Exploring Jellyfish as Natural Samplers of Environmental DNA

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Table of Contents

Chapter One: Introduction

Chapter Two: Methods of Exploring Jellyfish as Natural Samplers of Environmental DNA

Chapter Three: Results

Chapter Four: Discussion and future research

Tables and Figures

Tables

Figures

Abstract

Conservation management aimed at mitigating the ongoing biodiversity loss critically relies on monitoring of ecosystems, to enable accurate predictions of species population sizes and distributions. Environmental DNA (eDNA) analysis has become increasingly popular for noninvasive and high-throughput species detection and biodiversity assessments. Recent advancements in the field include the use of natural samplers of eDNA (nsDNA), which are organisms that trap genetic material from the environment in their tissues. In this study, the potential of jellyfish to serve as marine nsDNA samplers was investigated. This was explored using four jellyfish (phylum Cnidaria, Class Schyphozoa) species (*Aequorea forskalea, Aurelia solida, Rhizostoma pulmo*, and *Cotylorhiza tuberculata*) that were collected from the same location. The findings revealed that jellyfish collect eDNA in their tissues, with 23 fish species detected from 12 jellyfish individuals. However, the amount of eDNA collected in the jellyfish tissue was shown to vary between different species. This study highlights the potential of jellyfish as tools for enhancing biodiversity monitoring efforts, particularly in remote and inaccessible areas where conventional surveying techniques may be difficult to employ but jellyfish can be easily captured. By opportunistically collecting jellyfish as nsDNA samplers, it is possible to overcome logistical constraints and obtain valuable eDNA samples, providing information on the biodiversityof environments that are otherwise challenging to collect eDNA. However, careful consideration must be given to the selection of jellyfish species for such surveys. The results of this pilot study offer new insights into the use of jellyfish as nsDNA samplers and provide a foundation for further advancements in biodiversity monitoring.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Chapter One: Introduction

1.1 Environmental DNA

Environmental DNA (eDNA) analysis has emerged as a powerful and non-invasive technique that enables the extraction and analysis of DNA from environmental samples (Taberlet et al., 2012a). By capturing and analysing eDNA, it is possible to gain valuable insights into the taxonomic and functional aspects of an ecosystem without the need for direct capture or observation of organisms (Thomsen and Willerslev, 2015). Environmental DNA is derived from various organismal sources, including shed skin cells, faeces, urine, mucus, and sperm (Taberlet et al., 2012a). These sources contain genetic traces with species-specific DNA fragments which can be used to identify specific organisms, or for the assessment of the biodiversity within an ecosystem. The approach offers advantages such as the detection of elusive species (Ficetola et al., 2015), comprehensive community assessments, and monitoring of changes in biodiversity, making it an important tool for conservation efforts.

The process of eDNA analysis begins with sampling the environment. Environmental samples can be collected from different sources including water, sediment, soil and more recently, air (Lynggaard et al., 2022). Once obtained, eDNA extraction techniques are used to isolate the DNA from other biological and environmental material. This is followed using polymerase chain reaction (PCR) to amplify and detect the DNA of either a single target species or the simultaneous amplification of DNA from broader taxonomic groups (Thomsen et al., 2012a; Doi et al., 2015). The amplified DNA is subjected to Next-Generation Sequencing (NGS), which enables the highthroughput sequencing of the eDNA samples. This allows for the simultaneous sequencing of millions of DNA fragments extracted from the eDNA samples in a single experiment, enabling comprehensive sampling of the genetic material present in the environment. The resulting sequencing reads obtained from NGS platforms are subsequently processed using bioinformatics tools and compared to reference databases. This comparison enables the identification of the organisms present within the samples.

The PCR step makes use of specific primers that target DNA regions of interest, enabling identification of organisms present in the samples. Species-specific primers have been used to detect individual taxa such as rare and elusive species (Ficetola et al., 2008; Thomsen et al., 2012b). Species-specific primers have been designed to specifically target the DNA of invasive species, allowing for the identification of their presence in environmental samples. By detecting DNA fragments unique to these invasive organisms, eDNA analysis enables early detection and surveillance of invasive species, even at low population densities or in challenging environments where direct observation may be difficult (Dougherty et al., 2016; Klymus, Marshall and Stepien, 2017). This early detection enables the implementation of timely intervention strategies to prevent or mitigate the negative impacts of the invasive species. The same approach can be applied to endangered species, aiding in the rapid development of conservation strategies when their DNA is detected (Weltz et al., 2017). The use of species-specific primers in eDNA analysis enhances the sensitivity and applicability for both invasive species management and conservation efforts targeting endangered species.

DNA metabarcoding is an approach within eDNA analysis that can be used for resolving broader taxonomic assemblages and assessing biodiversity (Taberlet et al., 2012a). This makes it possible to identify multiple taxa found in a single environmental sample. The process makes use of DNA regions called metabarcodes which consist of short taxonomically unique DNA regions flanked by conserved regions. These conserved regions act as primer anchors during PCR. By sequencing the amplified DNA fragments, the diversity and composition of the taxa present in the sample can be resolved, providing a comprehensive picture of the ecosystem's biodiversity.

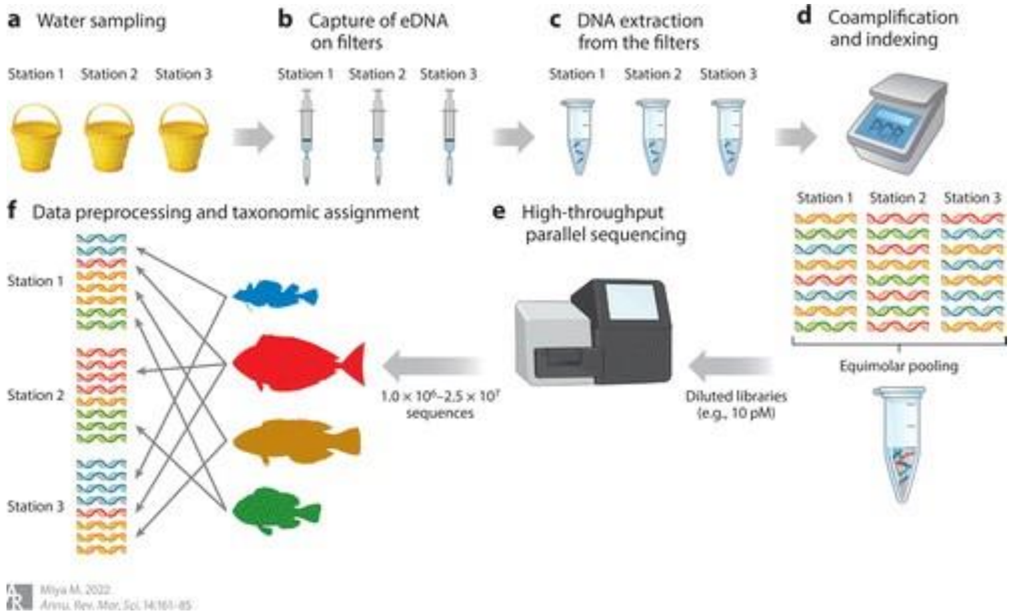


Figure 1 (Miya, 2022): The eDNA metabarcoding workflow for fish detection from water samples: (a) water sampled at three stations, (b) Water filtered on-site using a filter cartridge and syringe to concentrate and collect eDNA on the filter membrane, (c) eDNA extraction from the filter membrane, (d) amplification of fish eDNA fragments using PCR primers and library preparation through the addition of adapter and index sequences to the fragments, (e) high-throughput sequencing, and (f) bioinformatic analysis of DNA sequences for taxonomic assignment.

1.2 Marine eDNA Analysis

Environmental DNA analysis is particularly well-suited for aquatic environments, where the DNA released by organisms is well mixed and diffused in the water column. By collecting water samples, it is possible to extract the species-specific DNA fragments that capture the functional diversity of marine ecosystems (Aglieri et al., 2021). A comprehensive comparison of four different methods, namely underwater visual census strip transects (UVCt), baited underwater videos (BUV), small-scale fishery catches (SSFc), and eDNA metabarcoding suggested that eDNA analysis is comparable with traditional methods in capturing taxonomic diversity (Aglieri et al., 2021). Secondly, among the evaluated methods, eDNA stood out as the only approach capable of encompassing the entire range of considered functional traits, making it the most functionally diversified and least redundant in assessing fish assemblages. Lastly, it was revealed that eDNA exhibited high effectiveness in describing functional structure due to its lack of selectivity towards specific functional traits, underscoring its broad applicability and potential for providing comprehensive insights. These findings provide evidence supporting the advantages of eDNA metabarcoding, which has been particularly useful in marine research, where large-scale and multitaxa biodiversity surveys are challenging and expensive.

Mitochondrial DNA (mtDNA) is often targeted in marine eDNA analysis due to its higher copy number per cell compared to nuclear DNA, meaning it exists in higher proportions in the water making it more detectable (Hebert and Gregory, 2005). The choice of target gene depends on the taxonomic group or individual species of interest. Fragment lengths of the targeted DNA typically range from 79 to 285 base pairs (Ficetola et al., 2008), allowing for efficient amplification and detection. An extensively studied mtDNA gene for eDNA analysis is cytochrome c oxidase 1 (COI), which has led to a vast database with sequences representing a wide range of Metazoa (Hebert et al., 2003). There has been emphasis on the curation of a specific fish COI database (Ward, Hanner and Hebert, 2009), and indeed the COI primers have been used to successfully capture fish and invertebrate diversity in marine habitats (Nguyen et al., 2020). A study into the biodiversity of fish and invertebrate communities in a tropical coastal system found eDNA analysis using COI primers revealed community patterns consistent with visual surveys and known distribution records, demonstrating that COI primers can be used to unveil distribution patterns of fish and invertebrate taxa that may have been missed or underreported by visual methods (Nguyen et al., 2020). However, when specifically analysing fish communities, the COI fragment has been shown to perform poorly (Collins et al., 2019). This is due to the low proportion of chordate and fish reads amplified compared to non-specific amplification of prokaryotic and non-target eukaryotic DNA. In contrast, the mitochondrial 12S ribosomal RNA (rRNA) gene, another commonly targeted gene demonstrates better performance for vertebrate detection (Miya et al., 2015). These primers target a variable region of the 12S rRNA gene, which contains sufficient information to identify fishes to taxonomic family, genus and species. Both COI and 12S primers have been compared to traditional visual survey and 12S has shown greater congruence to surveys as well as greater detection of fish species (Collins et al., 2019). Therefore, the selection of the appropriate primer is crucial depending on the specific research objective of the study.

1.3 Marine Management

Genetic monitoring can enhance environmental management and policy decision making for Marine Protected Areas (MPAs) by providing accurate estimates of biodiversity and species distributions (Rees et al., 2014). Traditional marine monitoring protocols typically rely on underwater visual census surveys using SCUBA which are costly and labour-intensive. These are constrained by weather conditions and impacted by inaccuracies introduced by changes in marine organism's behaviour towards divers (Lindfield et al., 2014). Trawl surveys, or bottom trawl surveys, are commonly employed by fisheries to gather data on fish population dynamics and stock assessments. These surveys rely on specialised nets called trawls, towed behind research vessels to sample fish populations in specific areas. The invasive nature of trawling brings forth several negative impacts on marine ecosystems and fisheries. The method of bottom trawling causes rapid and substantial reductions in deep-sea stocks, removes benthic fauna and biogenic habitats, and leads to long-lasting impacts on biodiversity (Clark et al., 2016). The slow growth and limited recovery capacity of deep-sea ecosystems mean that the negative effects of bottom trawling persist for years, making it detrimental to the health and sustainability of these fragile environments. Bycatch, the unintended capture of non-target species, poses a significant concern, resulting in the unnecessary loss of marine life and biodiversity depletion. Furthermore, trawling exacerbates overfishing by depleting fish stocks and disrupting marine food webs. Thus, it is crucial to implement improved regulations and sustainable fishing practices to mitigate the adverse effects of trawling on our oceans.

Environmental DNA analysis has been shown to detect species not observed using traditional methods (Thomsen et al., 2012a). It offers advantages over traditional morphological identification methods, such as the ability to identify cryptic or elusive species that are difficult to identify using morphology alone (Elbrecht et al., 2017). Additionally, eDNA metabarcoding can circumvent many of the current logistical limitations of visual monitoring, increasing sampling frequency possibilities (Gold et al., 2021).

1.4 Constraints of current eDNA sampling methods

Marine eDNA is traditionally obtained from the ocean water by employing filtration techniques using artificial membranes (McQuillan and Robidart, 2017). However, these methods often present challenges as they can be cumbersome, requiring manual filtering of bottles using syringes or pumps, which not only consume significant amounts of plastic but also demand careful workflow management to minimise the risk of contamination. This can require a considerable budget for study design (Ficetola et al., 2015) or significant investment in advanced technologies such as integrated eDNA sampling systems (Thomsen et al., 2012a) and deep-sea robotic samplers (McQuillan and Robidart, 2017). Consequently, these solutions may pose constraints for small research groups and impede environments that are difficult to access.

To address these limitations, passive eDNA sampling has offered a simplified approach using costeffective capture media. One technique employed involved the use of adsorbent-filled sachets to collect and preserve eDNA (Kirtane et al., 2020). These sachets provide a convenient and affordable alternative to traditional mechanical filtration systems, allowing for easier and more accessible sampling. Another technique involves submerging filter membranes directly into the water column, eliminating the need of labour intensive water filtration (Bessey et al., 2021). This approach streamlines the sampling process and reduces the reliance on complex equipment. These passive sampling methods offer alternatives to mechanical filtration systems and provide simpler and more accessible approaches to eDNA sampling.

While passive eDNA sampling techniques have shown promise in providing simplified and costeffective alternatives to traditional filtration methods, they are still subject to limitations. One notable drawback is their reduced sampling efficiency compared to active sampling approaches. Passive sampling relies on the natural flow of water to bring eDNA into contact with the capture media, resulting in variable and unpredictable levels of eDNA capture influenced by factors such as water currents and depth. Moreover, the extended deployment durations required for passive sampling to achieve sufficient eDNA collection can pose logistical challenges in field studies. Longer exposure periods also increase the risk of cross-contamination and degradation of eDNA samples. Degradation of eDNA in the water column occurs rapidly, limiting the detection window within a range of 18.2 to 71.1 hours in marine systems (Collins et al., 2018). This emphasises the need to explore alternative eDNA sampling methods that may increase contact with eDNA and extend the timeframe for successful detection.

1.5 Natural eDNA Samplers

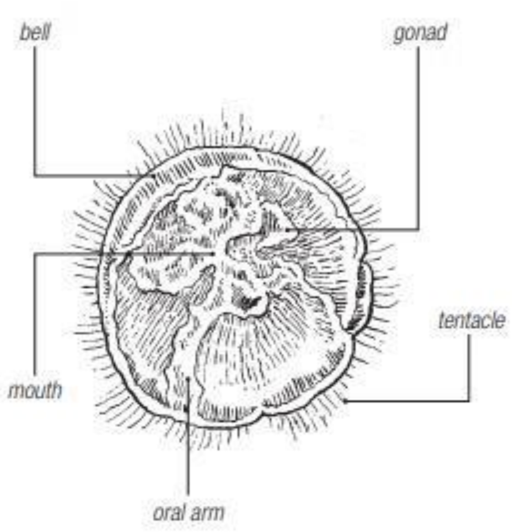
A novel approach to eDNA collection involves harnessing the eDNA accumulated by natural samplers (Mariani et al., 2019). It has been proposed that the use of nsDNA samplers could plastic waste, simplify sample collection, and be cost-efficient alternative, potentially overcoming the limitations of conventional sampling methods. Analysis of gut contents from shrimp has been used to assess fish diversity in pelagic ecosystems (Siegenthaler et al., 2019). By analysing the eDNA within the shrimp's digestive system, it was demonstrated that fish species were identifiable based on the consumed DNA, offering valuable insights into the composition of the fish community. However, the active predatory behaviour of shrimp may introduce biases and uneven representation of eDNA, underlining the necessity to identify alternative pelagic natural samplers.

A wide range of marine fish and mammals in Mediterranean and Antarctic waters have been successfully detected by extracting eDNA accumulated in the tissue of sponges (phylum Porifera) (Mariani et al., 2019). Sponge-based nsDNA metabarcoding has been shown to provide a loweffort and successful approach for detecting diverse fish species in Southeast Asian coral reef communities (Turon et al., 2020). The results reveal significant differences in fish communities between eutrophic and well-preserved areas within the Nha Trang Marine Protected Area, highlighting the capability of sponge-based nsDNA sampling to monitor and assess fish community composition. These studies present a novel perspective that expands the possibilities for practical, cost-effective, and technologically accessible biodiversity monitoring approaches. Another study aimed to compare vertebrate eDNA detection from different sources: water samples, bivalve gill-tissue dissections, sponge tissue, and ethanol in which filter-feeding organisms were stored (Jeunen et al., 2023). SCUBA divers provided concurrent observations to validate eDNA detections. Results showed that both water and sponge eDNA accurately reflected spatially specific eDNA signals within a vertical transect, while bivalve gill-tissue dissections were ineffective. Limited success was achieved in obtaining diversity information from ethanol-stored specimens.

Overall, eDNA sources, except for bivalve gill-tissue dissections, detected a greater number of vertebrate taxa compared to diver surveys. However, these aforementioned nsDNA samplers are benthic organisms that may not be suitable for pelagic biodiversity assessments.

1.6 Jellyfish as nsDNA Samplers

Jellyfish (phylum Cnidaria, class Scyphozoa) are abundant pelagic organisms found in diverse marine habitats (Barnes, 1982). There are currently around 200 recognised species of Scyphozoa (Dawson, 2004). The feeding process in medusae, the adult form of jellyfish, exhibits species specific differences due to their distinct anatomical features, leading to variations in capture and ingestion modes (Gershwin, 2016; Camacho-Pacheco et al., 2022). Some species, such as Aurelia and Aequorea species, for instance, utilise tentacles to capture their prey, attaching them and subsequently transporting them to the oral lobes for digestion. In contrast other species, such as those of the Rhizostomae family, including Cotylorhiza species and Rhizostoma species, lack marginal tentacles. Instead, they rely on structures like the manubrium, oral arms, and scapulets, which maintain contact with food through the production of mucus and the presence of nematocysts. Prey organisms are captured and subsequently paralysed before being transported to the oral-arm canals through millimeter-wide pores (Nagata and Morandini, 2018).



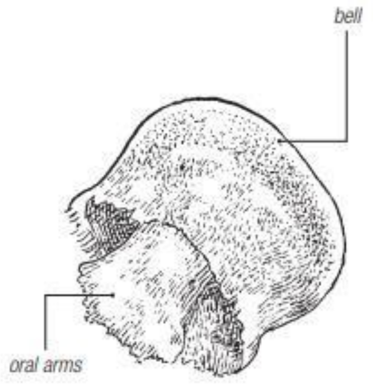


Figure 2 (Gershwin, 2016). Diagram of Aurelia sp (left) and Rhizostomae (right) morphology.

The feeding process unintentionally leads to the accumulation of various particles, potentially including eDNA, within the jellyfish's mucus or tissues. By collecting tissue samples from jellyfish, it may be possible to extract and identify eDNA from the organisms present in their surrounding waters. Given the diverse feeding interactions and tissue structures, different jellyfish tissues might harbor distinct eDNA compositions due to the varied interactions each tissue has with the marine environment and its potential to trap and retain eDNA.

One notable advantage of using jellyfish as eDNA samplers is their ability to cover extensive areas as they move through seawater masses, both horizontally and vertically (Gershwin, 2016). This attribute proves advantageous in studying expansive water where traditional eDNA sampling methods may be challenging or time-consuming. If jellyfish collect eDNA, they may offer the opportunity to collect a diverse range of genetic material from pelagic environments, which could lead to a framework for monitoring pelagic organisms, which may be well beyond the reach of sessile benthic filter-feeders currently associated with the "natural sampler DNA" approach.

Metabarcoding has been successfully applied to whole polyp and ephyra samples of *Aurelia coerulea* to study their diet composition (Sun et al., 2021). Universal eukaryotic primers were used to target the V4 region of the 18S rDNA of samples. This allowed more detailed detection of species preyed upon to reveal the diet compositions of polyps and ephyra compared with traditional methods of diet examination. Despite the high water content of jellyfish, it was shown to be possible to extract targeted trace DNA directly from jellyfish tissue, and amplify it using eukaryotic primers. However, there are no previously documented studies examining the possibility to extract trace DNA from medusa stage jellyfish using a metabarcoding assay targeting fish species (hence not necessarily DNA originating from feeding processes). Therefore, this study represents the first attempt to use jellyfish as natural eDNA samplers.

1.7 Thesis outline

The primary objective of this study was to assess the feasibility of using jellyfish as natural samplers of eDNA to capture teleost biodiversity. To achieve this, the objectives were as follows:

1. To evaluate and compare the ability of multiple jellyfish species as natural samplers of eDNA using metabarcoding techniques. This objective aimed to determine whether different jellyfish species collected in the same area exhibited different performances in capturing and representing the teleost biodiversity through eDNA analysis.

2. To investigate whether specific tissues within the jellyfish specimens were more effective in capturing and preserving biodiversity eDNA. By examining three different tissue types, umbrella, gut contents and oral arms, this objective aimed to identify the most suitable tissue for eDNA sampling.

By addressing these objectives, this study aimed to contribute to the advancement of eDNA research and facilitate the development and expansion of more effective and efficient biodiversity monitoring methods.

Chapter Two: Methods Exploring Jellyfish as Natural Samplers of Environmental DNA

2.1 Data collection

Jellyfish specimens were collected from the Miramare Marine Protected Area (MPA) located off Trieste, Italy (45.7057N, 13.7121E) (Figure 3), during two sampling events. The species selected for this study, *Aurelia solida, Aequorea forskalea, Rhizostoma pulmo*, *and Cotylorhiza tuberculate*, were used based on their accesbility for sampling and different feeding behaviours and morphologies.*Aurelia solida* captures planktonic prey using tentacles, subsequently directing them to its oral lobes for digestion (Gershwin, 2016). In comparison, *Aequorea forskalea*, while sharing feeding strategies with Aurelia species, predominantly consumes zooplankton, including smaller cnidarians (Dawson, 2004). In contrast, Rhizostoma pulmo from the Rhizostomae family, lacks marginal tentacles and instead uses structures like the manubrium and oral arms, supplemented by mucus and nematocysts, for prey capture and immobilization (Nagata and Morandini, 2018). Another Rhizostomae member, Cotylorhiza tuberculata, follows similar prey capture but is uniquely identified by its symbiotic relationships with photosynthetic organisms. Together, these species capture some of the diverse feeding and anatomical differences present in Scyphozoans (Barnes, 1982; Camacho-Pacheco et al., 2022)

The first sampling took place on May 31, 2022 involving the collection of *Aurelia solida, Aequorea forskalea* and *Rhizostoma pulmo.* These samples were selected opportunistically using capture apparatus from land of samples close to the shore. The second sampling occurred on July 15, 2022 and it collected *Cotylorhiza tuberculata* specimens. In total, 12 jellyfish specimens were obtained, with three individuals representing each species. Following collection, the jellyfish samples were immediately preserved in 100% ethanol and stored at a temperature of -20°C.

Jellyfish specimens were separated into parts based on their size and ease of handling and dissection at the National Institute of Oceanography and Experimental Geophysics in Trieste. For *R. pulmo* and *A. solida* specimens, the gut contents, oral arms, and umbrellas were isolated as distinct body parts. In the case of *A. forskalea,* the specimens were separated into a core section containing the gut and oral arms, and the umbrellas. *C. tuberculata* specimens were processed whole without separation. Specimens were then transported on ice and stored at -20°C until the extraction of eDNA was performed.

Figure 3. Map of the Miramare Marine Protected Area (MPA) in red within the geographical location of the Miramare MPA in Italy where jellyfish collection took place.

2.2 Laboratory work

2.2.1 DNA extraction

Upon removal from the storage ethanol, each jellyfish sample was carefully blotted dry using tissue paper within a sterile Petri dish. Subsequently, the oral arms, umbrellas, and whole samples were delicately cut into smaller pieces to facilitate the extraction process. Each dissected part was then transferred to a separate 1.5 ml microcentrifuge tube, which would serve as the vessel for DNA extraction. In the case of gut contents, a partial drying step was performed. The gut contents were spread out in Petri dishes and allowed to partially dry. Once partially dried, the remaining residual was collected and transferred to the 1.5ml tube. Any remaining ethanol from the gut contents was allowed to evaporate. For the extraction of DNA from all samples, the modular mu-DNA protocol (Sellers et al., 2018) was followed. Chemical component of stock solutions are listed in appendix 1. To facilitate lysis, 260 µL of Lysis Solution, 20 µL of Tissue Lysis Additive, and 20 µL of PK were added to the tube. The tissue was then vortexed briefly to ensure thorough mixing. Subsequently, the tube was incubated at 55°C with occasional vortexing until complete tissue dissolution was achieved, which typically took more than 3 hours or overnight.

After the incubation period, the samples were centrifuged at 10,000g for 1 minute at room temperature making sure not to disturb the pellet. Following centrifugation, the supernatant, containing the dissolved tissue components was carefully transferred to a fresh 1.5 mL tube, while avoiding any transfer of the pellet. This resulted in 300 µL of transferred supernatant. Next, 600 µL of Tissue Binding Solution, equivalent to twice the volume of the supernatant, was added to the sample. The mixture was then briefly vortexed to ensure thorough mixing. Subsequently, 650 µL of the mixed solution was transferred to a spin column, and the column was centrifuged at a minimum speed of 10,000g for 1 minute at room temperature. The resulting flow-through was discarded, and this process of transferring and centrifuging was repeated until all the mixture had passed through the spin column.

Moving on to the wash step, 500µL of Wash Solution was added to the spin column. Following the addition of the wash solution, the spin column was again centrifuged at 10,000g for 1 minute at room temperature, and the flow-through discarded. To ensure thorough washing, the washing step was repeated once more, with the spin column being centrifuged at the same conditions for 2 minutes. The collection tube was then replaced with a fresh 1.5 mL tube.

For the elution of DNA, 200 µL of Elution Buffer was directly added to the spin column membrane, and the mixture was incubated for 1 minute at room temperature. Subsequently, the spin column was centrifuged at 10,000g for 1 minute at room temperature. These elution steps were then repeated to obtain a higher DNA yield.

2.2.2 DNA Amplification, Library preparation, and Sequencing

PCR amplifications were performed using the Tele02 primers (Taberlet et al., 2018), specifically designed to amplify a ~169 bp fragment of the mitochondrial 12S rRNA gene using the forward sequence Tele02-F (5'-AAACTCGTGCCAGCCACC- 3') and the reverse sequence Tele02-R (3'GGGTATCTAATCCCAGTTTG-5'). The PCR reactions were prepared in 20 µl volumes, consisting of 10 µl of 2× MyFi Mix (Meridian Bioscience), 1 µl of each forward and reverse primer, 0.16 µl BSA (Bovine Serum Albumin Solution, Thermo Fisher Scientific), 5.84 µl of molecular grade water (Invitrogen), and 2 µl of DNA template.

Each sample was amplified in triplicate (to reduce stochasticity of the PCR process) under the following cycling conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 60°C for 45 s, 72°C for 30 s, and finishing at 72°C for 5 min. Positive and no-template PCR controls were included in each PCR run. The positive PCR controls contained DNA extract (0.05 ng/µl) from the iridescent shark catfish, *Pangasianodon hypophthalmus*, which was not present in the sampling area. After PCR amplification, the triplicate PCR products for each sample were pooled together, and 1µl of the pooled PCR product was visualised on 2% agarose gels stained with SYBRsafe (Invitrogen) to confirm successful amplification of the target fragment. Gel images were captured and analysed using Image Lab Software (Bio-Rad

Laboratories). The PCR products were then purified using Mag-Bind Total Pure NGS magnetic beads (Omega BioTek) with a ratio of 1x magnetic beads to 20µl PCR product. The purified PCR products were quantified using a Qubit 4.0 fluorometer (Invitrogen) and the Qubit dsDNA High Sensitivity Assay kit. To create two libraries, one for *A. solida, R. pulmo and A. forskalea* and another for *C. tuberculata* species (due to different sampling periods), the samples were normalised and pooled in equimolar amounts. These pooled PCR products were subjected to another round of purification using magnetic beads, followed by library preparation using the NEXTFLEX Rapid DNA-Seq Kit for Illumina (PerkinElmer), following the manufacturer's instructions.

The libraries were quantified using quantitative PCR (qPCR) with the NEBNext Library Quant Kit for Illumina (New England Biolabs) on a Rotor-Gene instrument (Qiagen). Fragment size distribution was assessed using the Tape Station 4200 (Agilent). Finally, the libraries were pooled together at equimolar concentrations with a final molarity of 60 pM, including 10% PhiX control. Sequencing of the libraries was performed on an Illumina iSeq100 instrument at Liverpool John Moores University using iSeq i1 Reagent version 2 (300 cycles) to generate the raw sequencing data required for subsequent data analysis and interpretation.

2.3 Bioinformatic analysis

Bioinformatic analysis of the sequencing data followed the OBITools v1.2.11 metabarcoding pipeline (Boyer et al., 2016). Initially, the read quality was assessed using FASTQC. The raw sequencing data were then subjected to trimming using obicut to remove low-quality ends. Paired reads were merged using the 'illuminapairedend' tool, excluding alignments with low quality scores (<40). The demultiplexing of alignments was performed using 'ngsfilter'. Sequences outside the expected length range of 130 to 190 bp and those with ambiguous bases were filtered out using OBIGREP. Dereplication was carried out using 'obiuniq', and chimeric sequences were identified and removed using the de novo chimera search function in vsearch 2.4.3 (Rognes et al., 2016). MOTUs were delimited using the aggregation clustering algorithm implemented in SWARM v2.4.3 (Mahé et al., 2015), with a clustering threshold of "-d 3". Taxonomy assignment was performed using 'ecotag' against a custom-made 12S vertebrate reference database, which was constructed by in-silico PCR for Tele02 primers against the EMBL database (Release version r143) using 'ecoPCR'. The resulting MOTU table was formatted using R scripts available at the following GitHub repository: https://github.com/metabarpark/R_scripts_metabarpark. Taxonomic assignments were further validated by manual BLAST searches to remove ambiguous MOTUs, while non-target reads (non-teleosts) were excluded from downstream analysis.

2.4 Statistical analysis

All statistical analyses were conducted using R v4.2.3 (R Core Team, 2023). Venn diagrams were generated to examine the overlap across different species of jellyfish, and body parts within each jellyfish species. These were created using the ggvenn package which is an extension of the ggplot2 packacke in R (Wickham, 2019). The normality of the number of MOTUs and logtransformed read counts in the samples was assessed using the Shapiro-Wilk test. Homogeneity of variance of the log-transformed read counts within each species was examined using Levene's test. To investigate potential differences in the number of reads and the number of detected MOTUs among the different species, a combination of parametric (ANOVA) and non-parametric (KruskalWallis) tests was employed. To further assess the statistical significance of differences in MOTU detection between species and body parts, PERMANOVA tests were performed. The PERMANOVA tests were based on Jaccard distances calculated from a binary presence-absence dataset that included all taxa. The adonis function from the vegan package in R (Okansen et al.,2019) was utilised for the PERMANOVA analysis (1000 permutations). To visualise the dissimilarity between samples, non-metric multidimensional scaling (nMDS) plots were generated. The nMDS plots were created using the metaMDS function from the vegan package in R (Okansen et al., 2019).

Chapter Three: Results

3.1 Bioinformatic processing

A total of 2,386,952 reads were obtained from the first Illumina iSeq run, which included samples of *A. solida, R. pulmo and A. forskalea*, as well as controls and samples from another project. Out of these reads, 1,560,266 were assigned to the jellyfish samples (Table 1). Quality filtering was performed to remove non-teleost MOTUs and sequences with low percentage identity. Since no contaminating fish sequences were found in the controls, the presence of false positives was deemed negligible, and only MOTUs with a read count of one were removed. After filtering, 186,584 reads remained for further analysis belonging to 15 samples. Among the remaining reads, a total of 15 MOTUs were identified. Of these, six were classified at the species level, three at the genus level, two at the subfamily level (Gobiinae, Macrourinae), and four at the family level (Serranidae, Callionymidae, Scophthalmidae, Blennidae). Within the *A. forskalea* samples, teleost sequences were detected in three samples, totalling 445 reads across six OTUs. In the case of the *A. solida* samples, teleost sequences were found in six samples, comprising 648 reads across four MOTUs. The *R. pulmo* samples yielded the highest number of reads, with seven samples containing 185,491 reads belonging to 13 MOTUs.

	A. solida	*A. forskalea*	*R. pulmo*	*C. tuberculata*
Number of fish MOTUs	4	6	13	10
Target fish reads	648	445	185491	30321
Mammal reads	896	0	4630	0
Bird reads	18808	3	18011	0
Human reads	492218	255523	479723	1328
Total reads	512747	256588	789631	31649
Target fish reads (%)	0.13	0.17	23.49	95.8

Table 1: The number of reads and MOTUs across each species of jellyfish.

In the second Illumina iSeq run, library 2 consisted of three *C. tuberculata* samples alongside samples from another project, which together produced a total of 1,769,198 reads. Among these reads, 31,683 were assigned to the *C. tuberculata* samples. After sample assignment and quality filtering, 30,321 reads remained for analysis. All three *C. tuberculata* samples contained teleost sequences, with a total of 10 OTUs detected, all resolved at the species level, and only two of which that were also detected across the three other species.

3.2 Detection of MOTUs

The number of MOTUs detected in every tissue sample for the four jellyfish species is visualised in a boxplot (n=15) (Figure 4). The number of MOTUs detected is highest in *C. tuberculata,* followed by *R. pulmo, A. forskalea* and *A. solida* which contained the lowest number of MOTUs. The normality of the number of MOTUs detected in each species was assessed using the Shapiro-Wilk test, which revealed a significant deviation from normality (n=4, W= 0.88945, p = 0.03776). To further examine potential differences in the number of MOTUs across the different species, a

Kruskal-Wallis rank sum test was conducted. The test indicated a statistically significant difference among the species (n=4, $\chi^2 = 9.9895$, df = 3, p = 0.01866) The log-transformed number of reads was also plotted as a box plot for each sample (Figure 5). The plot shows both *C. tuberculata* and *R. pulmo* samples showed similar median log read numbers, while *A. forskalea* and *A. solida* had lower medians that were comparable to each other. The normality of the data was assessed using the Shapiro-Wilk test (n=15, W = 0.97449, p = 0.8766). The Levene's test for homogeneity of variance indicated that there was no significant difference in the variances of the log number of reads among the species (F(3, 14) = 0.7671, p = 0.5313). Therefore, an analysis of variance (ANOVA) was performed to evaluate the differences in the log number of reads among the four jellyfish species. The ANOVA results revealed a significant effect of species on the log number of reads (n=15, F = 12.87, p < 0.001).

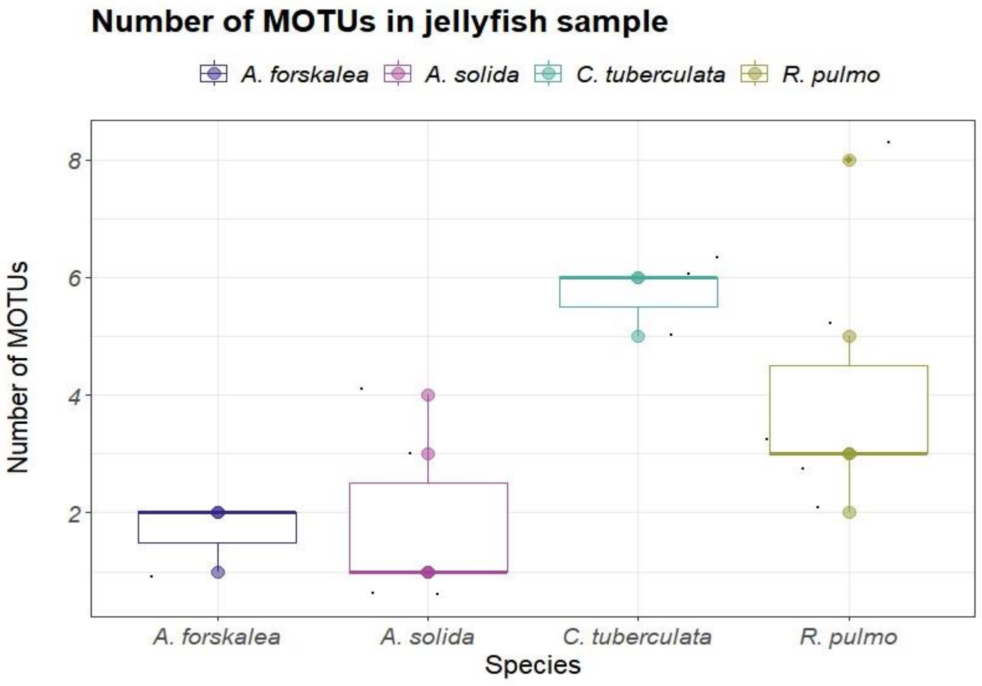


Figure 4. Comparison of number of MOTUs detected in all 15 samples for the four species of jellyfish.

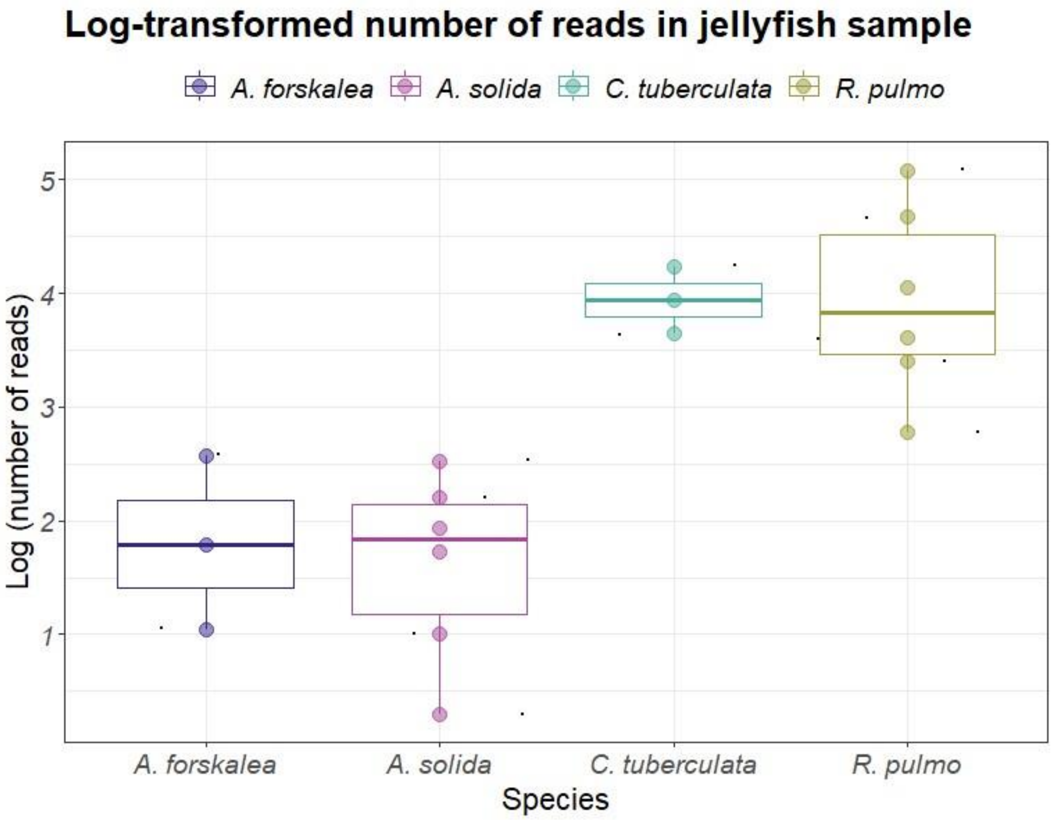
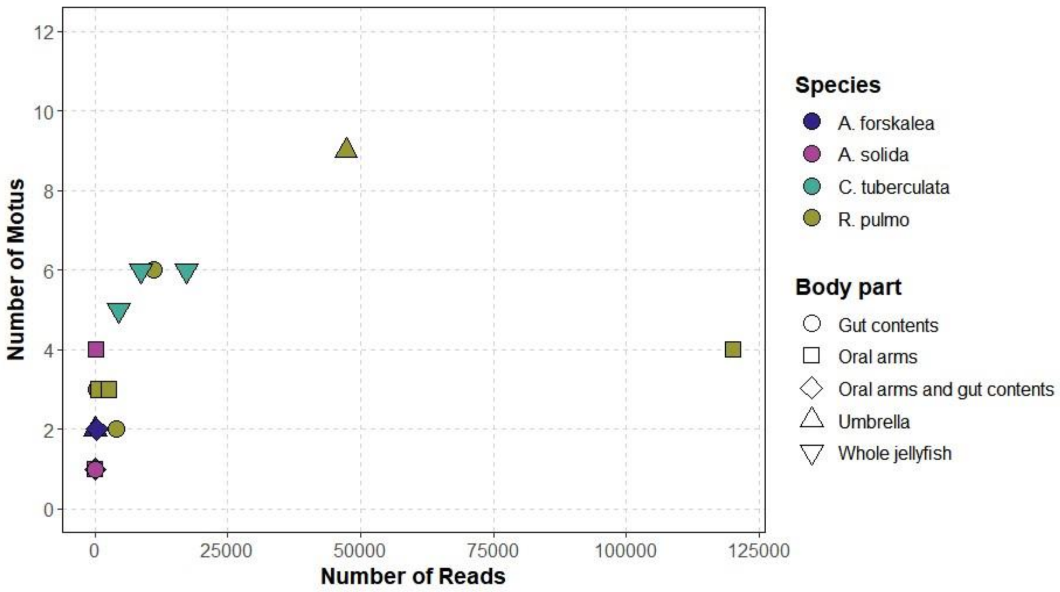


Figure 5. Comparison of log-transformed number of reads across 15 tissue samples for four species of jellyfish.

The scatter plot analysis (Figure 6A) visually examined the potential relationship between the number of MOTUs detected and the number of reads obtained per sample. The results showed that an increase in the number of reads was associated with a higher number of MOTUs detected, suggesting that a sequencing depth of 50,000 reads per sample was required to capture diversity in the study area. However, the *R. pulmo* oral arm data point suggested that any further increase in read depth does not yield further information. A more balanced curve is shown after the removal of that outlier (Figure 6B).

A.



B.

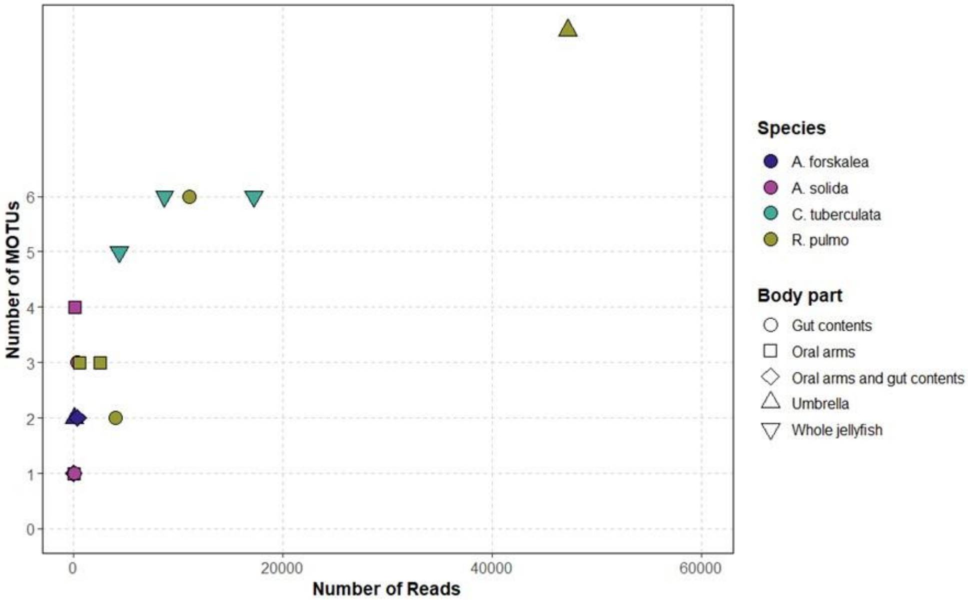


Figure 6. The relationship between the number of reads (x-axis) and the number of motus (y-axis) based on different body parts (represented by shape) and species (represented by fill colour) for A. all samples and B. all samples with a read count <60000.

The variation in MOTU composition detected across different species of jellyfish and body parts is visualised using a non-metric multidimensional scaling (nMDS) plot (Figure 7). The plot provides a visual representation of the relationships and groupings among the samples based on their dissimilarities. To ensure clarity of the plot, an outlier (*A. forskalea*, gut contents and oral arms) that rendered the plot unreadable was excluded. The nMDS plot reveals distinct separation of *C. tuberculata* samples (sampled in August) from all other samples, with substantial overlap observed among the other three species (sampled in May). Supporting the nMDS findings, pairwise comparisons demonstrate significant differences in MOTU composition both between the four species (PERMANOVA, Jaccard, $p < 0.001$) (Table 2A) and among the five different body parts (PERMANOVA, Jaccard, $p < 0.002$) (Table 2B). Analysis of the three jellyfish species collected in May indicated significant differences in MOTU composition (PERMANOVA, Jaccard, $p = 0.047$) (Table 3A). However, when examining the variation in composition across different body parts for the three species, a trend towards significance was observed but did not reach statistical significance (PERMANOVA, Jaccard, $p = 0.09$) (Table 3B).

2A.

term	**df**	**SumOfSqs**	***R^2***	**F**	***p***
Jellyfish species	3	2.270114	0.3006772	1.863137	**0.001**
Residual	13	5.279891	0.6993228		
Total	16	7.550005	1.0000000		

B.

term	**df**	**SumOfSqs**	***R^2***	**F**	***p***
Body part	4	2.669819	0.353618	1.64122	**0.002**
Residual	12	4.880186	0.646382		
Total	16	7.550005	1		

Table 2. Results of PERMANOVA used to compare variation in MOTU composition between A. jellyfish species and B. between body parts.

3A.

term	**df**	**SumOfSqs**	***R*** ^{2}	**F**	***p***
Jellyfish species	2	1.1975	0.19599	1.3407	**0.047**
Residual	11	4.9123	0.80401		
Total	13	6.1098	1.0000000		

B.

term	**df**	**SumOfSqs**	***R*** ^{2}	**F**	***p***
Body part	3	1.5972	0.26141	1.1798	**0.09**
Residual	10	4.5126	0.73859		
Total	13	6.1098	1		

Table 3. Results of PERMANOVA used to compare variation in MOTU composition between A. jellyfish species and B. between body parts for the three species found in May: *R. pulmo, A. solida* and *A. forskalea*.

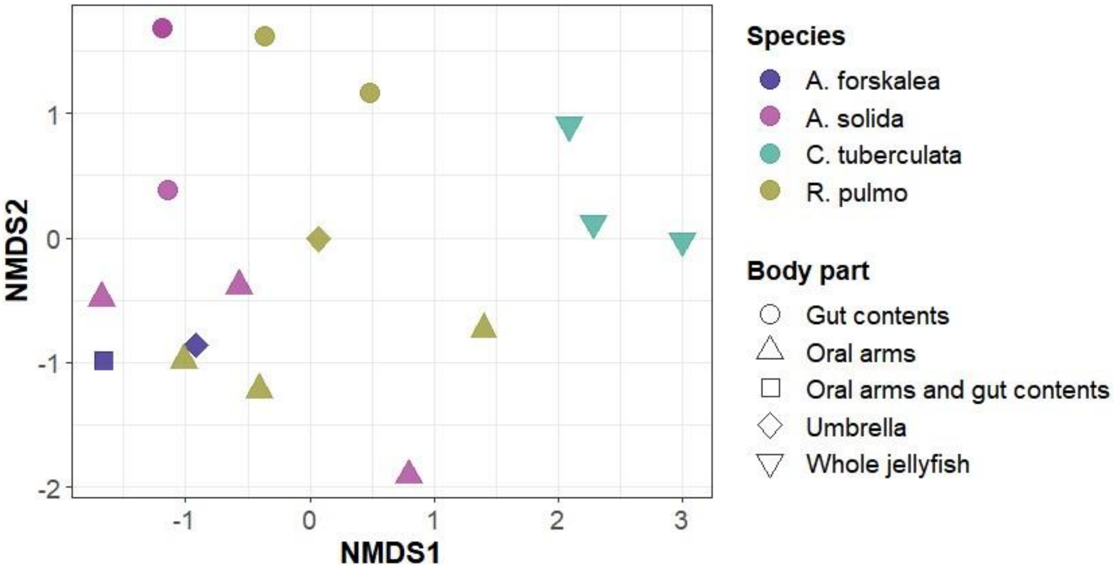
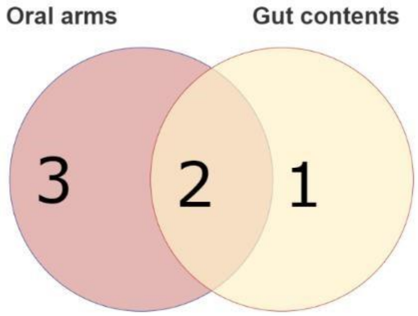


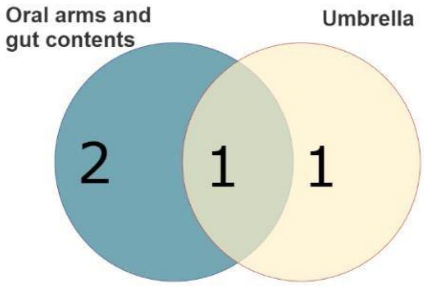
Figure 7. nMDS plot illustrating dissimilarity between samples. Colours denote different species, while shapes indicate different body parts.

Venn diagrams were used to show the overlap among the various body parts for *A. solida, R. pulmo and A. forskalea* jellyfish samples (Figure 8). Comparisons between body parts for *C. tuberculata* samples were not conducted since samples were processed whole. The Venn diagrams demonstrate only little overlap between the different body parts assessed. This finding highlights the distinct composition and potentially reflects specialised functions of each body part within these jellyfish species.

Figure 8A. *A. forskalea*



B. *A. solida*



C. *R. pulmo*

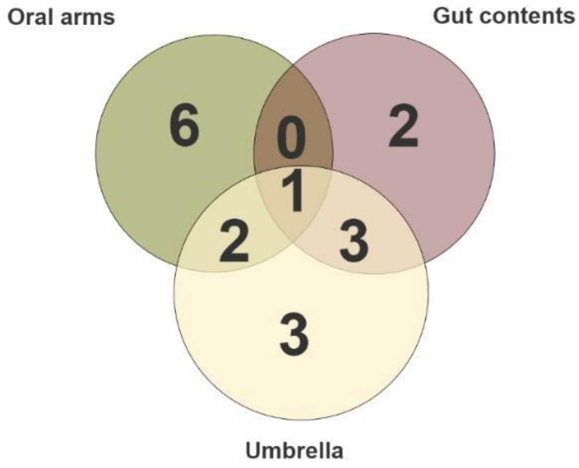


Figure 8: Venn diagrams showing the distribution of detected MOTUs across different body part for three jellyfish species: A. *A. forskalea, B. A. solida* and C. *R. pulmo* jellyfish samples.

The relative abundance of each MOTU in each species of jellyfish was visualised in a bubble plot (Figure 9). MOTUs from individual jellyfish samples were pooled to give an overview of each species. In this plot, each bubble represents a MOTU, and its size corresponds to the relative abundance. The relative abundance of each MOTU in each species of jellyfish was determined by dividing the number of reads corresponding to a specific MOTU by the total number of reads across all samples within each jellyfish species. This normalisation step allows for a comparison of the relative abundance of different MOTUs within and across species.

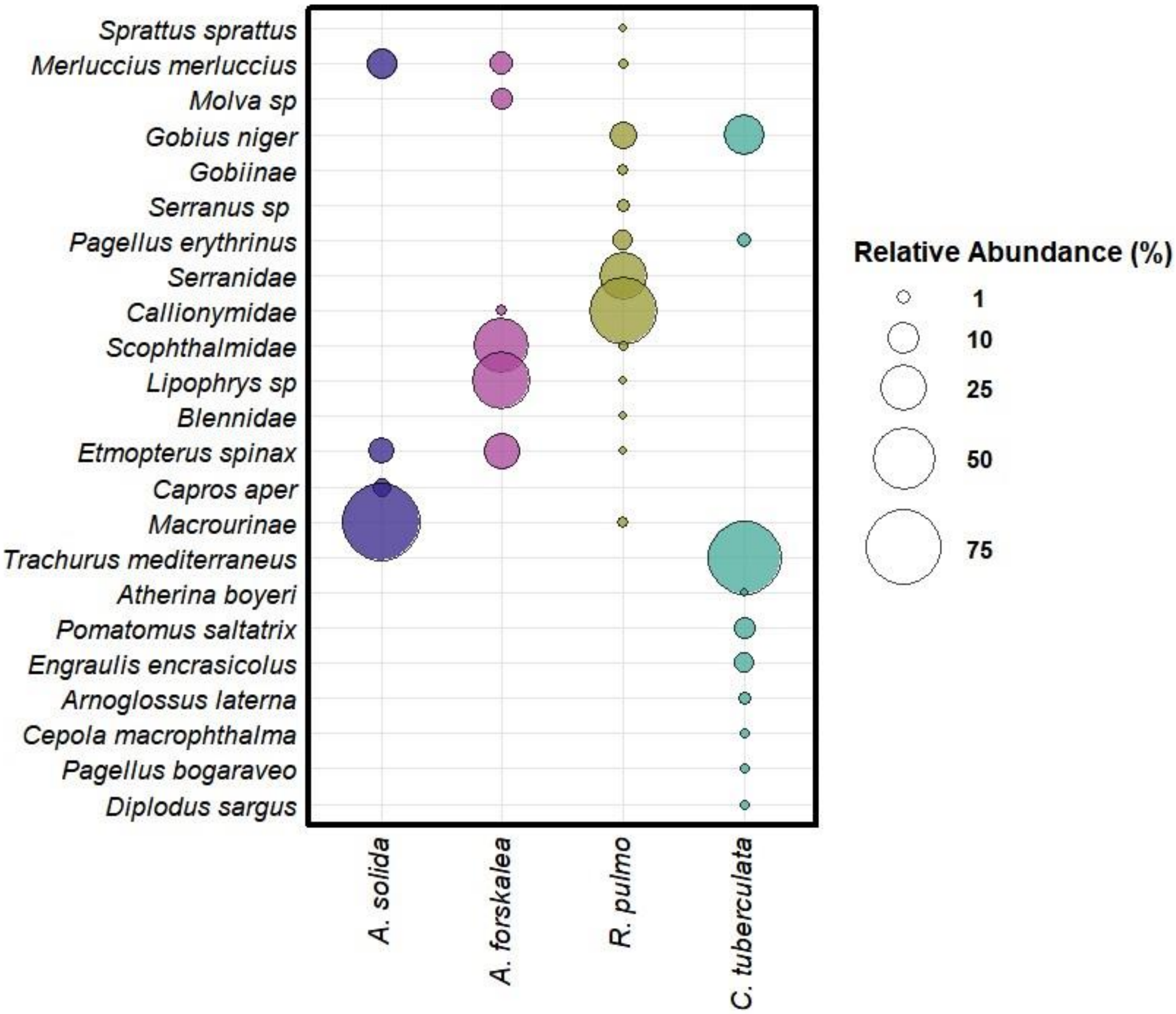


Figure 9. Bubble plot showing the relative abundance of each MOTU within each of the four species of jellyfish.

Chapter Four: Discussion and Future Research

4.1 Discussion

In recent years, eDNA analysis has emerged as a valuable tool in ecological research, enabling non-invasive and high-throughput species detection and biodiversity assessments (Taberlet et al., 2012b; Valentini et al., 2016). Recent advancement in the field involves harnessing genetic material from the environment using nsDNA samplers offering an efficient and cost-effective approach (Mariani et al., 2019). These samplers enable the detection of a wide range of organisms while circumventing some of the challenges associated with traditional eDNA sampling methods, such as the need for direct sampling or disturbance. This eliminates the requirement for manual filtering of water, reducing plastic consumption and the risk of contamination. However, it is important to acknowledge a limitation associated with the use of natural samplers, which is the potential for variable eDNA capture efficiency. This variability arises from factors such as species specific traits, environmental conditions, and the abundance of target organisms. Therefore, to optimise and standardise the use of natural samplers, it is crucial to explore and compare the effectiveness of different organisms.

This study explored the capability of four jellyfish species, *A. solida, A. forskalea, R. pulmo,* and *C. tuberculata,* to serve as nsDNA samplers of teleost biodiversity. The results have demonstrated that jellyfish could serve as nsDNA samplers; however, they have also highlighted that the choice of jellyfish species and body part introduce stochasticity, leading to variation in the composition of eDNA in the samples.

The analysis, including species richness and nMDS analyses, revealed a distinct separation of C. *tuberculata* samples from other species, with A. *solida*, A. *forskalea*, and R. *pulmo* showing substantial overlap. The significant differences in MOTU diversity among these species, as confirmed by PERMANOVA tests and Venn diagrams, indicate that the choice of species is crucial in jellyfish-based eDNA sampling. Notably, the segmentation of jellyfish into separate body parts for eDNA extraction might lead to a loss of MOTUs. Thus, employing the entire jellyfish specimen for eDNA analysis is recommended to capture greater diversity, offering a more complete representation of the sampled environment.

This approach is further supported by the fact that pooling body parts reduces differences among the species collected in May, while *C. tuberculata* collected in July remains distinct. This suggests that the variance in our samples may be partly due to the different composition of pelagic eDNA across the sampled periods. Moreover, *R. pulmo* and *C. tuberculata* generated substantially more DNA reads than *A. forskalea* and *A. solida*, resulting in a higher number of detected MOTUs. This variability highlights that the selection of a particular jellyfish species as an eDNA sampler can significantly impact MOTU diversity.

The observed differences in MOTU composition among jellyfish species can be attributed to several factors, including the species' morphology, feeding behaviors, and habitat preferences. These distinctions are important in selecting more effective species for environmental DNA sampling. Jellyfish species employ distinct feeding strategies, with some utilising nematocysts in their tentacles to actively hunt and consume prey, while others rely on their tentacles to filter feed on plankton from the water column (Gershwin, 2016). *R. pulmo* and *C. tuberculata*, both of the order Rhizostomeae, lack true tentacles and instead concentrate nematocysts on the large surface area of their oral arms, where prey are captured and subsequently paralysed before being transported to the oral-arm canals through millimeter-wide pores (Nagata and Morandini, 2018). In contrast, *A. solida* and *A. forskalea* employ similar feeding strategies using their tentacles to capture and filter small planktonic organisms from the water (Pitt et al., 2008). *Aurelia* species and *Aequorea* species generally have fewer nematocytes in their tentacles compared to *Cotylorhiza* and *Rhizostoma* species, relying more on the passive capture of small planktonic organisms rather than actively capturing larger prey. The differences in feeding behaviours among jellyfish species may contribute to variations in MOTU composition, highlighting the importance of considering species-specific characteristics when using jellyfish as natural environmental DNA samplers.

Given the disparities in MOTU composition observed among the different jellyfish species, it becomes evident that both species selection and method of sampling are critical for effective eDNA collection. The analysis clearly shows that *R. pulmo* and *C. tuberculata* stand out in terms of the quantity of DNA reads and the diversity of MOTUs detected. Their morphological features, particularly the expansive surface area of their oral arms equipped with nematocysts, appear to enhance their capability as eDNA samplers (Nagata and Morandini, 2018). In contrast, *A. solida* and *A. forskalea*, with their different feeding strategies and fewer nematocytes, yield different MOTU profiles (Pitt et al., 2008). Therefore it is advisable to consider using entire specimens of species such as *R. pulmo* and *C. tuberculata* or those with a similar morphology for more comprehensive eDNA sampling. This recommendation, however, also underscores the need for further research to explore the full range of eDNA capture capabilities across various jellyfish species.

It should be noted that this study did not develop a bespoke, optimised protocol for the retrieval of eDNA from jellyfish tissues, and rather relied on previous protocols developed for other organisms (Harper et al., 2023). This may have contributed to inconsistent DNA yields, potentially leading to incomplete or biased representation of species in the metabarcoding dataset, and the observed high variance in MOTU detection among *A. solida, R. pulmo and A. forskalea* and *C. tuberculata* samples. Future studies should undertake an evaluation of different extraction approaches. Implementing robust and efficient DNA extraction procedures will facilitate improved resolution of MOTUs and enhance the accuracy of species identification in metabarcoding analyses. Another possible reason for the presence of samples assigned to higher taxonomic levels is the incompleteness of reference sequences or comprehensive reference databases for certain species or groups of interest. The success of metabarcoding relies on the availability and accuracy of reference databases (Curd et al., 2018). Reference databases may not contain adequate representation of specific species, resulting in samples being assigned to higher taxonomic levels instead of being identified at the species level (Dowle et al., 2015). Additionally, sequencing errors, such as base misincorporations or low-quality reads, can contribute to inaccurate taxonomic assignments (Andres et al., 2023). These errors may arise during DNA extraction, PCR amplification, or the sequencing process itself. The presence of sequencing errors can introduce noise or misinterpretation of the obtained data, leading to less precise taxonomic classifications. However, the bioinformatic filtering process employed in this study should have effectively mitigated the influence of such errors. As a result, it is unlikely that the results of this study were significantly affected by sequencing errors, providing confidence in the accuracy of the taxonomic assignments obtained.

The bubble plot analysis presented in this study offers insights into the fish assemblages of the study area by depicting the relative abundance of each MOTU within different jellyfish species, and revealing some expected findings, along with a few more puzzling ones. The most obvious pattern is the major differences in species compositions between the jellyfish samples collected in May and July, which may be attributed to factors such as timing of spawning or other biological characteristics specific to the species present in the region. Many observed species exhibit seasonal variations in their abundance, behaviour, and distribution, which are often driven by environmental factors like temperature, food availability, and reproductive cycles (Palomera et al., 2007; Carbonara et al., 2019). Therefore, the presence or absence of certain species during specific months can be influenced by their unique spawning patterns, migration patterns, or other ecological factors, which has been shown to leave signatures in eDNA analysis (Collins et al., 2022).

It was initially hypothesised that jellyfish could be used as samplers of eDNA to capture pelagic diversity. However, most of the detected species are either benthic or have established associations with nearshore habitats, particularly during the spring/summer seasons when samples were collected. During this period, numerous fish species engage in spawning activities near the coast, taking advantage of favourable conditions such as increased food availability and optimal temperatures (Jørgensen et al., 2008; Tsikliras et al., 2010). The species composition observed in May could reflect the species that are more active or abundant during that time, potentially influenced by their spawning or reproductive behaviours. Conversely, the different species composition detected in July may indicate a shift in the community structure, with species that have different spawning or ecological requirements becoming more prevalent during that period. Understanding the spawning and ecological characteristics of the fish species detected in the study can provide insights into their seasonal occurrence and help explain the observed variations in species composition between May and August samples. By incorporating an understanding of the life history traits of detected species, more meaningful interpretations can be derived from the results, enabling the identification of potential correlations with environmental factors such as temperature, food availability, and habitat preferences.

Upon examining the eDNA results, a distinction emerges between pelagic species and those originating from benthic or deeper marine habitats. Expected contributors like bluefish (*Pomatomus saltatrix*), scad (*Trachurus trachurus*), and anchovy (*Engraulis encrasicolus*) from pelagic environments were identified. Yet, a considerable portion of the eDNA detected belongs to benthic and deep-sea species, such as flatfishes (*Pleuronectiformes*), gobies (*Gobiidae*), blennies (*Blenniidae*), and others not typically found in coastal habitats. The origin of these signals is of interest, particularly since adult individuals of deep-sea species are not likely to be in the immediate vicinity of the sampled jellyfish.

A plausible explanation for this pattern is the dispersal of eDNA from reproductive events. For instance, spawning activities from deep-sea species like the hake (*Merluccius merluccius*), which predominantly inhabit deeper waters away from coastal regions, can introduce significant amounts of eDNA into the marine environment. With hake's reproductive behavior as a batch spawner with notable fecundity and year-round spawning tendencies (Carbonara et al., 2019), it is probable that the detected eDNA is associated with eggs or larvae, rather than mature individuals. This interpretation aligns with the understanding that oceanic currents and tidal actions can transport these DNA fragments from offshore spawning grounds to coastal areas where the jellyfish samples were collected.

Among the pelagic species detected was *European sprat (Sprattus sprattus).* The observed detection of *S. sprattus* in the jellyfish samples collected only in May can be attributed to their typical spring spawning period, which occurs from December to May (Teskeredžić, 1983). During this time, water temperatures and other environmental cues are conducive to reproduction for this species. While it is possible for some individuals to spawn later into the summer months, including July, the majority of spawning activity for the European sprat is observed earlier in the season The temporal pattern observed in detection aligns with the expected biological behaviour of the species, providing a plausible explanation for the findings in this study.

Among the MOTUs identified, the dragonet family (*Callionymidae*), including genera *Callionymus*, *Protogrammus*, and *Synchiropus* (Vagenas et al., 2021), displayed one of the highest read counts. However, limited research has been conducted on this family in the Mediterranean, resulting in scarce information regarding their spawning behaviors, which could shed light on their presence specifically in the May samples (Dimarchopoulou, Stergiou and Tsikliras, 2017). A study on the spawning period of Mediterranean marine fishes (Tsikliras et al., 2010) that the majority of species spawn between April and August, with some species exhibiting spawning periods from September to March. Therefore, the findings of this study suggest that the spawning period for Callionymidae in the Mediterranean might occur in the spring, thus explaining their detection in May rather than July.

The association between *C. tuberculata* and the pelagic fish species Mediterranean horse mackerel *(Trachurus mediterraneus)*is noteworthy, as the most abundant MOTU detected in all three *C. tuberculata* samples belonged to *T. mediterraneus*. *T. mediterraneus*, is a semipelagic species that inhabits the continental shelf and is distributed throughout the Mediterranean (Mir-Arguimbau, Sabatés and Tilves, 2019). The juvenile stage of *T. mediterraneus* is known to form associations with both *R. pulmo* and *C. tuberculata* jellyfish species, where the jellyfish act as passive hosts, providing protection from predators and serving as a potential food source through direct consumption or utilisation of captured plankton (Tilves et al., 2018). Interestingly, *T. mediterraneus* spawning occurs in the spring and summer seasons. It is intriguing that *T. mediterraneus* was only detected in the July samples, despite known associations with *R. pulmo* species caught in May. This suggests that jellyfish may provide valuable insights into the spawning times of T. mediterraneus and other species known to associate with jellyfish. Another intriguing species to note is the presence of bluefish (*Pomatomus saltatrix*), a voracious predator that tends to migrate closer to coastal areas towards the end of summer (Sanchez-Jerez et al., 2008). This temporal pattern aligns with the collection period of the *C. tuberculata* samples, which were obtained in July. In contrast, the other three jellyfish species were sampled in May and did not detect bluefish.

The variable detection of bluefish in jellyfish samples, potentially linked to migration timing, suggests that jellyfish sampling might offer insights into the migration patterns of this species. This potential relationship underscores the possibility that jellyfish could serve as indicators of certain ecological dynamics, but this interpretation is tentative and requires further validation. For a more robust understanding, additional research is essential. This should encompass long-term data collection and the inclusion of environmental variables to better interpret the relationship between jellyfish sampling, species composition, and significant biological events such as fish spawning.

The detection of pelagic species common to the Miramare MPA such as European sprat, European hake, and Mediterranean horse mackerel, along with benthic species like black goby and blennies, demonstrates the efficacy of jellyfish as eDNA samplers. However, the absence of species typically abundant in the area, such as the European sardine (*Sardina pilchardus*) and Atlantic mackerel (*Scomber scombrus*), indicates potential limitations in capturing the full spectrum of local marine biodiversity (Angelini et al., 2021).

It's also worth noting that the scope of this study was limited to nearshore habitats. The samples, predominantly collected from shallow coastal waters, might exhibit a detection bias towards benthic organisms. By extending sampling efforts to the open ocean, we could potentially access a broader spectrum of pelagic biodiversity. Such efforts would help in understanding the nuances of jellyfish as natural samplers in various habitats. Capturing samples from pelagic environments might reveal information on species with different spawning behaviors, life cycle strategies, and ecological roles compared to those from nearshore habitats. While the current findings provide a foundation, there's an intriguing avenue for future studies to explore whether the dynamics of pelagic ecosystems can be understood through jellyfish sampling in broader oceanic contexts.

In our study, the detection of a notable quantity of human and other non-teleost DNA reads highlights the sensitivity of the methods to non-specific amplification (Ruiz-Villalba et al., 2017). To enhance the specificity of our approach, we propose the adoption of blocking primers in the PCR mix, a technique proven to selectively inhibit non-target DNA sequences (Boessenkool et al., 2011).

4.2 Limitations

As a pilot study, this investigation successfully detected teleost eDNA in the tissue of jellyfish, providing initial insights into the potential of jellyfish as natural eDNA samplers. However, the study was constrained by its limited sampling effort, covering only two collection occasions. The small sample restricted the statistical power of the analyses and thus ability to draw definitive conclusions regarding the use of jellyfish as eDNA samplers. Additionally, the jellyfish species collected were not consistent across the sampling periods, with different species collected in May and July. This variation in species could introduce detection biases. For example, each jellyfish species might have different preferences for prey, which in turn could influence the types of eDNA present in their tissues.

To advance this, future studies should prioritise increasing the sampling effort by collecting jellyfish samples of the same species periodically and in larger quantities. Furthermore, a direct comparison between the eDNA detected in jellyfish tissue and water collected alongside the jellyfish through traditional filtration methods should be conducted to comprehensively assess the effectiveness and biases of jellyfish as natural eDNA samplers.

There are other issues to consider before applying jellyfish as nsDNA samplers. The morphology of jellyfish, with their gelatinous and delicate bodies, presents challenges for eDNA extraction. Optimal extraction methods need to be developed to ensure efficient recovery of DNA from these organisms. Furthermore, the preservation of eDNA within jellyfish bodies may differ from that in the surrounding water. Environmental DNA is subject to rapid degradation, with half-life of eDNA in marine systems ranging from 18.2 to 71.1 h in seawater (Collins et al., 2018). A longer half-life of eDNA in jellyfish compared to the water may provide a temporal advantage in detecting historical presence or occupancy of species. However, further research is needed to investigate the stability and degradation rates of eDNA within jellyfish bodies, to allow for accurate interpretation of results. It is also important to consider the abundance of the selected species of jellyfish. The species selected in this study are abundant in the sampled area, however, different oceans will have differing populations of jellyfish. Selecting an abundant species is important to ensure reproducibility of the analysis to better accommodate long-term conservational goals of the tool.

Furthermore, the effectiveness of jellyfish as nsDNA samplers may be influenced by the inherent unpredictability associated with the pelagic environment. The vastness and dynamic nature of the pelagic environments contributes to diverse environmental conditions and biological communities, resulting in significant heterogeneity in eDNA patterns (Liu et al., 2022). This variability introduces challenges in accurately predicting and quantifying eDNA presence and abundance when relying on jellyfish as samplers. Therefore, the effectiveness of jellyfish as eDNA samplers may be influenced by the inherent unpredictability associated with the pelagic environment, and only an increased sampling effort can circumvent or reduce this environmental stochasticity.

The limitations associated with sequence reference data bases is common to every metabarcoding application – not just in the specific field of natural sampler DNA. The expansion and curation of reference databases, with closer interaction between molecular ecologists and morphological taxonomists, remains a major avenue of research for the broader eDNA community, towards the enhancement of the accuracy of taxonomic identifications.

4.3 Applications in biodiversity monitoring

Jellyfish populations exhibit seasonal fluctuations, making their availability for sampling unpredictable. However, there are instances where jellyfish can be inadvertently captured during surveys, presenting an opportunity to leverage their presence as natural samplers of eDNA. This potential becomes useful in surveys conducted in remote or inaccessible areas where logistical constraints hinder regular sampling or deployment of traditional equipment. By opportunistically collecting jellyfish in such regions, eDNA samples can be obtained, overcoming the limitations of conventional techniques.

Jellyfish as nsDNA samplers have the potential to play a role in monitoring and assessing marine ecosystems in coastal areas or marine habitats that experience frequent jellyfish blooms (Mills, 2001). These blooms, characterised by the rapid proliferation of jellyfish populations, can have substantial impacts on the surrounding environment. By harnessing jellyfish as natural samplers of eDNA, researchers can potentially obtain valuable information about the genetic diversity present, enabling more effective assessments of the dynamics of the affected ecosystem. This approach could help identifying marine organisms associated with jellyfish blooms, which can contribute to a better understanding of the factors driving these blooms.

Moreover, jellyfish frequently exhibit vertical migration patterns, navigating different water depths throughout the day (Kaartvedt et al., 2007; Gershwin, 2016). By sampling jellyfish at multiple depths, researchers may be able to investigate the vertical gradients of eDNA and examine how species composition and biodiversity vary with depth. This approach would enable the exploration of the vertical distribution of organisms. Consequently, utilising jellyfish as eDNA samplers may allow for the investigation of ecological patterns along the vertical axis.

The findings of this study shed light on the variability in eDNA capture capabilities among different species of jellyfish. There are around 200 recognised species of Scyphozoa (Dawson, 2004), and many will differ in their ability to collect eDNA from the pelagic realm. Many of them, however, have wide distributions, and many of them may assist us in the process of gathering a representation of the biodiversity present in our oceans, if not as a primary target source of information, perhaps as a serendipitous, opportunistic one.

4.4 Conclusions

Continued advancements in the field of natural samplers will enhance our ability to gather valuable eDNA information and contribute to a better understanding of ecological dynamics in challenging environments. The concept of natural samplers is still in its early stages, and further research is necessary to optimise jellyfish-based eDNA sampling protocols and evaluate their performance compared to other methods. This pilot study has demonstrated that some species of jellyfish can be used as natural samplers of eDNA. Species with a larger surface area on their oral arms accumulate more eDNA in their tissues and are better suited for eDNA analysis. Overall, jellyfish show promise as non-invasive and cost-effective tools for eDNA analysis in ecological research. With continued advancements and collaboration between scientists, policymakers, and conservation practitioners, we can unlock the full potential of natural samplers, making significant strides in the conservation and sustainable management of our marine environments.

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