BLANK-03 | PCR BLANK | PCR Negative | *Balaenoptera bonaerensis* | 1 |
| lib2_D10 | Smpl076 | PCR-BLANK-03 | PCR BLANK | PCR Negative | *Electrona antarctica* | 1 |
| lib2_E04 | Smpl029 | EXT_01 | EXT BLANK | Extraction negative | *Krefftichthys anderssoni* | 1 |
| lib2_F08 | Smpl062 | BAS-2017.01.06-E170-03 | NEG | Field Negative | *Balaenoptera bonaerensis* | 1 |
| lib2_F08 | Smpl062 | BAS-2017.01.06-E170-03 | NEG | Field Negative | *Balaenoptera physalus* | 1 |
| lib2_F08 | Smpl062 | BAS-2017.01.06-E170-03 | NEG | Field Negative | *Electrona antarctica* | 1 |
| lib2_F08 | Smpl062 | BAS-2017.01.06-E170-03 | NEG | Field Negative | *Krefftichthys anderssoni* | 1 |
| lib2_F08 | Smpl062 | BAS-2017.01.06-E170-03 | NEG | Field Negative | *Notothenia coriiceps* | 1 |
| lib2_F08 | Smpl062 | BAS-2017.01.06-E170-03 | NEG | Field Negative | *Protomyctophum bolini* | 1713 |
| lib2_F08 | Smpl062 | BAS-2017.01.06-E170-03 | NEG | Field Negative | *Trematomus lepidorhinus* | 3 |
| lib2_G03 | Smpl023 | BAS-2017.01.03-E134-03 | NEG | Field Negative | *Arctocephalus australis* | 1 |
| lib2_G03 | Smpl023 | BAS-2017.01.03-E134-03 | NEG | Field Negative | *Balaenoptera physalus* | 2 |
| lib2_G03 | Smpl023 | BAS-2017.01.03-E134-03 | NEG | Field Negative | *Electrona antarctica* | 2 |
| lib2_G03 | Smpl023 | BAS-2017.01.03-E134-03 | NEG | Field Negative | *Gymnoscopelus fraseri* | 1 |
| lib2_G03 | Smpl023 | BAS-2017.01.03-E134-03 | NEG | Field Negative | *Krefftichthys anderssoni* | 1 |
| lib2_G03 | Smpl023 | BAS-2017.01.03-E134-03 | NEG | Field Negative | *Protomyctophum bolini* | 1 |
| lib2_G03 | Smpl023 | BAS-2017.01.03-E134-03 | NEG | Field Negative | *Trematomus lepidorhinus* | 1 |
| lib2_G06 | Smpl047 | BAS-2017.01.05-E150-04 | NEG | Field Negative | *Arctocephalus gazella* | 1 |
| lib2_H10 | Smpl080 | EXT_02 | EXT BLANK | Extraction negative | *Arctocephalus australis* | 1 |
| lib2_H10 | Smpl080 | EXT_02 | EXT BLANK | Extraction negative | *Balaenoptera bonaerensis* | 1 |
| lib2_H10 | Smpl080 | EXT_02 | EXT BLANK | Extraction negative | *Balaenoptera physalus* | 1 |
| lib2_H10 | Smpl080 | EXT_02 | EXT BLANK | Extraction negative | *Gymnoscopelus fraseri* | 1 |
| lib2_H10 | Smpl080 | EXT_02 | EXT BLANK | Extraction negative | *Notothenia rossii* | 1 |
| lib2_H10 | Smpl080 | EXT_02 | EXT BLANK | Extraction negative | *Trematomus lepidorhinus* | 3 |
| lib2_H12 | Smpl096 | BAS-2016.12.22-Und-01 | NEG | Field Negative | *Arctocephalus gazella* | 5 |

**Supplementary Information Table S 3.5.** List of Antarctic fish species, from Parkes G. (1992) Fishes of the Southern Ocean. *Reviews in Fish Biology and Fisheries* 2, 344–345.

| Scientific Name | Family | Order | Class | In the reference |
|---|---|---|---|---|
| *Acanthodraco dewitti* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Achiropsetta tricholepis* | Achiropsettidae | Pleuronectiformes | Actinopteri | NO |
| *Aethotaxis mitopteryx* | Nototheniidae | Notothenioidei | Actinopteri | GENBANK |
| *Akarotaxis nudiceps* | Bathydraconidae | Notothenioidei | Actinopteri | GENBANK |
| *Alepisaurus brevirostris* | Alepisauridae | Alepocephaliformes | Actinopteri | NO |
| *Alepocephalus australis* | Alepocephalidae | Alepocephaliformes | Actinopteri | NO |
| *Alepocephalus bicolor* | Alepocephalidae | Alepocephaliformes | Actinopteri | GENBANK |
| *Alepocephalus productus* | Alepocephalidae | Alepocephaliformes | Actinopteri | GENBANK |
| *Alepocephalus tenebrosus* | Alepocephalidae | Alepocephaliformes | Actinopteri | SRA |
| *Amblyraja georgiana* | Rajidae | Rajiformes | Elasmobranchii | GENBANK |
| *Amblyraja taaf* | Rajidae | Rajiformes | Elasmobranchii | NO |
| *Anotopterus pharao* | Anotopteridae | Aulopiformes | Actinopteri | NO |
| *Antimora rostrata* | Moridae | Gadiformes | Actinopteri | GENBANK |
| *Arctozenus risso* | Paralepididae | Aulopiformes | Actinopteri | NO |
| *Argyropelecus aculeatus* | Sternoptychidae | Stomiiformes | Actinopteri | GENBANK |
| *Argyropelecus affinis* | Sternoptychidae | Stomiiformes | Actinopteri | GENBANK |
| *Argyropelecus gigas* | Sternoptychidae | Stomiiformes | Actinopteri | GENBANK |
| *Argyropelecus hemigymnus* | Sternoptychidae | Stomiiformes | Actinopteri | NO |
| *Argyropelecus sladeni* | Sternoptychidae | Stomiiformes | Actinopteri | GENBANK |
| *Artedidraco glareobarbatus* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Artedidraco lonnbergi* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Artedidraco mirus* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Artedidraco orianae* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Artedidraco shackletoni* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Artedidraco skottsbergi* | Artedidraconidae | Notothenioidei | Actinopteri | GENBANK |
| *Bathydraco antarcticus* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Bathydraco joannae* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Bathydraco macrolepis* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Bathydraco marri* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Bathydraco scotiae* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Bathylagus antarcticus* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Bathylagus gracilis* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Bathylagus tenuis* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Bathyraja eatonii* | Arhynchobatidae | Rajiformes | Elasmobranchii | GENBANK |
| *Bathyraja irrasa* | Arhynchobatidae | Rajiformes | Elasmobranchii | NO |
| *Bathyraja maccaini* | Arhynchobatidae | Rajiformes | Elasmobranchii | NO |
| *Bathyraja meridionalis* | Arhynchobatidae | Rajiformes | Elasmobranchii | NO |
| *Bathyraja murrayi* | Arhynchobatidae | Rajiformes | Elasmobranchii | NO |
| *Benthalbella elongata* | Scopelarchidae | Aulopiformes | Actinopteri | NO |
| *Benthalbella macropinna* | Scopelarchidae | Aulopiformes | Actinopteri | NO |
| *Borostomias antarcticus* | Stomiidae | Stomiiformes | Actinopteri | NO |
| *Bovichtus angustifrons* | Bovichtidae | Notothenioidei | Actinopteri | GENBANK |
| *Bovichtus argentinus* | Bovichtidae | Notothenioidei | Actinopteri | GENBANK |
| *Bovichtus chilensis* | Bovichtidae | Notothenioidei | Actinopteri | NO |
| *Bovichtus diacanthus* | Bovichtidae | Notothenioidei | Actinopteri | GENBANK |
| *Bovichtus oculus* | Bovichtidae | Notothenioidei | Actinopteri | NO |
| *Bovichtus psychrolutes* | Bovichtidae | Notothenioidei | Actinopteri | NO |
| *Bovichtus variegatus* | Bovichtidae | Notothenioidei | Actinopteri | NO |
| *Bovichtus veneris* | Bovichtidae | Notothenioidei | Actinopteri | NO |
| *Ceratias tentaculatus* | Ceratiidae | Lophiiformes | Actinopteri | NO |

| | | | | |
|---|---|---|---|---|
| *Ceratoscopelus warmingii* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Chaenocephalus aceratus* | Channichthyidae | Notothenioidei | Actinopteri | GENBANK |
| *Chaenodraco wilsoni* | Channichthyidae | Notothenioidei | Actinopteri | GENBANK |
| *Champsocephalus esox* | Channichthyidae | Notothenioidei | Actinopteri | GENBANK |
| *Champsocephalus gunnari* | Channichthyidae | Notothenioidei | Actinopteri | GENBANK |
| *Channichthys aelitae* | Channichthyidae | Notothenioidei | Actinopteri | NO |
| *Channichthys bospori* | Channichthyidae | Notothenioidei | Actinopteri | NO |
| *Channichthys irinae* | Channichthyidae | Notothenioidei | Actinopteri | NO |
| *Channichthys mithridatis* | Channichthyidae | Notothenioidei | Actinopteri | NO |
| *Channichthys panticapaei* | Channichthyidae | Notothenioidei | Actinopteri | NO |
| *Channichthys rhinoceratus* | Channichthyidae | Notothenioidei | Actinopteri | GENBANK |
| *Channichthys richardsoni* | Channichthyidae | Notothenioidei | Actinopteri | NO |
| *Channichthys rugosus* | Channichthyidae | Notothenioidei | Actinopteri | NO |
| *Channichthys velifer* | Channichthyidae | Notothenioidei | Actinopteri | NO |
| *Chionobathyscus dewitti* | Channichthyidae | Notothenioidei | Actinopteri | GENBANK |
| *Chionodraco hamatus* | Channichthyidae | Notothenioidei | Actinopteri | GENBANK |
| *Chionodraco myersi* | Channichthyidae | Notothenioidei | Actinopteri | GENBANK |
| *Chionodraco rastrospinosus* | Channichthyidae | Notothenioidei | Actinopteri | GENBANK |
| *Coelorinchus fasciatus* | Macrouridae | Gadiformes | Actinopteri | GENBANK |
| *Coelorinchus fasciatus* | Macrouridae | Gadiformes | Actinopteri | NO |
| *Coelorinchus marinii* | Macrouridae | Gadiformes | Actinopteri | NO |
| *Coryphaenoides armatus* | Macrouridae | Gadiformes | Actinopteri | GENBANK |
| *Coryphaenoides ferrieri* | Macrouridae | Gadiformes | Actinopteri | NO |
| *Coryphaenoides lecointei* | Macrouridae | Gadiformes | Actinopteri | NO |
| *Cottoperca gobio* | Bovichtidae | Notothenioidei | Actinopteri | NO |
| *Cottoperca trigloides* | Bovichtidae | Notothenioidei | Actinopteri | NO |
| *Cryodraco antarcticus* | Channichthyidae | Notothenioidei | Actinopteri | GENBANK |
| *Cryodraco atkinsoni* | Channichthyidae | Notothenioidei | Actinopteri | NO |
| *Cryodraco pappenheimi* | Channichthyidae | Notothenioidei | Actinopteri | NO |
| *Cryothenia amphitreta* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Cryothenia peninsulae* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Cyclothone acclinidens* | Gonostomatidae | Stomiiformes | Actinopteri | NO |
| *Cyclothone braueri* | Gonostomatidae | Stomiiformes | Actinopteri | NO |
| *Cyclothone microdon* | Gonostomatidae | Stomiiformes | Actinopteri | NO |
| *Cyclothone pallida* | Gonostomatidae | Stomiiformes | Actinopteri | NO |
| *Cydothone pseudopallida* | Gonostomatidae | Stomiiformes | Actinopteri | NO |
| *Cygnodraco mawsoni* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Cynomacrurus piriei* | Macrouridae | Gadiformes | Actinopteri | NO |
| *Dacodraco hunteri* | Channichthyidae | Notothenioidei | Actinopteri | NO |
| *Diaphus hudsoni* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Diaphus ostenfeldi* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Diastobranchus capensis* | Synaphobranchidae | Anguilliformes | Actinopteri | GENBANK |
| *Dieidolycus leptodermatus* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Dissostichus eleginoides* | Nototheniidae | Notothenioidei | Actinopteri | GENBANK |
| *Dissostichus mawsoni* | Nototheniidae | Notothenioidei | Actinopteri | GENBANK |
| *Dolloidraco longedorsalis* | Artedidraconidae | Notothenioidei | Actinopteri | GENBANK |
| *Echiodon cryomargarites* | Carapidae | Ophidiiformes | Actinopteri | NO |
| *Electrona antarctica* | Myctophidae | Myctophiformes | Actinopteri | In this study |
| *Electrona carlsbergi* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Electrona paucirastra* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Electrona subaspera* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Eleginops maclovinus* | Eleginopidae | Notothenioidei | Actinopteri | GENBANK |
| *Etmopterus lucifer* | Etmopteridae | Squaliformes | Elasmobranchii | GENBANK |
| *Genioliparis lindbergi* | Liparidae | Cottoidei | Actinopteri | NO |
| *Geotria australis* | Geotriidae | Petromyzontiformes | Petromyzonti | NO |
| *Geotria australis* | Geotriidae | Petromyzontiformes | Petromyzonti | NO |
| *Gerlachea australis* | Bathydraconidae | Notothenioidei | Actinopteri | GENBANK |

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|---|---|---|---|---|
| *Gobionotothen acuta* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Gobionotothen angustifrons* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Gobionotothen barsukovi* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Gobionotothen gibberifrons* | Nototheniidae | Notothenioidei | Actinopteri | GENBANK |
| *Gobionotothen marionensis* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Gvozdarus balushkini* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Gvozdarus svetovidovi* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Gymnodraco acuticeps* | Bathydraconidae | Notothenioidei | Actinopteri | GENBANK |
| *Gymnoscopelus bolini* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Gymnoscopelus braueri* | Myctophidae | Myctophiformes | Actinopteri | In this study |
| *Gymnoscopelus fraseri* | Myctophidae | Myctophiformes | Actinopteri | In this study |
| *Gymnoscopelus fraseri* | Myctophidae | Myctophiformes | Actinopteri | GENBANK |
| *Gymnoscopelus hintonoides* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Gymnoscopelus microlampas* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Gymnoscopelus nicholsi* | Myctophidae | Myctophiformes | Actinopteri | GENBANK |
| *Gymnoscopelus opisthopterus* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Gymnoscopelus piabilis* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Gyrinomimus andriashevi* | Cetomimidae | Beryciformes | Actinopteri | NO |
| *Gyrinomimus grahami* | Cetomimidae | Beryciformes | Actinopteri | NO |
| *Halaphritis platycephala* | Bovichtidae | Notothenioidei | Actinopteri | NO |
| *Halargyreus johnsonii* | Moridae | Gadiformes | Actinopteri | GENBANK |
| *Halosauropsis macrochir* | Halosauridae | Notacanthiformes | Actinopteri | NO |
| *Harpagifer andriashevi* | Harpagiferidae | Notothenioidei | Actinopteri | NO |
| *Harpagifer antarcticus* | Harpagiferidae | Notothenioidei | Actinopteri | GENBANK |
| *Harpagifer bispinis* | Harpagiferidae | Notothenioidei | Actinopteri | NO |
| *Harpagifer crozetensis* | Harpagiferidae | Notothenioidei | Actinopteri | NO |
| *Harpagifer georgianus* | Harpagiferidae | Notothenioidei | Actinopteri | NO |
| *Harpagifer kerguelensis* | Harpagiferidae | Notothenioidei | Actinopteri | NO |
| *Harpagifer macquariensis* | Harpagiferidae | Notothenioidei | Actinopteri | NO |
| *Harpagifer marionensis* | Harpagiferidae | Notothenioidei | Actinopteri | NO |
| *Harpagifer nybelini* | Harpagiferidae | Notothenioidei | Actinopteri | NO |
| *Harpagifer palliolatus* | Harpagiferidae | Notothenioidei | Actinopteri | NO |
| *Harpagifer permitini* | Harpagiferidae | Notothenioidei | Actinopteri | NO |
| *Harpagifer spinosus* | Harpagiferidae | Notothenioidei | Actinopteri | NO |
| *Helcogrammoides antarcticus* | Tripterygiidae | Blenniiformes | Actinopteri | NO |
| *Hintonia candens* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Histiobranchus bathybius* | Synaphobranchidae | Anguilliformes | Actinopteri | NO |
| *Histiodraco velifer* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Holcomycteronus brucei* | Ophidiidae | Ophidiiformes | Actinopteri | NO |
| *Icichthys australis* | Centrolophidae | Scombriformes | Actinopteri | NO |
| *Idiacanthus atlanticus* | Stomiidae | Stomiiformes | Actinopteri | NO |
| *Indonotothenia cyanobrancha* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Krefftichthys anderssoni* | Myctophidae | Myctophiformes | Actinopteri | In this study |
| *Krefftichthys anderssoni* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Laemonema kongi* | Moridae | Gadiformes | Actinopteri | NO |
| *Lamna ditropis* | Lamnidae | Lamniformes | Elasmobranchii | NO |
| *Lamna nasus* | Lamnidae | Lamniformes | Elasmobranchii | NO |
| *Lampanyctus achirus* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Lampanyctus ater* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Lampanyctus australis* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Lampanyctus intricarius* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Lampanyctus macdonaldi* | Myctophidae | Myctophiformes | Actinopteri | GENBANK |
| *Lampichthys procerus* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Lampris guttatus* | Lampridae | Lampriformes | Actinopteri | GENBANK |
| *Lampris immaculatus* | Lampridae | Lampriformes | Actinopteri | NO |
| *Lepidion ensiferus* | Moridae | Gadiformes | Actinopteri | GENBANK |
| *Lepidonotothen kempi* | Nototheniidae | Notothenioidei | Actinopteri | NO |

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|---|---|---|---|---|
| *Lepidonotothen larseni* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Lepidonotothen squamifrons* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Leptoderma lubricum* | Alepocephalidae | Alepocephaliformes | Actinopteri | GENBANK |
| *Leptoderma retropinna* | Alepocephalidae | Alepocephaliformes | Actinopteri | GENBANK |
| *Lindbergichthys mizops* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Lindbergichthys nudifrons* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Lycenchelys antarctica* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Lycenchelys aratrirostris* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Lycenchelys argentina* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Lycenchelys bellingshauseni* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Lycenchelys hureaui* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Lycenchelys nanospinata* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Lycenchelys nigripalatum* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Lycenchelys tristichodon* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Lycenchelys wilkesi* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Lycodapus antarcticus* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Lycodapus pachysoma* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Lycodichthys antarcticus* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Lycodichthys dearborni* | Zoarcidae | Zoarcoidei | Actinopteri | GENBANK |
| *Lycodichthys dearborni* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Macrourus carinatus* | Macrouridae | Gadiformes | Actinopteri | GENBANK |
| *Macrourus holotrachys* | Macrouridae | Gadiformes | Actinopteri | NO |
| *Macrourus whitsoni* | Macrouridae | Gadiformes | Actinopteri | GENBANK |
| *Magnisudis prionosa* | Paralepididae | Aulopiformes | Actinopteri | NO |
| *Mancopsetta maculata* | Achiropsettidae | Pleuronectiformes | Actinopteri | NO |
| *Mancopsetta milfordi* | Achiropsettidae | Pleuronectiformes | Actinopteri | NO |
| *Melamphaes microps* | Melamphaidae | Beryciformes | Actinopteri | GENBANK |
| *Melanocetus rossi* | Melanocetidae | Lophiiformes | Actinopteri | NO |
| *Melanonus gracilis* | Melanonidae | Gadiformes | Actinopteri | GENBANK |
| *Melanonus zugmayeri* | Melanonidae | Gadiformes | Actinopteri | GENBANK |
| *Melanostigma bathium* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Melanostigma gelatinosum* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Melanostigma vitiazi* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Metelectrona ventralis* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Muraenolepis marmoratus* | Muraenolepididae | Gadiformes | Actinopteri | NO |
| *Muraenolepis microcephalus* | Muraenolepididae | Gadiformes | Actinopteri | NO |
| *Muraenolepis microps* | Muraenolepididae | Gadiformes | Actinopteri | NO |
| *Muraenolepis orangiensis* | Muraenolepididae | Gadiformes | Actinopteri | GENBANK |
| *Myxine australis* | Myxinidae | Myxiniformes | Myxini | NO |
| *Nansenia antarctica* | Microstomatidae | Argentiniformes | Actinopteri | NO |
| *Narcetes stomias* | Alepocephalidae | Alepocephaliformes | Actinopteri | NO |
| *Neoachiropsetta milfordi* | Achiropsettidae | Pleuronectiformes | Actinopteri | GENBANK |
| *Neopagetopsis ionah* | Channichthyidae | Notothenioidei | Actinopteri | GENBANK |
| *Notocetichthys trunovi* | Cetomimidae | Beryciformes | Actinopteri | NO |
| *Notolepis annulata* | Paralepididae | Aulopiformes | Actinopteri | NO |
| *Notolepis coatsi* | Paralepididae | Aulopiformes | Actinopteri | NO |
| *Notoliparis kurchatovi* | Liparidae | Cottoidei | Actinopteri | NO |
| *Notoliparis macquariensis* | Liparidae | Cottoidei | Actinopteri | NO |
| *Notoscopelus resplendens* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Notothenia angustata* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Notothenia coriiceps* | Nototheniidae | Notothenioidei | Actinopteri | GENBANK |
| *Notothenia microlepidota* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Notothenia neglecta* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Notothenia rossii* | Nototheniidae | Notothenioidei | Actinopteri | GENBANK |
| *Notothenia trigramma* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Nototheniops larseni* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Nototheniops nybelini* | Nototheniidae | Notothenioidei | Actinopteri | NO |

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| *Nototheniops tchizh* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Oidiphorus brevis* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Oidiphorus mcallisteri* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Oneirodes notius* | Oneirodidae | Lophiiformes | Actinopteri | NO |
| *Ophthalmolycus amberensis* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Ophthalmolycus bothriocephalus* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Pachycara brachycephalum* | Zoarcidae | Zoarcoidei | Actinopteri | SRA |
| *Pagetopsis macropterus* | Channichthyidae | Notothenioidei | Actinopteri | GENBANK |
| *Pagetopsis maculata* | Channichthyidae | Notothenioidei | Actinopteri | NO |
| *Pagothenia borchgrevinki* | Nototheniidae | Notothenioidei | Actinopteri | GENBANK |
| *Pagothenia brachysoma* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Parachaenichthys charcoti* | Bathydraconidae | Notothenioidei | Actinopteri | GENBANK |
| *Parachaenichthys georgianus* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Paradiplospinus gracilis* | Gempylidae | Scombriformes | Actinopteri | NO |
| *Paraliparis anarthractae* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis andriashevi* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis antarcticus* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis cerasinus* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis copei* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis devriesi* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis diploprora* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis eltanini* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis fuscolingua* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis gracilis* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis incognita* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis kreffti* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis leobergi* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis leucogaster* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis leucoglossus* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis mawsoni* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis meganchus* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis monoporus* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis neelovi* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis operculosus* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis somovi* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis stehmanni* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis terraenovae* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis tetrapteryx* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis thalassobathyalis* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis trilobodon* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paraliparis valentinae* | Liparidae | Cottoidei | Actinopteri | NO |
| *Paranotothenia dewitti* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Paranotothenia magellanica* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Patagonotothen brevicauda* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Patagonotothen canina* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Patagonotothen cornucola* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Patagonotothen elegans* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Patagonotothen guntheri* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Patagonotothen jordani* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Patagonotothen kreffti* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Patagonotothen longipes* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Patagonotothen ramsayi* | Nototheniidae | Notothenioidei | Actinopteri | GENBANK |
| *Patagonotothen shagensis* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Patagonotothen sima* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Patagonotothen squamiceps* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Patagonotothen tessellata* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Patagonotothen thompsoni* | Nototheniidae | Notothenioidei | Actinopteri | NO |

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| *Patagonotothen wiltoni* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Pleuragramma antarctica* | Nototheniidae | Notothenioidei | Actinopteri | GENBANK |
| *Pogonophryne albipinna* | Artedidraconidae | Notothenioidei | Actinopteri | GENBANK |
| *Pogonophryne barsukovi* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne bellingshausenensis* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne brevibarbata* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne cerebropogon* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne curtilemma* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne dewitti* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne dolichobranchiata* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne eakini* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne favosa* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne fusca* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne immaculata* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne lanceobarbata* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne macropogon* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne maculiventrata* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne marmorata* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne mentella* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne neyelovi* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne orangiensis* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne orcadensis* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne pavlovi* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne permitini* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne phyllopogon* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne platypogon* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne sarmentifera* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne scotti* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne skorai* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne squamibarbata* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne stewarti* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne tronio* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne velifera* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Pogonophryne ventrimaculata* | Artedidraconidae | Notothenioidei | Actinopteri | NO |
| *Polyacanthonotus challengeri* | Notacanthidae | Notacanthiformes | Actinopteri | NO |
| *Poromitra crassiceps* | Melamphaidae | Beryciformes | Actinopteri | GENBANK |
| *Prionodraco evansii* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Protomyctophum andriashevi* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Protomyctophum bolini* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Protomyctophum choriodon* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Protomyctophum crockeri* | Myctophidae | Myctophiformes | Actinopteri | SRA |
| *Protomyctophum gemmatum* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Protomyctophum luciferum* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Protomyctophum normani* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Protomyctophum parallelum* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Protomyctophum tenisoni* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Protomyctophum thompsoni* | Myctophidae | Myctophiformes | Actinopteri | SRA |
| *Pseudaphritis undulatus* | Pseudaphritidae | Notothenioidei | Actinopteri | NO |
| *Pseudaphritis urvillii* | Pseudaphritidae | Notothenioidei | Actinopteri | GENBANK |
| *Pseudochaenichthys georgianus* | Channichthyidae | Notothenioidei | Actinopteri | GENBANK |
| *Pseudocyttus maculatus* | Oreosomatidae | Zeiformes | Actinopteri | GENBANK |
| *Pseudomancopsetta andriashevi* | Achiropsettidae | Pleuronectiformes | Actinopteri | NO |
| *Psilodraco breviceps* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Racovitzia glacialis* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Racovitzia harrissoni* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Rouleina attrita* | Alepocephalidae | Alepocephaliformes | Actinopteri | NO |
| *Rouleina maderensis* | Alepocephalidae | Alepocephaliformes | Actinopteri | GENBANK |

| | | | | |
|---|---|---|---|---|
| *Scopelosaurus hamiltoni* | Notosudidae | Aulopiformes | Actinopteri | NO |
| *Seleniolycus laevifasciatus* | Zoarcidae | Zoarcoidei | Actinopteri | NO |
| *Sio nordenskjdldii* | Melamphaidae | Beryciformes | Actinopteri | NO |
| *Somniosus microcephalus* | Somniosidae | Squaliformes | Elasmobranchii | NO |
| *Somniosus rostratus* | Somniosidae | Squaliformes | Elasmobranchii | NO |
| *Stomias boa* | Stomiidae | Stomiiformes | Actinopteri | GENBANK |
| *Stomias gracilis* | Stomiidae | Stomiiformes | Actinopteri | NO |
| *Symbolophorus boops* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Taaningichthys bathyphilus* | Myctophidae | Myctophiformes | Actinopteri | NO |
| *Thunnus maccoyii* | Scombridae | Scombriformes | Actinopteri | GENBANK |
| *Trematomus bernacchii* | Nototheniidae | Notothenioidei | Actinopteri | GENBANK |
| *Trematomus eulepidotus* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Trematomus hansoni* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Trematomus lepidorhinus* | Nototheniidae | Notothenioidei | Actinopteri | GENBANK |
| *Trematomus loennbergii* | Nototheniidae | Notothenioidei | Actinopteri | SRA |
| *Trematomus newnesi* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Trematomus nicolai* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Trematomus pennellii* | Nototheniidae | Notothenioidei | Actinopteri | GENBANK |
| *Trematomus scotti* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Trematomus tokarevi* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Trematomus vicarius* | Nototheniidae | Notothenioidei | Actinopteri | NO |
| *Vomeridens infuscipinnis* | Bathydraconidae | Notothenioidei | Actinopteri | NO |
| *Zanclorhynchus spinifer* | Congiopodidae | Scorpaenoidei | Actinopteri | NO |

3

4

# Chapter 4

# Nuclear environmental DNA resolves fine-scale population genetic structure in an African cichlid fish

An adapted version of this chapter is in preparation to be submitted to a peer-reviewed journal:

Liu, Z., Kishe, M.A., Gambagambi, P.N., Ngatunga, B.P., Shechonge, A.H., Smith, K., Collins, R.A., Saxon, A.D., Hudson, A.G., Lynderoth, T., Durbin, R., Turner, G.F. & Genner, M.J. Nuclear environmental DNA resolves fine-scale population genetic structure in an African cichlid fish.

Author contributions: Environmental DNA samples were collected by MJG, MAK and PNG. Fish genotype data were provided by TL and RD. Fish samples were collected and analysed over 10 years by MJG, GFT, Emilia Santos, HA, Gregoire Vernaz, RD, BPN, Semvua Mzighani and AHS. Light measurements were taken by AGH. eDNA extraction and PCR were conducted by ZL. eDNA library preparation and sequencing were carried out by ZL and ADS. Bioinformatics and downstream data analysis were conducted by ZL and MJG. Writing and presentation were undertaken by ZL and MJG.

**Abstract**

Environmental DNA (eDNA) has proven to be an effective tool for determining the presence and abundance of aquatic animals, and mitochondrial haplotype distributions of focal species derived from eDNA have been used to resolve population genetic structure. However, whether components of the nuclear genome present in environmental DNA can be used to quantify population structuring of target species has been unclear. Here we use information on allelic composition present in eDNA to resolve the fine scale spatial genetic structure of the Eastern happy cichlid (*Astatotilapia calliptera*) in crater Lake Masoko, Tanzania. In this lake the species is diverging into two genetically distinguishable ecomorphs along a 30 m depth gradient, separated by a thermo-oxycline at ~15 m, that separates biologically distinct water masses. We found we can quantify the spatial distribution of the fish ecomorphs using a targeted set of 71 SNPs present that are also reliably represented in eDNA. We also found that the spatial segregation of alleles in eDNA allowed us to identify population genetic structure, and that allelic frequencies in eDNA reflect with those found among the fish populations. Thus, by targeting known genetic variation among populations within aquatic eDNA, we can quantify population genetic structure of focal species. We conclude the genome-wide information present in eDNA could be used for non-destructive establish of patterns of biodiversity, both within and across species.

## 4.1 Introduction

There is growing interest in the use of aquatic eDNA for studying population genetics of focal species (Adams et al. 2019; Sigsgaard et al. 2020). To date much of this research has focussed on variation in target regions of the mitochondrial genome, and it has proven possible to resolve spatial population genetic structure in a range of vertebrate species (Sigsgaard et al. 2016, Gorički et al. 2017, Sigsgaard et al. 2020). Mitochondrial DNA has been employed for population genetic inference in this context as it can be reliably amplified and sequenced and has a relatively high evolutionary rate that can allow populations to be distinguished. Moreover, since it is possible to make extensive reference libraries of species in a geographic region, and since variable sections of mtDNA are often flanked by phylogenetically conserved regions, useful primers can be readily identified and eDNA-derived sequences reliably assigned to focal species.

Mitochondrial DNA, however, can be suboptimal for resolving population genetic structure, particularly among very recently diverged populations. The marker is typically maternally inherited and non-recombining, so the whole mtDNA genome acts as a single locus and primarily provides only information on the female portion of a species history (Galtier et al. 2009). Furthermore, nuclear insertions of mitochondrial origin (numts), or selection on the mitochondrial genome, could also confound population genetic inference (Galtier et al. 2009; Hlaing et al. 2009). Thus, there has been interest in the potential for nuclear eDNA to be used for population genetic analysis (Sigsgaard et al. 2020). To date, however, the evidence that environmental DNA from the nuclear genome of non-microbial species can be used for population genetic inference is limited. Nevertheless, there are promising signs that analyses may be insightful; in both mesocosms and the natural environment microsatellite allele frequencies of round goby (*Neogobius melanostomus*) derived from eDNA showed a strong correspondence with allele frequencies of source fish (Andres et al. 2021). Here we build on this research by investigating if population genetic structure can be determined over a small-spatial scale using single nucleotide polymorphism (SNP) variants from the nuclear genome within aquatic eDNA samples.

Our study system is Lake Masoko, a crater (maar) lake in southern Tanzania that has no surface connections with nearby rivers (Garcin et 2006; Malinsky et al. 2015; Fig. 4.1 a-b). The

lake is ~700 m in diameter, has a maximum depth of ~35 m, is stratified with a thermo-oxycline at approximately 15 m that is present from August-May, and has rapid attenuation of light with increasing depth (Fig. 1c-e; Delalande 2008). The lake contains a pair of ecomorphs of the cichlid fish *Astatotilapia calliptera*, with a littoral ecomorph with yellow males that feeds primarily on benthic invertebrates dominating the shallow waters (<5 m), and deep ecomorph with blue males that feeds primarily on zooplankton dominating the deeper waters (>20 m) (Malinsky et al. 2015; Carruthers et al. 2022; Vernaz et al. 2022). The ecomorphs show strong differentiation in ecologically relevant morphology, including body shape and the structure of lower pharyngeal jaws used principally for processing prey (Malinsky et al. 2015; Carruthers et al. 2022). Using whole genome-level sequence data of individuals collected from known depth strata, the ecomorphs have been shown to be clearly genetically differentiated, with that strong genetic differentiation apparent over the depth gradient (Munby et al. 2021).

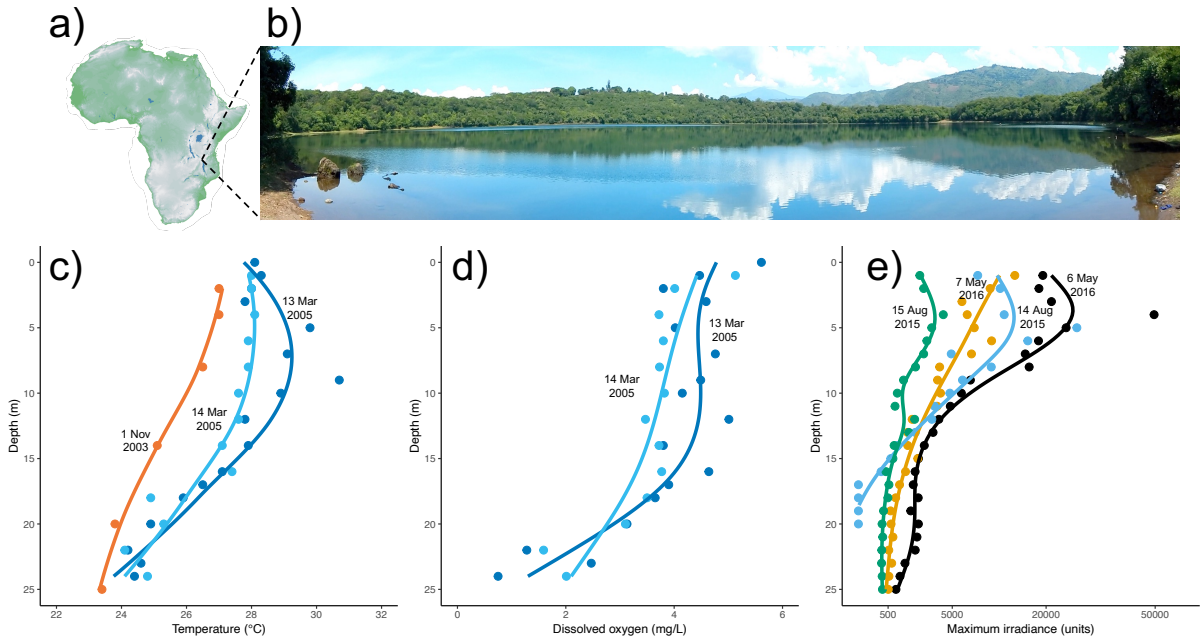



**Figure 4.1.** a-b) Lake Masoko crater lake in southern Tanzania. c-e) The lake has a vertical gradient of decreasing temperature, dissolved oxygen and light levels with increasing depth, with striking differences either side of the thermo-oxycline at 10-15 m, shown in grey. Temperature and oxygen data are from Delalande (2008). Light data are from Alan Hudson.

In this study we first test if the thermo-oxycline does reliably separate two water masses, by comparing the bacterial communities as resolved through nanopore sequencing of the

92 bacterial communities in water samples. We undertake then undertake a series of analyses
93 focussed on SNPs with known differences in allele frequencies between ecomorphs,
94 determining if they can be identified in eDNA derived sequence data, if they share common
95 allele frequencies with fish sampled from the same depth strata, and if the eDNA-derived SNP
96 data can be used to resolve population genetic structure observed in the fish populations.

## 4.2 Materials and Methods

### *4.2.1 Sampling eDNA in Lake Masoko*

Water samples were collected using SCUBA from five depths in Lake Masoko (3, 7, 12, 18 and 22 metres below the water surface) on 3 September 2019. Water samples were taken to the surface, and each was filtered through a 0.22 µm Sterivex-GP PES filter (Merck Millipore, MA) using repeated loadings of a 60 mL syringe. The total volume of water filtered was recorded (Tables 4.1 and 4.2). DNA within the filters was preserved by an addition of 0.3 mL of ATL buffer to the filter cartridge (Qiagen, Hilden, Germany), before each filter was sealed at both ends, and was placed individually in a whirlpak bag, labelled, and stored as cool as possible in the field. For longer term storage filters were placed in a -20 °C freezer.

### *4.2.2 DNA extraction*

DNA was extracted from sterivex filters using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany), in a dedicated trace DNA laboratory. The extraction protocol is described in Collins (2021).

**Table 4.1.** Samples used for nanopore sequencing of bacterial community composition, and outputs from the KrakenUniq assignment analysis.

| Sample Code | Collection depth (m) | Extraction ID | Volume filtered (mL) | Number of sequences generated | Number of reads assigned to bacteria orders |
|---|---|---|---|---|---|
| N-01 | 3 | 3mA | 720 | 136544 | 5668 |
| N-02 | 3 | 3mB | 720 | 120918 | 8074 |
| N-03 | 22 | 22mA | 1500 | 116424 | 2884 |
| N-04 | 22 | 22mB* | 1500 | 203788 | 5215 |
| N-05 | 3 | 3m-10 | 600 | 369208 | 8271 |
| N-06 | 7 | 7m-8 | 480 | 154701 | 3191 |
| N-07 | 12 | 12m-8 | 480 | 147284 | 4193 |
| N-08 | 18 | 18m-9 | 540 | 209429 | 2761 |
| N-09 | 22 | 22m-25 | 1500 | 151360 | 5910 |

*Sample was sequenced twice using the MinION.

**Table 4.2.** Samples used for the eDNA analysis targeting *A. calliptera* SNPs.

| Sample analysis code | Sample collection depth (m) | Extraction ID | Library ID | Volume filtered(ml) | Nanodrop concentration | Number of reads (half for paired end | Mapping success |
|---|---|---|---|---|---|---|---|
| 01 | 3 | 210907_01 | L07-01 | 840 | 55 | 20489542 | 41.09% |
| 02 | 3 | 210907_02 | L07-02 | 720 | 38 | 20196430 | 74.86% |
| 03 | 22 | 210907_03 | L07-03 | 480 | 17 | 18716758 | 72.20% |
| 04 | 22 | 210907_04 | L07-04 | 480 | 22 | 21917982 | 80.44% |
| 05 | 3 | 191216_01 | L16-01 | 720 | 41 | 26194419 | 89.50% |
| 06 | 22 | 191216_02 | L16-02 | 1560 | 99 | 12992931 | 62.94% |
| 07 | 3 | 200217_01 | L17-01 | 720 | 22 | 30436408 | 94.89% |
| 08 | 3 | 200217_02 | L17-02 | 720 | 32 | 16483929 | 26.91% |
| 09 | 7 | 200217_03 | L17-03 | 360 | 21 | 11292322 | 33.52% |
| 10 | 7 | 200217_04 | L17-04 | 360 | 14 | 13652153 | 24.86% |
| 11 | 12 | 200217_05 | L17-05 | 540 | 9 | 15460119 | 65.19% |
| 12 | 12 | 200217_06 | L17-06 | 600 | 16 | 12445640 | 52.36% |
| 13 | 18 | 200217_07 | L17-07 | 480 | 44 | 7368881 | 14.70% |
| 14 | 18 | 200217_08 | L17-08 | 600 | 19 | 10104935 | 28.63% |
| 15 | 22 | 200217_09 | L17-09 | 1320 | 10 | 19235065 | 43.97% |
| 16 | 22 | 200217_10 | L17-10 | 1020 | 81 | 16095402 | 34.96% |

### *4.2.3 Nanopore sequencing of bacterial communities in eDNA*

To confirm two water masses are present, we used MinION nanopore sequencing of nine samples of bulk environmental DNA (Table S1). Initially, the sample was passed through a OneStep PCR inhibitor removal column (Zymo Research, CA). The quantity of DNA in each sample was quantified using a Qubit 3.0 fluorometer (Invitrogen, MA) with a Broad Range Assay. Using a starting volume of 500ng of DNA in 10 µl, we added 15 µl of nuclease-free water, and used DNA repair (NEBNext FFPE DNA Repair Mix; NEB, MA) and end-prep (End repair/dA-tailing Module; NEB, MA) following the manufacture protocol. The samples were then subject to a bead-clean up (30 µL of sample, 30 µL AMPure XP beads, Beckman Coulter CA) using 75% fresh ethanol. After clean up, 1 µL was quantified using the Qubit fluorometer.

The DNA was then adaptor-ligated (NEBNext Quick Ligation Module; NEB, MA) and cleaned again using AMPure XP beads, following protocol specified by the manufacturer. A nanopore buffer (either SFB or LFB) was used to wash beads and enrich fragments. The sample was stored in a LoBind tube (Eppendorf) and 1 µl was quantified using the Qubit fluorometer, ensuring samples contained between 3 and 20 fmol. Samples were sequenced

on Flongle flow cells (Oxford Nanopore, Oxford, UK) following the manufacturers protocol, ensuring a minimum active pore number of 60 prior to loading.

*4.2.4 PCR amplification, and Illumina sequencing fish genomic variants in eDNA*

We used a set of 100 pairs of primers flanking regions containing SNPs, identified by Malinsky et al. (2015), to amplify target sequences using PCR. Primers were assigned to 26 different groups according to their loci and annealing temperatures, and PCR reactions were performed in multiplex. Three replicates were performed on each eDNA template with each primer group. Each PCR was conducted in a 10 µL volume comprising: 5 µL AmpliTaq Gold 360 Master Mix (Applied Biosystems, MA); 0.5 µL forward primer from each group (5 µM original concentration); 0.5 µL reverse primer from each group (5 µM original concentration); 3 µL molecular grade water; and 1 µL eDNA template. Thermocycling initially comprised a polymerase activation step at 95°C for 10 mins. This was followed by 40 cycles of: denaturation at 95 °C for 30 s, annealing (estimated annealing temperature plus 3-4 degree) for 30 s, extension at 72 °C for 60 s. The final extension was at 72 °C for 10 mins. Alongside the extracted 16 samples we include 2 extraction negative controls in the PCRs. The eDNA extractions, pre-PCR preparations, and post-PCR procedures were carried out in separate rooms.

PCR products were checked on gel, and then pooled and purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and the Oligo Clean & Concentrator kit (Zymo Research, CA) using a modified version of the manufacturer protocols (Supporting Information Text S4.1). Library preparation was conducted using xGen$^{TM}$ UDI-UMI adapters (IDT, IA), which contain Illumina adaptors, attached to the amplicons using the PCR-free KAPA HyperPrep Kits (Roche, Basal, Switzerland) following the manufacturer's protocol. A total of 16 libraries using unique indexes were created. Libraries were then quantified individually using a NEBNext qPCR assay (New England Biolabs; MA), standardised, and sequenced on an Illumina NextSeq 500 using v2.5 (2 × 75 bp paired-end) high output chemistry and 10% phiX spike-in. Sequence data are available at the Sequence Read Archive BioProject PRJNA985047.

### *4.2.5 Bioinformatic analyses of nanopore sequences of bacterial communities*

Base-calling and quality filtering were performed using Guppy software (Oxford Nanopore Technologies). Adaptors were removed using Cutadapt 4.1 (Martin, 2011). For assignment of reads to microbial taxa, we used KrakenUniq 0.7.3 (Breitwieser et al. 2018) employing the minikraken_20171019_8GB microbial database (https://ccb.jhu.edu/software/kraken/). Taxonomic assigned reads were filtered to only include bacteria assigned at the order level. To quantify community structure across the depth gradient we used a Canonical Correspondence Analysis using the R package vegan 2.5-7 (Oksanen et al. 2020), testing the association of the primary axes of variation with depth using the anova.cca function, with 10000 permutations.

### *4.2.6 Bioinformatic analyses of fish genomic variants in eDNA*

Demultiplex sequencing reads were trimmed to remove adaptors using cutadapt 4.1 (Martin 2011). They were then mapped to an indexed *Astatotilapia calliptera* reference genome (fAstCal1.2; GCA_900246225.3) using the mem function in bwa 0.7.17-r1188 (Li and Durbin 2009). Then, using samtools 1.9 (Danecek et al. 2021) the resultant sam files were converted to bam files using the view function, sorted using the sort of function, read groups added using the addreplacerg function, and the bam files were indexed using the index function. Mapping success was determined using the flagstat function in samtools.

We initially focussed the analysis on 98 primer pairs that could be located using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) within the fAstCal1.2 genome. Using the coordinates of the target sequences in a .bed file, we used vcftools 0.1.16 (Danecek et al. 2011) to filter a VCF file of 648 aligned Lake Masoko *A. calliptera* genomes, sourced from Munby et al. (2021). The resultant VCF file contained a set of 120 biallelic SNPs within the 98 target loci. The coordinates of these target 120 SNPs were used to generate a .bed file.

We counted the number of reads assigned to each allele in each SNP within the eDNA-derived bam files using the ASEReadCounter function in gatk 4.3.0 (McKenna et al. 2010). These allele count data files were then manually curated into a combined file including all samples, listing the reference allele and alternative allele. We retained 71 focal SNPs that were represented in at least 75% of 16 samples.

We tested if reference allele frequencies of SNPs in the eDNA were significantly associated with reference allele frequencies of SNPs in the fish, for each of five depth strata. Using eDNA-derived data we calculated the average reference allele frequency of each SNP in samples from each depth. We then filtered the VCF file of genome-wide SNPs from 648 aligned Lake Masoko *A. calliptera* individuals, to retain only those 71 focal SNPs. Next, we filtered the file of 648 individuals to generate five separate VCF files, grouping by the depth range that they were collected, using the view function in bctools 1.8 (Danecek et al. 2021). We then generated the allele frequency of individuals from each depth range using the freq2 function in vcftools 0.1.16 (Danecek et al. 2011). Using linear models in R (R Core Team 2020) we compared SNP reference allele frequencies of: 3 m eDNA to 0-5 m fish; 7m eDNA to 5-10 m fish; 12 m eDNA to 10-15 m fish; 18m eDNA to 15-20 m fish; 22 m eDNA to 20-25m fish.

We determined if the 71 SNPs were able to resolve genetic structure over the Lake Masoko gradient, using SNP data derived from sampled individual fish collected from known depths (530 of the 648 individuals). First, we pruned out SNPs in linkage disequilibrium from the main set (3107901 of 3881258 variants removed) using plink 1.90b6.2 (Purcell et al. 2007). Next, we used Admixture 1.3.0 (Alexander et al. 2009) to calculate individual ancestry scores for this set of 773,357 unlinked SNPs, assuming two populations (K = 2). We then calculate individual ancestry scores based on the 71 focal SNPs.

To summarise variation across the 71 SNPs in the 16 eDNA samples, we used Principal Component Analysis (PCA) using R package pcaMethods (Stacklies et al. 2007). Finally, we compared the shift in allele frequencies from shallow (3 m eDNA; 0-5 m fish) to deep (22 m eDNA, 20-25 m fish) habitats, between the two datasets, using the cor.test function in R.

## 4.3 Results

### *4.3.1 The separation of water masses*

Our analyses supported the concept that the thermo-oxycline separates Lake Masoko into two water masses. We found a significant association between the composition of the bacterial community and increasing depth (Canonical Correspondence Analysis, Anova $F_{1,7}$ = 4.582, $P$ = 0.009), with the major axis of variation (CCA1) capturing a switch in community composition between 10-20 m (Fig. 4.2a), coincident with the known position of the thermo-oxycline at approximately 15 m (Fig. 4.1c-d). The shift the functional composition of the bacterial community reflected the change in environment, with shallow, well oxygenated, warmer and more brightly lit waters possessing the greatest proportion of photosynthetic cyanobacteria (e.g. *Synechococcales*), and deeper, poorly oxygenated, cooler and darker waters possess the greatest dominance of anaerobic bacteria (e.g. *Enterobacterales*) (Fig. 4.2b).

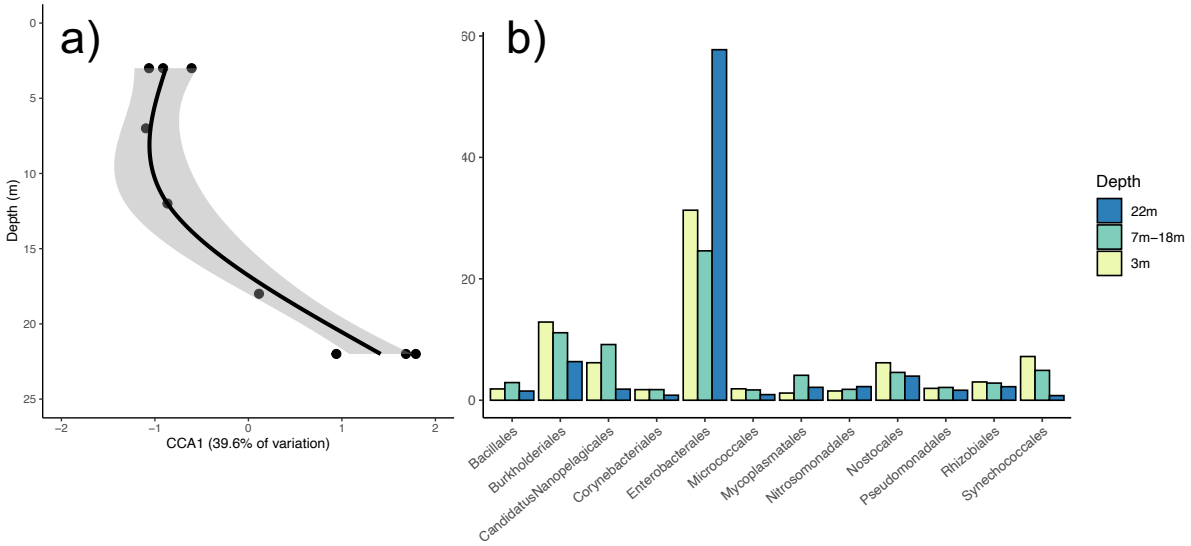



**Figure 4.2.** a) The primary axis of bacterial community composition (CCA axis 1) shows community change with increasing depth, a line fitted with a generalised additive model smooth with the shaded area representing one standard error. b) The mean proportion of reads assigned to each order of bacteria, within surface (3 m, n=3), central (7 m-18 m, n=3) and deep (22 m, n=3) waters of the lake.

### *4.3.2 Nuclear eDNA to identify SNPs*

Our analyses of eDNA samples from the five depths (3, 7, 12, 18 and 22 m) all provided evidence of target nuclear DNA reads. Average mapping success of reads to the *Astatotilapia calliptera* genome (fAstCal1.2) was 52.56% of reads (range 14.70 to 97.89%; Table 4.2). Within these reads we found 114 of these 120 SNP positions. Of these 114 SNPs, 102 were resolved as variables in the eDNA. We filtered the SNPs to only include those present in 12 or more of the 16 samples. This resulted in a final set of 71 SNPs, from 54 amplified loci.

### *4.3.3 Correlation of allele frequency in eDNA and fish*

We confirmed these 71 SNPs can resolve population genetic structure over the depth gradient in the fish samples with known collection depth subject to whole genome sequencing (n = 530 individuals from Mumby *et al.* (2021); 3,881,258 biallelic SNPs, 773,357 after filtering for linkage disequilibrium). The plotted individual ancestry estimates from the whole genome sequencing data showed a clear break in the population genetic structure between 12 m and 18 m, coincident with the location of the thermo-oxycline. Using the subset 71 SNPs, it was possible to resolve a similar pattern across the five depth strata, although with less clear-cut assignment of individuals to the two population genetic clusters (Figure 4.3).

Focussing on the 71 SNPs, at each depth, there was a strong positive association between the allele frequency observed in the fish, and the allele frequency observed in the environmental DNA (Fig. 4.4a; Table 4.3).

The Principal Component Analysis on allelic composition in of the 71 SNPs in the eDNA samples revealed clear differences in the allelic composition of the deepest and shallowest sample grouping (Fig. 4.4b). To determine if the SNPs that were contributing to variation in the fish corresponded to the SNPs that were contributing to variation in eDNA, we compared the change in SNPs allele frequency from the fish from 0-5 m to 20-25 m, with change in SNPs allele frequency from the eDNA from 0-5 m to 20-25 m. Overall, we found a positive significant association between change in allele frequency in the eDNA, and the change in allele frequency in the fish (Pearson's correlation, n=71, $r^2 = 0.268$, $p = 0.0237$). This suggests overall

that allelic variants of SNPs contributing to the population genetic structure were consistent between the fish and eDNA datasets. However, we note this pattern was driven by ~20 SNPs that showed the greatest contrast in reference allele frequencies between depths in the eDNA dataset (Fig. 4.4c).

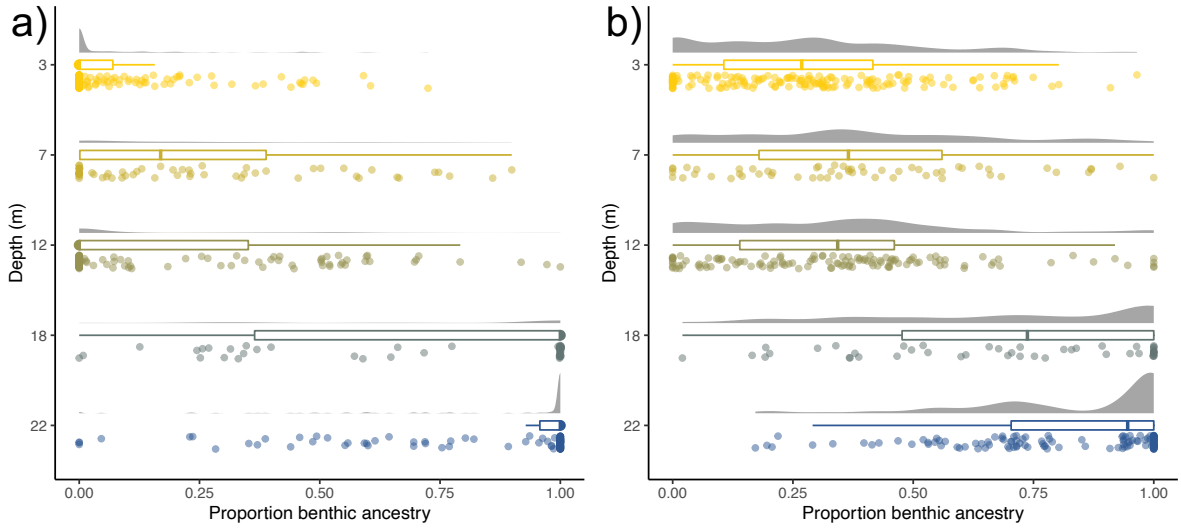
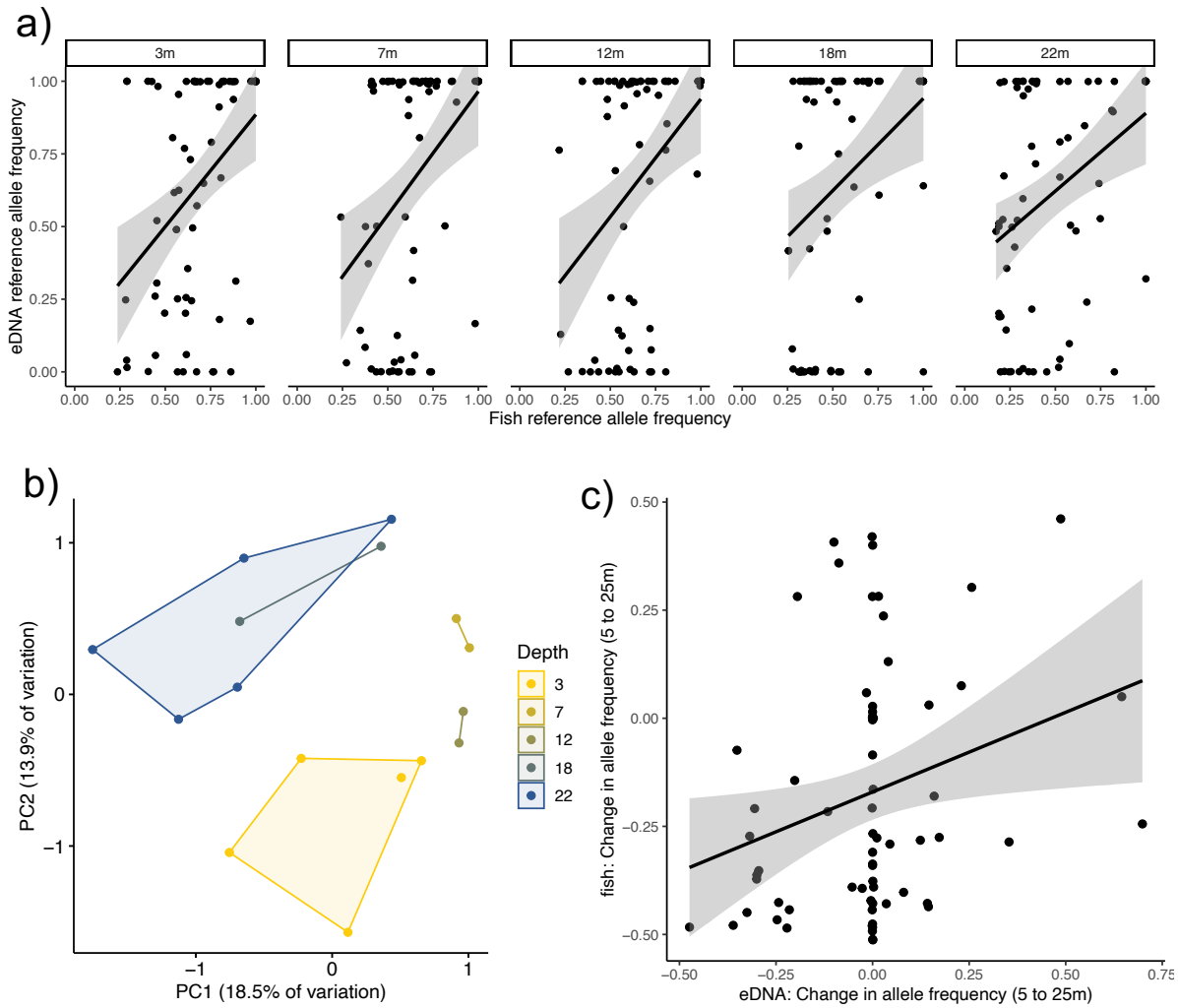



**Figure 4.3.** Results of admixture analyses of fish assuming two populations (K=2) using data from a) the whole genome (773,357 SNPs after filtering for linkage disequilibrium) and b) 71 focal SNPs. Each datapoint represents one individual fish.

**Table 4.3.** Linear models comparing allele frequencies in eDNA and allele frequencies in fish samples at the 71 focal SNPs.

| Depth (m) | Slope | Slope SE | *F* | $R^2$ | *P* |
|---|---|---|---|---|---|
| 3 | 0.21604 | 0.05814 | $F_{1,69}$ = 13.81 | 0.167 | 0.00041 |
| 7 | 0.19193 | 0.05274 | $F_{1,68}$ = 13.24 | 0.163 | 0.00053 |
| 12 | 0.18451 | 0.05298 | $F_{1,69}$ = 12,13 | 0.149 | 0.00086 |
| 18 | 0.18699 | 0.06183 | $F_{1,68}$ = 11.85 | 0.118 | 0.00352 |
| 22 | 0.27703 | 0.07985 | $F_{1,69}$ = 12.04 | 0.148 | 0.00090 |




273

274 **Figure 4.4.** a) Correspondence between allele frequencies in fish sampled from each depth
275 stratum, and mean allele frequencies from eDNA sampled from the same stratum. b)
276 Population genetic structure of fish inferred from eDNA allele frequencies in each sample
277 using the focal 71 SNPs. Each point represents one eDNA sample. c) Correspondence
278 between shifts in mean allele frequencies in eDNA samples (between 5 and 25 m) and shifts
279 in allele frequencies in fish sampled (between 5 and 25 m), for the 71 SNPs, line derived
280 from a linear model, shading = 1 standard error.

281

## 4.4 Discussion

Our study demonstrates that the clear depth differences in genetic structure of the Lake Masoko *Astatotilapia calliptera* ecomorphs are reflected in the aquatic eDNA. We suggest that this pattern has arisen due to strong adherence of the ecomorphs to specific shallow or deep-water habitats, with very little active movement between depths. Although to date the movements of these fish have not been tracked, there is clear support for habitat-specific adaptation in adult individuals of these cichlids, from phenotypic (Malinsky et al. 2015; Carruthers et al. 2022; Vernaz et al. 2022), genomic (Malinsky et al. 2015; Carruthers et al. 2022; Munby et al. 2021), transcriptomic (Carruthers et al. 2022; Vernaz et al. 2022) and epigenetic (Vernaz et al. 2022) evidence. Differences in environmental regimes, including light, oxygen and food resources may have led selection to favour philopatry, plausibly to strong negative effects on fitness for individuals with inappropriate phenotypes for specific habitats (Hendry et al. 2004). Notably, although work to date to indicates that strong differences in adaptive traits have been focussed on adult individuals, divergence in eDNA between the depth zones either side of the thermo-oxycline is suggestive of philopatry being present in all life history stages, including juveniles.

### *4.4.1 Vertical structure of alleles in eDNA*

Evidence of spatial structuring of allele frequencies within eDNA across an extremely small spatial scale of less than 20m matches the structure observed across the source fish populations. This work therefore clearly indicates the potential for eDNA to be used to describe fine scale spatial structure in biological assemblages, both at the community and species level. The spatial structure we observed is most plausibly driven by the restricted movement of the ecomorphs by the presence of thermo-oxycline. Studies in both freshwater and marine systems have also revealed the potential for fine scale differences in environmental DNA composition over narrow depth gradients. For example, in shallow Canadian lakes (13-30m deep), there was clear differentiation in the fish species resolved between five depth gradients when the lakes were thermally stratified – matching the distribution of the fish species, however those differences in community structure among depth zones were not present when the lakes were mixed (Littlefair et al. 2021). Similarly, in a New Zealand fjord, stratification with a halocline led to differentiation in fish, crustacean

and echinoderm community composition being resolved using eDNA over a depth of only 4 metres (Jeunen et al. 2020). Even in the absence of a clear water mass boundary, in Californian kelp forests differences in fish community structure have been resolved over a depth gradient of only 10 metres (Monuki et al. 2021). It is becoming clear therefore that aquatic eDNA may be useful to describe not only gradients in diversity across larger spatial scales as is commonly recognised (e.g. Holman et al. 2021), but also fine spatial scales in those systems, such as Lake Masoko, that are characterised by limited water movement and stratification.

### *4.4.2 Nuclear eDNA in population genetics*

This study, to our knowledge, is unique in demonstrating that sufficient information on nuclear SNP allelic composition can be gained from environmental DNA to enable inference of population genetic structure. Studies that have explored the capacity for population genetics using eDNA data until now have focussed on mtDNA (e.g. Sigsgaard et al. 2016), or nuclear microsatellites (e.g. Andres et al. 2021). We were able to benefit from *a-priori* knowledge of the SNPs that were present and segregating in our focal species within a well characterised environment. Future population genomic work taking a targeted metagenomic approach using eDNA would equally require knowledge of segregating SNPs of focal populations. This approach has a clear advantage to shotgun sequencing of eDNA, as it enables selective amplification of target alleles, and enables some taxonomic specificity, but may suffer from PCR biases. An alternative method would be to use DNA hybridization-capture techniques to select regions of interest from within bulk environmental DNA, as has been applied when targeting species-specific mitochondrial regions from eDNA (Aylward et al. 2018; Wilcox et al. 2018).

### *4.4.3 The future of eDNA application in population genetics*

Our study highlights the importance of a reference genome of the target species for enabling the identification and targeting of SNPs suitable for environmental DNA-based population genetics. More widespread availability of reference genomes from non-target species in communities would help in the identification of clear species-specific genomic variants that can be reliably used to study population genetic structure. In Lake Masoko, the dominant fish species is *Astatotilapia calliptera*, but three other fish species are present

including two cichlids (*Coptodon rendalli* and *Oreochromis squamipinnis*), and one non-cichlid (*Clarias gariepinus*) (Turner 2019). In addition, there are many domestic and wild terrestrial vertebrates in the Lake Masoko crater generating environmental DNA that will enter the water. It is possible that primers would also amplify fragments of eDNA from these or other sources, which may vary spatially in the lake, and this may explain the high variation in mapping success we observed (samples ranged from 14.7 to 89.5%). Ideally this study, and future work, requires the implementation of data filtering steps that remove reads that map more closely to heterospecifics in the environment. We suggest, however, that since we focussed on 71 known biallelic SNPs with known nucleotides, heterospecifics are unlikely to have had a major influence on the results presented.

### *4.4.4 Concluding remarks*

In principle, population-genomic methods using aquatic environmental DNA could have considerable value for research focussed on species that are technically hard to sample due to their intrinsic rarity, low catchability, or occupancy of sites that are difficult to access (Adams et al. 2019). It may also be valuable to studies of species where the capture and direct sampling of genetic material may be ethically questionable. This study has contributed to this field of research through a clear demonstration that nuclear loci can be reliably amplified from environmental DNA, and that it is possible to generate allele frequencies that can be used for population genetic inference. Certainly, the results will be influenced by a multitude of factors, including the methods used for eDNA collection, preservation, extraction, as well as the protocols used for PCR and sequencing. There are also issues raised about how effective eDNA-based methods may be able to inform us about population level genetic processes, such as selection, drift and mutation. The results nevertheless give a strong indication that future research aimed at refining eDNA-based population genomic methods has potential to improve our understanding of population structure of many species of commercial, ecological and conservation importance, within marine, estuarine and freshwater systems.

**Supporting Information**

**Supplementary Information Text S4.1.** Protocol for gel-based DNA cleanup of PCR amplicons

Before starting:

a. Prepare buffers and solutions following the manufacturer's instructions.
b. Preheat an incubator to 30 °C.
c. Store pure ethanol in -20 °C freezer for at least one hour.

Procedure

1. Add 4 μL (range 0-100 bp) gel ladder in a 2% agarose gel (120 mL 0.5% TBE buffer, 1X gel stain). Carefully add 20-30 μL PCR product into each well. Electrophoresis for 30 mins under 100 V.
2. Use a sharp blade to cut off the area of interest (here 50-100 bp), then transfer gel pieces into a 2 μL microcentrifuge tube.
3. Add 1 volume of QE buffer (QIAquick Gel Cleanup kit) (e.g. 100 mg gel, add 100 μL QE buffer) and incubate tubes in 35 °C for at least 15 mins until gel is fully dissolved.
4. Add 2 volumes of Oligo Binding buffer to the mixture (e.g. 200 μL mixture, add 400 μL OB buffer), then add 400 μL -20 °C 100% Ethanol and mix thoroughly by pipetting.
5. Place a Zymo-Spin IC column in a 2 mL collection tube, carefully transfer the mixture to the column, and centrifuge for 45 s at 14,000 rpm. Discard flow-through and collection tube. Repeat this step to make sure all mixture goes through the spin column.
6. Place the spin column in a new 2 mL collection tube, add 750 μL DNA Wash buffer, and centrifuge for 1 min at 14,000 rpm. Discard flow-through and collection tube. This step can be repeated (optional). And then place the spin column in a new 2 mL collection tube and centrifuge for 2 mins at 14,000 rpm to remove the residual ethanol.
7. Place the spin column into a clean 2 mL microcentrifuge tube, add at least 6 μ 10 nM Tris buffer (no EDTA) directly to the membrane of the spin column and elute at room temperature for 10 mins. Centrifuge for 45 s at 14,000 rpm. DNA will be eluted and preserved in the flow-through. Store the tubes in -20 °C.

# Chapter 5

# General discussion

## 5.1 Summary

This thesis presented research on environmental DNA from three different aquatic environments, providing new insights into eDNA-based approaches to studying biodiversity. Chapter 2 reports metabarcoding results from an eDNA survey of the Western English Channel over a period of 14 months, revealing the spatial and temporal pattern of elasmobranch community. These results enable us to conclude that eDNA metabarcoding can provide a non-invasive, sensitive and fairly accurate approach to elasmobranch monitoring, as well as helping to resolve the broader spatial and temporal pattern of their distributions in the marine environment. Chapter 3 reports the results from a metabarcoding study of eDNA samples collected across depth and latitudinal gradients in the Scotia Sea region of the Southern Ocean. The results focus on the mesopelagic fish community and demonstrate a disconnect between the biomass of fish in survey trawls, and numbers of eDNA reads assigned to those species. The results also indicate the potential application of eDNA-based methods in the mapping and monitoring of marine mammal diversity in Antarctic environments. Chapter 4 reports an analysis of nuclear allele frequencies of *Astatotilapia calliptera* within a ~30m depth African crater lake, comparing data derived from sampled fish to the data derived from sampling eDNA. The results indicate a non-destructive way of studying population genetic structure of focal species within aquatic environments.

## 5.2 Environmental DNA in elasmobranch conservation

Elasmobranchs (sharks, rays, skates and sawfish) are undergoing a global-scale extinction crisis caused primarily by overfishing (Dulvy et al., 2021; Simpfendorfer, 2022). In total 32.6% of elasmobranch species are now listed as threatened on the IUCN Red List of Threatened Species (either Critically Endangered, Endangered or Vulnerable) (Dulvy et al., 2021). A lack of long-term monitoring data is a major challenge in elasmobranch conservation, in part because of the highly mobile and pelagic nature of many species. The

study presented in Chapter 2, based in the Western English Channel, successfully recovered 13 elasmobranch species from eDNA samples, including threatened species including tope shark and undulate ray. It proved more successful at locating species than trawl surveys conducted at the same locations over the same period, which yielded specimens of only three species. The study also found significant spatial differences of elasmobranch community structure over a 30 km scale, and demonstrated a clear seasonal shifts of elasmobranch composition, possibly linked to seasonal differences in breeding activity and overwintering locations. There was also a significant positive correlation between metabarcoding reads assigned to species from eDNA samples, and catch per unit effort resolved from over a century of trawl survey (1911-2018). This indicates the potential of eDNA to resolve the presence of species that may be uncommon in a region, and shows how eDNA metabarcoding can be considered to be a semi-quantitative approach. However, challenges of employing eDNA analysis were also highlighted in this study. For example, we are unsure of the extent that the results have been influenced by eDNA transportation and mixing (Harrison et al. 2019) and it is also unclear how the results were influenced by differences in rates of eDNA production across species, space or time, perhaps related to life stage or activity levels (Danziger et al. 2022).

Although we recovered a high proportion of the elasmobranch species known to be in the Western English Channel, it is notable that eDNA failed to recover two species: shagreen ray and angelshark, which were present in records of historical trawls undertaken by the Marine Biological Association of the UK. Moreover, several species, such as basking shark (*Cetorhinus maximus*) and blue shark (*Prionace glauca*), which have been encountered in the study area, were also absent from eDNA analysis. The absence of these species in eDNA analysis may be because they were absent or extremely rare in the study area at the time of sampling. It may also be that the species resolution we obtained was impaired by primer biases, sequencing depth, bioinformatic filtering or insufficient sampling effort. Future research would benefit from (a) a detailed evaluation of local oceanographic influences on patterns of eDNA movement, (b) evaluation of primer efficiencies and biases in the laboratory, (c) assessing sampling efforts to determine if these were optimal for the target species assemblage, and (d) determining if there are opportunities to further optimise and standardise the bioinformatic analyses. The raw sequencing data and metadata for this

chapter are freely available, so researchers can examine the reproducibility of the analyses presented.

### 5.3 Environmental DNA in the Southern Ocean

Ecosystems in Antarctica and its surrounding Southern Ocean are facing challenges from climate change and other anthropogenic impacts (Howell et al. 2021), and it is possible that biodiversity loss is already ongoing due to climate stress (Chown et al., 2017). We know relatively little about many of the organisms living in these ecosystems, because of the extreme weather and inaccessible environments that make them challenging to study. Hence, molecular biology approaches could help to generate a more comprehensive understanding of their ecology (Howell et al., 2021; Ragot & Villemur, 2022).

Chapter 3 reported an eDNA metabarcoding survey of the mesopelagic fish assemblage of the Scotia Sea in the Southern Ocean. While 29 fish species were recovered from 127 eDNA samples, the data were patchily distributed across samples, possibly because the sampling volume was too low for the targeted organisms, or because of unknown issues related to DNA preservation. Moreover, we found no evidence of a significant association between the abundance of fish captured in survey trawl nets and eDNA abundance, possibly because of a substantial influence of the genetic material present in eggs or larvae, because of transport or mixing of eDNA across the water column, or because of diel vertical migration of adults that means DNA is actively deposited across the water column. The study also provided strong evidence of the capacity of eDNA to capture information on marine mammal diversity and distribution in Antarctic seas. Therefore, eDNA sampling that targets marine mammals using taxon-specific metabarcoding primers, may have the capacity to provide comprehensive temporal and spatial habitat use information, informing conservation initiatives.

To further study fish distributions and behaviour, we suggest that specific sampling to target key species using qPCR may be valuable, for example the myctophid *E. antarctica*. This approach may give new insights into the compositions of mesopelagic fish species aggregations, the extent of vertical migration that these species undertake across the region, and the extent of carbon transfer by the key species within the water column. Linking these data with the results of active acoustic surveys would enable comprehensive

estimates of biomass. Finally, a key observation of the research undertaken for Chapter 3 was the clear absence of a comprehensive fish and marine mammal sequence reference library, which will be imperative for future eDNA metabarcoding studies of Antarctic vertebrate communities.

### 5.4 Environmental DNA in population genetics

Although environmental DNA has been widely employed for studying the presence or abundance of species, there have been few attempts to study population genetic structure using eDNA as a substrate. Those studies that have explored population genetic structure using eDNA have focussed on mitochondrial (Dugal et al., 2022) or nuclear microsatellite (Andres et al., 2021) markers. In Chapter 4, it was shown that information on nuclear SNP allelic frequencies can be gained from environmental DNA, enabling inference of population genetic structure. A clear vertical pattern of allele frequencies was found within the eDNA and this broadly matched the structure observed in fish samples, which indicates that eDNA-based approaches can describe intraspecific population genetic structure on a fine geographical scale.

The ability of eDNA to capture population genetic structure will vary among aquatic ecosystems and be dependent on the extent of water movement and stratification. In addition, there are other considerations when determining if eDNA can reliably be used to study population genetic differences. First, any study would benefit from pre-existing knowledge of genetic variation within the study region, possibly from genome-wide sequencing. Second, it would be beneficial to know about genetic variation in closely related species, that may be inadvertently sequenced alongside the eDNA of target species. In our study, it is possible that some sequences of heterospecific fish species may have been amplified and mapped to the Eastern happy cichlid genome. It would be useful to determine the specificity of our target primers, from *in-silico* testing, laboratory tests of amplification success against known DNA templates, or through careful evaluation of our derived sequences.

Due to the rarity of nuclear DNA from targeted species, our approach was to use PCR-amplification, which may have led to possible PCR biases and errors, and therefore mismatches between eDNA-based allele frequencies and those that we observed in the fish.

It may also explain why there was considerable variation among samples in the SNPs that were recovered. It is possible that alternative PCR-free sequence capture methods could be used in future. There are also opportunities for the development of new bioinformatic pipelines that are specifically able to exclude heterospecific sequences, and enable mapping and scoring of genetic variation in eDNA from target species.

### 5.5 The future of aquatic eDNA-based research

The limitations of eDNA-based sequencing approaches primarily focus on three topics: sampling challenges, PCR biases and sequencing errors. In studies of aquatic eDNA, water volume, sampling location and replicates should be decided based on local ecological knowledge and can be helped by eDNA sampling optimising models. For example, in freshwater ecosystems, Erickson et al. (2019) proposed a sampling design based on occurrence model results, while Carraro et al. (2021) demonstrated optimal sampling locations in river networks based on elevation and watersheds. In marine environments, Andruszkiewicz et al. (2019) showed that models can be used to determine rates of eDNA transportation, and in principle such models could be used to inform optimal sampling plans, particularly if rates of eDNA persistence are known.

Compared to other survey methods, sampling effort for eDNA-based analyses can be relatively modest (Peterson et al. 2022). Nevertheless, researchers are still required to spend time on water collection, filtering, eDNA extraction and processing. As technology advances, it is possible to envisage water sampling equipment that reduces or eliminates issues associated with filter clogging, and can readily be deployed across a range of aquatic environments (Turner et al., 2014; Hunter et al., 2019). In addition, straightforward extraction protocols may be designed to reduce the risk of contamination, while saving extraction time, and elevating the DNA concentrations available to work within downstream analyses. Ideally, inexpensive automatic extraction systems would be developed (Adams et al., 2019).

In general, there is a strong expectation among scientists, policy makers and the public that eDNA-based methods can transform our understanding of the natural environment. Developing inexpensive and rapid sampling accessories and sequencing technologies will certainly increase the accuracy and efficiency of eDNA approaches. In return, eDNA may be

able to provide rapid information on the species diversity in habitats, and possibly also the genetic diversity within those species. This rich source of biological and ecological data will help humans to tackle future ecological challenges, including pollution, defaunation and climate change.

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