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FROM NANO TO MACRO: (e)DNA FOR MARINE BIOTECHNOLOGY IN AN UPWELLING ZONE OF THE SOUTH ATLANTIC OCEAN

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JÚLIA DA LUZ BUENO

FROM NANO TO MACRO: (e)DNA FOR MARINE BIOTECHNOLOGY IN AN UPWELLING ZONE OF THE SOUTH ATLANTIC OCEAN

Tese apresentada ao Programa de Pós-Graduação em Biotecnologia Marinha do Instituto de Estudos do Mar Almirante Paulo Moreira e da Universidade Federal Fluminense, como parte dos requisitos para obtenção do título de Doutora em Biotecnologia Marinha.

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Arraial do Cabo/RJ, **31 de Julho de 2023**.

Aos meu pais amados Maria de Fátima da Luz Bueno e André Duarte Bueno
e à minha irmã Sofia da Luz Bueno

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“decomposition is cellular work“
(Delia Owens, 2019)

PREFACE

This document is divided into six chapters. The first chapter provides the context in which this work was developed as an introduction for the thesis. The second one approaches a review paper entitled "Environmental DNA in Marine Ecosystems", which is a critical compilation of the factors that influence the dynamics of eDNA in marine environments. The third chapter relies on a comparison between marine eDNA sources (sediment and water) regarding their identification potential and sampling distances. Furthermore, as a biotechnological application of the tool, we evaluate if detecting *Tubastrae* using eDNA is possible and discuss the importance of producing genetic information to feed databases. Moreover, the fourth chapter provides genetic information about an introduced species (*Balanus trigonus*) and discuss if it represents a distinct genetic lineage or population from its native distribution. In the fifth chapter, we make considerations on how DNA molecules are integrated into marine biotechnology and the conservation of marine environments. Finally, we have a succinct sixth chapter gathering the main conclusions of this work.

1 INTRODUCTION

The Cabo Frio upwelling system promotes a shift in the general oligotrophic conditions of the South Atlantic Ocean in Southeastern Brazil into a rich nutrient environment (FERNANDES; FAGUNDES NETTO et al., 2017; VALENTIN; COUTINHO, 1990). The upwelling phenomenon is characterized by wind patterns that push surface waters offshore and thus pump (i.e., 'upwell') nutrient-rich deeper waters into the illuminated surface layers where they become available to support photosynthesis (BAKUN et al., 2010). The fertilization of biological productivity in upwelling zones is responsible for about 50% of the total productivity of world fishing, even representing less than 1% of the total surface of the oceans (NOAA, 2017).

Most upwelling zones are arranged along the temperate eastern boundaries of the Pacific and Atlantic Oceans. Yet, special topography features in the Brazilian coastline lead to this phenomenon (RAMOS, 2011). The abrupt shift in the coastline orientation from north-south to west-east and the small extension of the continental shelf platform set the permanent attributes that, combined with Northwest wind patterns, promote a burst of the South Atlantic Central Water (SACW) mass over the superficial Tropical Water (FERNANDES; QUINTANILHA et al., 2012; VALENTIN; COUTINHO, 1990). The SACW upwelling prompts a high nutrient input, causing a phytoplankton bloom that has a direct impact on the structure of the benthic and nektonic communities (VALENTIN; ANDRE; JACOB, 1987; VALENTIN, 2001; COELHO-SOUZA, Sérgio A et al., 2013; FERNANDES; FAGUNDES NETTO et al., 2017).

The burst's intensity, timing and spatial structure influence the geographical distribution of marine biodiversity and establish strong linkages between upwelling and marine ecosystems (WANG et al., 2015). Valentin, Andre e Jacob (1987) has shown that the dynamic of different water masses prevailing in the Cabo Frio region induces spatial variation in the composition and abundance of the marine community. Thus, the marine environment in the region reflects the hydrodynamics of a heterogeneous marine community (VALENTIN; COUTINHO, 1990; COELHO-SOUZA, Sergio Augusto et al., 2012). It creates a unique ecosystem on the South Atlantic Ocean with the co-occurrence of tropical and subtropical species in proximity (LABOREL, 1969; VALENTIN, 1984; FERREIRA, 2003; LANARI; COUTINHO, 2014). Laborel Laborel (1969) described the influence of the upwelling on the region's high biodiversity and considered it a transition zone between the tropical and subtropical regions of the West Atlantic Ocean. Due to this small-scale spatial gradient in temperature, the region displays a high diversity of reef-associated organisms.

Studying this region's marine environmental DNA (eDNA) can provide valuable

insights into its biodiversity and ecological processes. The hydrodynamics of the marine community, as influenced by factors such as water currents, temperature, and salinity, can affect the distribution and transport of eDNA in the environment. Moreover, by analyzing the eDNA present in different areas of the region, it is possible to understand better the spatial distribution of marine organisms and their interactions with the environment. This information can be used to inform conservation efforts, monitor changes in the marine ecosystem over time, and develop more effective management strategies for marine resources.

Considering the combination of factors such as being a tropical and subtropical environment due to upwelling, Arraial do Cabo thus serves as a recipient of both tropical and subtropical non-indigenous species (NIS) (FERREIRA, 2003). Ferreira, Gonçalves e Coutinho (2006) suggested that the port activities were probably the primary source of the introduction of the NIS because of ships and platforms that in the past used Port of Forno inlet as an anchoring area for repairs or maintenance. Currently, the Forno Port supports domestic and low traffic but occasionally receives supply ships, tugboats, and other vessels from commercial and offshore oil trades from the Campos Basin (BATISTA et al., s.d.). These factors combined may have led to Arraial do Cabo becoming a *hotspot* for NIS (GRANTHOM *et al*., in progress).

Zoobenthic species represent 70% of the exotic species on the Brazilian coast (RUBENS, 2009). Of the nine invasive species with evident and reported impact in the country, six of them are found in Arraial do Cabo (RUBENS, 2009). An example of an introduced species is the scleractinian coral known as *Tubastraea coccinea* Lesson, 1830. Originally from the South-Pacific Ocean in Fiji, this coral was first discovered in Brazil near the Campos Basin oil field in the mid-1980s (CASTRO; PIRES, 2001). It was found attached to oil drilling platforms in the northern region of the Campos basin and later detected in Arraial do Cabo in 1998 (FERREIRA, 2003). Since then, it has spread along more than 130km of the Brazilian southwest coast (PAULA; CREED, 2004; MANTELATTO et al., 2012). Another species of scleractinian coral, *Tubastraea tagusensis*, from the East Pacific in the Galapagos, was also reported to occur in the southern region of the Campos Basin oil field in the mid-1990s (CASTRO; PIRES, 2001; FERREIRA, 2003). However, the taxonomy of the *Tubastraea* genus can be confusing due to overlapping morphological characteristics between species. Recent molecular studies by Bastos et al. (2022) showed that the morphotype of *Tubastraea* found in the South of the Campos Basin oil field was misidentified and proposed to be *Tubastraea sp*. In addition, another introduced species in the Atlantic, the barnacle *Balanus trigonus* Darwin, 1854, a distinctive and easily recognized species, was collected no later than 1864 in southern Brazil by Müller (1868), who, in a pattern characteristic of the nomenclatural history of many alien species, inadvertently re-named it as a new taxon (*Balanus armatus*) (CARLTON; NEWMAN; PITOMBO, 2011). The species is also

present in Arraial do Cabo.

NIS are the biggest cause of biodiversity decline along with climate change (TRISOS; MEROW; PIGOT, 2020; CLAVERO; GARCÍA-BERTHOU, 2005; URBAN, 2015). Early detection and monitoring of NIS in susceptible areas provide a way to protect marine ecosystems against unbalanced biological interactions (DUARTE et al., 2021). However, monitoring is often impacted by the large number of habitats present in a port, the seasonal variability, and the time-consuming morphological approach used for taxonomic identification (TAMBURINI et al., 2021). Often, they are also imprecise due to the large number of very diverse taxa to be identified and present difficulties for the adequate survey of benthic organisms (DARLING; BLUM, 2007). The development and implementation of rapid, sensitive and accurate detection techniques is an essential step towards the early identification and control of marine invasive species (BISHOP et al., 2015).

A molecular tool that represents a promising alternative for comprehensive port biological surveys is environmental DNA (REY; BASURKO; RODRIGUEZ-EZPELETA, 2020). Environmental DNA refers to the DNA material that can be extracted from environmental samples without first isolating any target organisms and comprises molecules that are continuously released into the environment by the shed of metabolic waste or gametes (TABERLET et al., 2012). This continual release means that organisms almost inevitably leave a DNA signature behind when in an environment, and presence can be inferred from these environmental signatures (FREELAND, 2017). This tool offers advantages over traditional techniques, such as needless to see or handle the organism itself, ease of species identification, lower sampling costs, faster and more accurate detection even at presumably low population densities (JERDE; CHADDERTON et al., 2013; GOLDBERG; PILLIOD et al., 2011; DARLING; MAHON, 2011). Thus, considering the recent reduction in sequencing costs, molecular methods are becoming a viable approach for biomonitoring established species and early detection (ARDURA; PLANES, 2017; ELBRECHT et al., 2017).

Metabarcoding represents a promising alternative for comprehensive port biological surveys (REY; BASURKO; RODRIGUEZ-EZPELETA, 2020). Metabarcoding surveys are performed using environmental DNA (eDNA) and have provided important insights for monitoring contemporary biodiversity in marine ecosystems (DEINER et al., 2017). It has been used for surveys and monitoring different ecosystems with several objectives. eDNA is the genetic material obtained directly from environmental samples (soil, sediment, water, etc.) without any obvious signs of biological source material (THOMSEN; WILLERSLEV, 2015). The amplification of certain genetic markers over eDNA generates a massive amount of DNA. Coupled with technologies like high-throughput sequencing (HTS) platforms and bioinformatics processing, the genetic

imprinting left by the organisms can provide the detection of high-resolution community composition data (SHOKRALLA et al., 2012; ZAIKO; SAMUILOVIENE et al., 2015; ZAIKO; SCHIMANSKI et al., 2016).

Currently, eDNA studies can be categorized into two main groups: species-specific and semi-directed approaches (SEYMOUR, 2019). Species-specific methodologies use techniques such as quantitative PCR (qPCR) and digital drop PCR (ddPCR) and have been frequently used in studies to monitor invasive species (JERDE; MAHON et al., 2011; GOLDBERG; PILLIOD et al., 2011; JERDE; CHADDERTON et al., 2013; SANTAS et al., 2013; NATHAN et al., 2015; JANOSIK; JOHNSTON, 2015). These PCR techniques require the design of a specific primer for a given species and the execution of specific assays for its validation (SIMMONS et al., 2016). Thus, although the species-specific approach is useful for monitoring established invasive species, these techniques are not suitable for detecting incipient invasions (SEYMOUR, 2019). On the other hand, the semi-directed approach is broader, and with it, it is possible to track all the DNA strands of a given sample and associate them with their species of origin (RUBENS, 2009; SEYMOUR, 2019; LOPES et al., 2021). In a comparison between the advantages and disadvantages of the two possibilities for monitoring aquatic invaders, Simmons et al. (2016) suggest the semi-directed approach to be used for the early detection of invasive species. Furthermore, the author argues that this technology can simultaneously assess the presence of potentially threatened species and monitor changes in community composition. The semi-directed approach includes metabarcoding, which is used to amplify, sequence and analyze a specific gene region from different organisms (ZAIKO; SAMUILOVIENE et al., 2015). To perform it, high-throughput sequencing equipment is therefore required (ELBRECHT et al., 2017).

Based on the first studies carried out to identify invading organisms, the main source for extracting eDNA is water (FICETOLA et al., 2008; STAT et al., 2017)). However, a study by Turner, Uy e Everhart (2015) showed that environmental DNA can be preserved in sediments for longer periods and that, per milliliter, sediments tend to contain three orders of magnitude more DNA than seawater. Besides the environmental source to use for eDNA extraction, other biotic and abiotic factors may affect the detection of species in a marine environment (ANDRUSZKIEWICZ; SASSOUBRE; BOEHM, 2017). Although progress has been made, eDNA studies on tropical areas remain scarce (NGUYEN et al., 2020). Except for studies conducted in areas at the East Side of Australia (Pacific Ocean) and the Caribbean Region (North Atlantic Ocean), other tropical areas are still neglected (STAT et al., 2017; BAKKER et al., 2019). For example, marine environments in the South Atlantic Ocean have not been explored using this approach yet (BAKKER et al., 2019). This fact can partly be explained by South America's lack of highly equipped infrastructure, facilities and well-trained people to support this kind of study (THOMPSON; KRUGER; THOMPSON, 2017). Yet the region

plays an important role in the world's marine biodiversity and holds great biotechnological potential (FERNANDES; FAGUNDES NETTO et al., 2017; THOMPSON; KRÜGER et al., 2018). In addition, the implementation of a reliable community-wide approach to assessing marine biodiversity across greater spatial scales provides a more holistic picture of ecosystem health and function by establishing patterns of biodiversity and possible community linkages between habitats (BOHMANN et al., 2014; NGUYEN et al., 2020).

Taking all exposed into consideration, it is proposed in this study to make a biodiversity assessment in the South Atlantic Ocean via environmental DNA in a region under the influence of the Cabo Frio upwelling phenomenon. The region is a good model for exploration because it is considered a transition between tropical and subtropical zones and a *hotspot* for NIS, which is important in terms of exploring the biotechnological applicability of the tool. Moreover, despite recent progress, the eDNA technique still needs optimization to produce knowledge on what is the best way to access the state and distribution not yet explored biodiversity (THOMSEN; WILLERSLEV, 2015; NGUYEN et al., 2020). Therefore, in this work, we perform eDNA metabarcoding for eukaryotic organisms using an 18S rRNA marker in an area of the South Atlantic Ocean to make a comparison on the assessment of different eDNA sources and explore its potential to identify NIS present in his area. Additionally, we evaluate the genetic lineage for an introduced species (*Balanus trigonus*).

OBJECTIVES

GENERAL OBJECTIVE

The main goal of this study was to perform eDNA metabarcoding for eukaryotic organisms in an area of the South Atlantic Ocean under the influence of the Upwelling System of Cabo Frio - RJ and produce methodological information regarding the usage and application of this tool (approach?).

SPECIFIC OBJECTIVES

- Access and critically compile information on which factors module the eDNA dynamics in marine ecosystems;
- Compare eDNA sediment and water sources regarding their identification potential for 18S rRNA genetic marker;
- Compare eDNA sediment and water sources regarding sampling distances;
- Determine if it is possible to detect non-indigenous species using eDNA;
- Verify if an introduced species (*Balanus trigonus*) in Southeastern Brazil represents a distinct genetic lineage or population from its native distribution.

ENVIRONMENTAL DNA (eDNA) DYNAMICS IN MARINE ECOSYSTEMS: A COMPLEX ISSUE IMPACTING BIODIVERSITY STUDIES AND SPECIES DETECTION

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ABSTRACT

Environmental DNA, or eDNA, is the genetic material obtained directly from environmental samples. The taxonomic information encrypted in eDNA, an efficient tool for biodiversity assessments, has revealed important ecological information in marine ecosystems. However, utilizing eDNA in a conservation context requires explicit understanding of its determinants in marine ecosystem. The presence of eDNA are influenced by biology, physics and chemistry regulating the entrance, integrity, and permanence in marine environments. Despite the increasing research using marine eDNA, such factors are often overlooked, hindering data interpretation and comparison between studies. Understanding the determinants of eDNA in marine environments will allow for monitoring marine ecosystems, distinguishing between localized and widespread populations of marine organisms, identifying the presence of invasive or endangered species, and optimizing sampling and ecological interpretations of eDNA detection. Through an unprecedented literature review, we present a comprehensive overview of the factors affecting the eDNA dynamics in marine environments, identify knowledge gaps, suggest future research directions, and offer a framework for developing standardized protocols and integrated studies in the field.

Keywords: Marine biodiversity, dynamics, decay, biotic, abiotic factors.

Graphic Abstract .

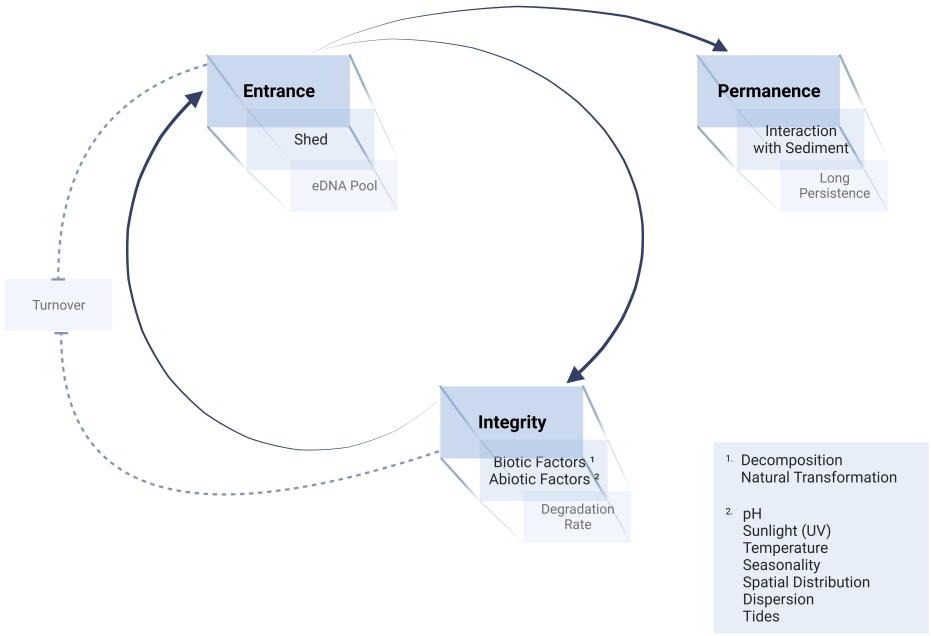

FIGURE 1 – eDNA dynamics in marine environments.

1.1 INTRODUCTION

Environmental DNA (eDNA) refers to the genetic material extracted from environmental samples such as soil, water, air, or sediment without any prior isolation or identification of biological source material (TABERLET et al., 2012; BOHMANN et al., 2014; THOMSEN; WILLERSLEV, 2015). Since the first study in 1987, more than one hundred articles have been published on the topic of eDNA, revolutionizing biodiversity estimates and providing opportunities to test new ecological hypotheses (**ogram1987extraction**; WILLERSLEV; HANSEN et al., 2003; STAT et al., 2017; DEAGLE et al., 2010; DJURHUUS et al., 2020). eDNA is a powerful tool that allows scientists to study the presence or absence of specific species, making it an effective method for monitoring species density and distribution. The use of eDNA has gained significant popularity in biodiversity and conservation studies due to its non-destructive nature and ability to detect a wide range of species (GOLDBERG; TURNER et al., 2016). Its efficiency in detecting low species densities is particularly valuable for identifying rare, cryptic, or endangered species, and for early detection of invasive species (JERDE; MAHON et al., 2011). Additionally, eDNA can accurately identify organisms across different life stages and environments, offering a broad taxonomic breadth for simultaneous biodiversity assessment (GREY et al., 2018; SAWAYA et al., 2019; THOMSEN; SIGSGAARD, 2019; ZHANG et al., 2020)b. Current ecological and conservation questions addressed using eDNA include estimating species distribution, biomonitoring ecosystem health and dynamics, studying diet and trophic interactions, investigating

spawning ecology, and monitoring overall biodiversity (SAHU et al., 2023; CRISTESCU; HEBERT, 2018). All organisms shed DNA into the environment, but the degradation and transport of environmental DNA (eDNA) can result in misleading interpretations when detecting species (BUXTON et al., 2021). These processes can also hinder inferences regarding spatiotemporal species distribution (SONG; SMALL; CASMAN, 2017; HARRISON; SUNDAY; ROGERS, 2019). The marine environment is characterized by complex physicochemical processes that modulate the dynamics of eDNA in seawater and sediment (LACOURSIÈRE-ROUSSEL; DEINER, 2021; LAMB et al., 2022; HOLMAN; CHNG; RIUS, 2022). Nonetheless, the factors involved in the entrance, persistence, and integrity of eDNA in the marine environment are largely overlooked. Adopting a multidisciplinary framework for studying how these factors interact and control eDNA is essential for sampling design and data interpretation (LACOURSIÈRE-ROUSSEL; DEINER, 2021; HOLMAN; CHNG; RIUS, 2022). Recently, numerous methods have been developed for the extraction, sequencing, and analysis of eDNA. Most studies rely on metabarcoding, metagenomics, or quantitative PCR (qPCR), and the appropriate method should be carefully selected based on the study's objective and the available financial resources (NGUYEN et al., 2020). For example, when studying large-scale processes like the environmental determinants of community composition, eDNA can be combined with metabarcoding tools (STAT et al., 2017). Through the amplification of taxonomic markers, this approach enables the simultaneous identification of species across multiple trophic levels and domains of life and provides insights into complex biotic interactions associated with ecosystem changes (DJURHUUS et al., 2020; GARLAPATI et al., 2019). A potential drawback of metabarcoding is the uncertainty of whether all taxa can be evenly amplified since it is PCR-dependent (PEDERSEN et al., 2015). In contrast, metagenomic shotgun sequencing of complex biological material offers the advantage of partially bypassing PCR limitations (TRINGE; RUBIN, 2005) and providing information on the community's taxonomic and functional profile. Metagenomic shotgun also enables community studies using sedimentary ancient eDNA, as it allows for the sequencing of highly fragmented DNA molecules (PEDERSEN et al., 2015). However, it is essential to achieve adequate sequencing coverage to uncover ecologically significant patterns through metagenomics, which implies higher costs compared to metabarcoding (ZIMMERMANN et al., 2023). Finally, if the objective of the study is to detect or quantify the abundance of one or a few species, qPCR is the method of choice. By providing species-specific information (NGUYEN et al., 2020) this method is frequently applied in population genetics (SIGSGAARD; NIELSEN; BACH et al., 2016), and to monitor the spread of non-indigenous species (NIS) (FICETOLA et al., 2008). This review comprehensively analyzes the literature on eDNA in marine environments, providing a detailed understanding of the main processes involved in eDNA dynamics. As such, the present work contributes for future research on the topic by highlighting the

relevant issues that should be considered for study design and results interpretation.

1.2 ENTRANCE OF eDNA INTO THE MARINE ENVIRONMENT

Environmental DNA is ubiquitous in marine ecosystems (SASSOUBRE et al., 2016; ANDRUSZKIEWICZ ALLAN et al., 2021; MAUVISSEAU et al., 2022). It enters the environment through its release by living organisms or by the deterioration of dead organisms. Depending on its state, the eDNA can be classified as organismal or extra-organismal (RODRIGUEZ-EZPELETA et al., 2021). Organismal eDNA is sourced from whole individuals, which are likely alive at the time of sampling, such as viruses and microorganisms (MAUVISSEAU et al., 2022). On the other hand, extraorganismal eDNA is the genetic material shed by an organism throughout its metabolism (BARNES; TURNER, 2016), which includes egestion, exfoliation, tissue replacement, decomposition, lises or cellular extrusion, egestion and active propagules, (PIETRAMELLARA et al., 2009; ANDRUSZKIEWICZ ALLAN et al., 2021). It can be found in the form of tissue, cellular or organelle, or extracellular eDNA (TORTI; LEVER; JØRGENSEN, 2015).

This last form of eDNA can be dissolved in water (D-DNA) (MAUVISSEAU et al., 2022) and is acknowledged by passing through a 0.2 or 0.22 µm pore-size filter (DEFLAUN; PAUL; DAVIS, 1986; KARL; BAILIFF, 1989). According to Brum (2005), D-DNA can be enzymatic hydrolyzable DNA, viruses DNA, or bound DNA (B-DNA), where the DNA is bound to minerals or organic particles. However, the bound DNA can also be attached to larger mineral and organic molecules or particles, mainly extracted from soil and sediment samples. It is worth noting that most studies on marine environments use filters ranging from 0.2 µm to 0.22 µm. Therefore, dissolved eDNA is not included in the analysis for those studies. DNA extracted from soil or sediment may contain all types of eDNA, including dissolved eDNA (Fig. 2).

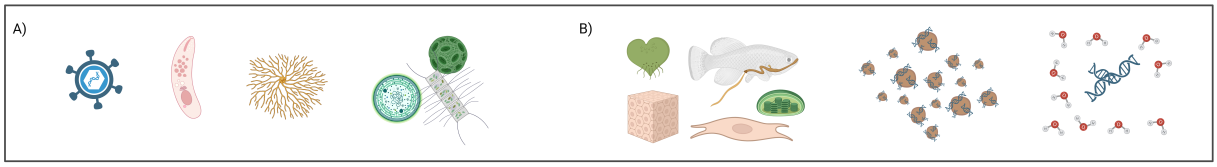

FIGURE 2 – States of eDNA. A) Organismal: whole individuals. E.g. viruses, prokaryotes, unicellular eukaryotes, and zooplanktonic organisms. B) Extraorganismal: Active propagules or gametes and metabolical residue - egestion, exfoliation, tissue substitution, decomposition, lises or cellular extrusion. Found within tissue, cell and organelle or free eDNA bound to organic matter and minerals or dissolved.

1.2.1 eDNA Shed

Understanding eDNA shedding requires knowing an organism's size, age, and biological activity (BARNES; TURNER, 2016). These three factors are intimately connected and play a pivotal role in eDNA shedding. For instance, the high success rates in detecting the invasive American Bullfrog (*Rana catesbiana*) in the first study ever made extracting eDNA from water samples were attributed to the limy coatings produced by such organism (FICETOLA et al., 2008). Based on a study conducted on seawater mesocosms, it was found that eDNA shedding differs among various species such as Northern Anchovy (*Engraulis mordax*), Pacific Sardine (*Sardinops sagax*), and Pacific Chub Mackerel (*Scomber japonica*s), as measured by qPCR (SASSOUBRE et al., 2016). Similarly, Yamamoto et al. (2016) aimed to determine if the distribution of eDNA concentration for Jack Mackerel fish (*Trachurus japonicus*) correlated with its biomass in the Maizuru Bay, Sea of Japan. The research revealed that the estimated eDNA concentration from surface water samples was associated with echo intensity, indicating the potential use of eDNA concentration as a reflection of the local fish biomass. Similar results were obtained for the freshwater Bluegill Sunfish (*Lepomis macrochirus*) (MARUYAMA et al., 2014). However, it was discovered that juvenile bluegill sunfish released eDNA faster than adult ones after size correction. Additionally, the fish's diet played a role consistent with the hypothesis that sloughed gut cells shed constitute a significant source of eDNA since it differed regarding the diet. Maruyama and colleagues concluded that ontogenetic factors, such as changes in behavior, life stage and metabolism also impact eDNA production.

Toshiaki Jo et al. (2019) investigated Japanese Jack Mackerel (*Trachurus japonicus*) eDNA shedding response according to four temperature levels and three fish biomass levels in tanks. The results showed that the eDNA shedding rate increased at higher water temperatures and larger fish biomass. However, the authors didn't expect that the eDNA shedding rates per fish body weight at some temperature levels were also positively correlated with fish biomass, as the surface area per fish body weight was negatively correlated with fish body weight. Other studies run for freshwater species didn't find a correlation between eDNA shedding and temperature (KLYMUS et al., 2015; TAKAHARA et al., 2012). However, these studies did not estimate the true eDNA shedding rate (i.e., estimated the accumulated amount of eDNA) and thus could not divide the effects of eDNA shedding and degradation. This highlights the importance of measuring the degradation rates rather than just the total accumulated amount of eDNA in the tanks when conducting this kind of study (JO, T. et al., 2019). Additionally, when studying three economically important fish species, Kirtane et al. (2021) didn't find a correlation between temperature and eDNA shedding. Still, they stress that environmental conditions such as temperature and biological activity were

likely altered due to physical, chemical, and biological treatment and water circulation in their experiments and may have influenced the results. Therefore, it is reasonable to state that these variables should be kept stable or monitored during experiments on the dynamics of shedding of eDNA.

1.2.2 eDNA Pool in the Marine Environment

The eDNA pool encompasses the genetic material of pelagic and benthic communities and terrestrial and riverine sources (TORTI; LEVER; JØRGENSEN, 2015). The input of eDNA to the deposit occurs through particle sedimentation from the photic layer (DELL'ANNO; DANOVARO, 2005). When associated with large particles, like marine snow, fecal pellets, and massive falls of phytoplankton material after surface blooms, the eDNA molecule sinks to the seafloor (HERNDL; REINTHALER, 2013). The downward flux of particulate eDNA is estimated to deposit up to 550 mg DNA m3 year1 in the continental margin and 10 mg in open-ocean sediments (DNA m^{-3}/$year^{-1}$) (DELL'ANNO; FABIANO; MEI et al., 1999; DELL'ANNO; CORINALDESI et al., 2005). According to DeFlaun, Paul e Jeffrey (1987), D-DNA concentrations decrease as a function of distance from shore and depth in the water column. In contrast, the concentration of B-DNA increases with depth. As a result, in deep-sea sediments around the world, eDNA is 6-8 times higher than the DNA contained within all benthic prokaryotes inhabiting the top 10 cm layers of marine sediment, making it the largest reservoir of DNA in the oceans (DELL'ANNO; DANOVARO, 2005). Moreover, extracellular DNA concentrations in sediment are 3 to 4 orders of magnitude higher than those in the water column in all aquatic ecosystems (CORINALDESI; DANOVARO; DELL'ANNO, 2005).

1.3 INTEGRITY

Degradation rates for the bioavailable (not bound) extracellular DNA in sediments can be 7 to 100 times higher than those for the water column (DELL'ANNO; CORINALDESI, 2004). However, when considering absolute measures, the eDNA turnover time (the rate that a pool of substance is depleted and replaced) in seawater is relatively short, lasting about 10 hours. In the open ocean, eDNA turnover time on seawater can vary from as little as 0.97 h to 6.2 h (BRUM, 2005). Meanwhile, it can take much longer in sediments, lasting 29 to 93 days. This prolonged duration is primarily due to the lower concentration of extracellular dissolved DNA in sediments (DELL'ANNO; CORINALDESI, 2004). Extended turnover times of extracellular DNA in marine sediments are believed to result from the unbalanced ratio between its supply production and degradation rates. More than 80% of the sedimentary DNA is not part of the live biomass in the marine environment (DELL'ANNO; FABIANO; DUINEVELD et al., 1998; DANOVARO; DELL'ANNO et al., 1999). Furthermore, a fraction higher than 90%

is extracellular DNA (CORINALDESI; DELL'ANNO; DANOVARO, 2007). Turnover times of extracellular DNA are approximately threefold shorter in coastal benthic systems than in deep-sea sediments (CORINALDESI; DELL'ANNO; DANOVARO, 2007).

As pointed out by Hyunbin Jo et al. (2019), analyzing the ecological traits of coastal ecosystems can be challenging due to their complexity and nonlinearity. Unlike freshwater ecosystems, which have been more extensively studied, eDNA in coastal marine ecosystems is exposed to more significant variation in abiotic conditions, which can impact its transport/dispersion and degradation ((FOOTE et al., 2012; THOMSEN; KIELGAST; IVERSEN; WIUF et al., 2012). The movement of eDNA in marine environments is influenced by currents, advection, mixing, and river influx (LACOURSIÈRE-ROUSSEL; CÔTÉ et al., 2016). Moreover, factors such as UV radiation, temperature, and pH can directly impact the denaturation of DNA (EICHMILLER; BEST; SORENSEN, 2016; TSUJI et al., 2017; STRICKLER; FREMIER; GOLDBERG, 2015). These abiotic features may also indirectly affect microbial metabolism and enzyme kinetics (TSUJI et al., 2017). Therefore, it is essential to consider these factors when analyzing eDNA from coastal ecosystems, given their significant impact on eDNA transport and degradation.

1.3.1 Biotic Influence

1.3.1.1 Microbial Degradation

Environmental DNA can undergo two biological fates, namely the action of DNAses and natural transformation (LEVY-BOOTH et al., 2007). The decomposition of detrital organic matter is primarily carried out by organotrophic microorganisms, which play a crucial role in biogeochemical cycling in sediments (ARNOSTI, 2014). These microorganisms actively secrete DNAse, which catalyzes the hydrolysis of the phosphodiester bond between the phosphate and deoxyribose moieties of DNA (VARELA-RAMIREZ et al., 2017). Upon eDNA entry, DNase production and excretion occur rapidly, increasing the accessibility of eDNA and promoting microbial growth (BLUM; LORENZ; WACKERNAGEL, 1997). The ubiquitous nature of these enzymes in various environments, such as soil, marine waters, and sediments (CORINALDESI; BEOLCHINI; DELL'ANNO, 2008; PAUL et al., 1989), makes them the most immediate cause of DNA degradation and the primary mechanism for extracellular DNA degradation (BLUM; LORENZ; WACKERNAGEL, 1997).

Following DNA degradation, the nucleotides can be uptake and utilized into new nucleic acids through the DNA salvage pathway (TURK et al., 1992; KROER; JØRGENSEN; COFFIN, 1994). Alternatively, they can be broken down further to provide carbon, nitrogen, and phosphorous (JØRGENSEN; KROER et al., 1993; JØRGENSEN; JACOBSEN, 1996). DNA as an energy source might therefore be a widespread energy source. Given that its composition comprises 40% C, 20% N, and 10% P, DNA repre-

sents a valuable source of nutrients for microbial metabolism. For example, Corinaldesi, Dell'Anno e Danovaro (2007) and Dell'Anno, Corinaldesi et al. (2005) found that eDNA supplies benthic heterotrophic communities with 4%, 7%, and 47% of their daily C, N, and P demand in deep-sea sediments. In comparison, these proportions are 2%, 4%, and 20% in coastal sediments.

1.3.1.2 Natural Transformation

On the other hand, competent microbial cells can incorporate eDNA by natural transformation. Although earlier investigations found that efficient DNA uptake only occurred with kilobase-long molecules (NIELSEN; JOHNSEN et al., 2007), recent studies demonstrated that molecules as short as 20 bp of damaged DNA from a 43,000-year-old mammoth bone could become incorporated in the host's genome by simply acting as primers for the synthesis of the Okazaki fragments during genome replication (OVERBALLE-PETERSEN; WILLERSLEV, 2014). This could contribute to microbial evolution through natural transformation regardless of their source and structural integrity. However, the extent of gene flow across microbial cells via eDNA transformation in marine sediments and the evolutionary impact on environmental microbial communities remain unaddressed (OVERBALLE-PETERSEN; WILLERSLEV, 2014).

1.3.2 Abiotic Influences

1.3.2.1 Spatial Heterogeneity

One consistently observed pattern of community spatial heterogeneity is that communities close to one another tend to be more similar than those farther apart (NEKOLA; WHITE, 1999). Port et al. (2016) reported the results of a 2.5 km eDNA transect surveying the vertebrate fauna present along a gradation of diverse marine habitats associated with a kelp forest ecosystem in Monterey Bay. The study generated eDNA sequence data targeting marine fishes and mammals compared to simultaneous visual dive surveys. The study found spatial concordance between individual species' eDNA and visual survey trends and that eDNA can distinguish vertebrate community assemblages from habitats separated by as little as 60 m. Moreover, eDNA reliably detected vertebrates with low false-negative error rates (1/12 taxa) compared to the surveys and revealed cryptic species known to occupy the habitats but overlooked by visual methods.

The degree to which eDNA community similarity can be predicted by physical proximity and diversity distribution from eDNA data was accessed by O'Donnell et al. (2017) and compared to distributions of macrobial communities. The study describes that offshore communities tend to be richer but less even than those inshore, though the experiments did not spatially correlate diversity. Besides that, there was evidence

for multiple, discrete eDNA communities in these habitats and that these communities decrease in similarity as they become further apart. Similarly, to determine the aquatic community taxonomic composition of the area of Gwangyang Baycoastal ecosystem Hyunbin Jo et al. (2019) used eDNA along with a broad range marker for eukaryotes in a well-known area divided into the inner bay, the bay's main channel, and the outer bay. Detailed spatial species distribution patterns were possible despite these areas' connectedness. In another study, the ability of eDNA metabarcoding surveys to distinguish localized signals obtained from a small spatial scale (<5 km) subject to significant tidal and along-shore water flow was assessed (JEUNEN et al., 2019). Ordination and cluster analyses for taxonomic and OTU data sets showed distinct eDNA signals between the sampled habitats. Individual taxa with strong habitat preferences displayed localized eDNA according to their respective habitat, whereas taxa known to be less habitat-specific generated ubiquitous signals.

These data reveal that eDNA metabarcoding surveys detect a broad range of spatially discrete taxa in marine environments. The results suggested that eDNA dispersal is limited among the studied habitats. On a broader work, DiBattista et al. (2022) compared eDNA-derived communities of bony fishes and invertebrates, including corals and sponges, from 15 locations spanning through a variety of habitats, including coral and rocky reefs, and covering three distinct marine ecoregions. The survey supports a known biogeographic break in fish communities between the north and the south of Oman; however, the eDNA data highlighted that this faunal break is mostly reflected in schooling baitfish species (e.g., sardines and anchovies), whereas reef-associated fish communities were more homogeneous along the coastline. The study provided compelling support that eDNA sequencing and associated analyses may serve as powerful tools to detect community differences across biogeographic breaks and ecoregions, particularly in places where there is significant variation in oceanographic conditions or anthropogenic impacts.

1.3.2.2 Dispersion Processes

Despite the ability of eDNA to detect spatially discrete taxa in marine environments, It is crucial to consider the influence of dispersion to accurately classify a detected sign as either autochthonous or allochthonous (SASSOUBRE et al., 2016). To acknowledge that, a study conducted by Yamamoto et al. (2016) aimed to assess the effectiveness and limitations of eDNA detection of Jack Mackerel eDNA (*Trachurus japonicus*) and determine its distribution in areas with physical disturbance. The results showed that eDNA detection of the fish was most accurate within a sampling distance of less than 150 meters from their location. This suggests that obtaining reliable eDNA signs may require closer proximity to the species, even in areas with physical disturbance. Dispersion processes were also investigated by Murakami et al. (2019) who

used Striped Jack (*Pseudocaranx dentexas)* in cages in Maizuru Bay, Sea of Japan, as this species is not found there. The researchers used quantitative PCR analyses and found that the targeted eDNA was detectable only while the cage was present and up to an hour after its removal. Additionaly, 79% of the amplified samples were collected from locations within a 30-meter distance from the cage, indicating that eDNA dispersion in the sea is limited to a narrow area and its presence lasts for a short time when emitted from a specific source. These findings suggest that eDNA can provide a snapshot of organisms in a coastal marine environment on a small scale of time and space.

Another approach by Andruszkiewicz, Sassoubre e Boehm (2017) considered advection, mixing, gravitational settling processes and decay under a Lagrangian model for the transport of eDNA particles in the coastal area of Monterey Bay. The modeling showed that under a moderate degradation rate (0.055 per hour), the eDNA sign could have been shed up to 40 km towards the south within four days. Besides that, the study revealed that as a response to a deeper thermocline, the vertical displacement of eDNA increases while horizontal displacement decreases. Moreover, it was found that horizontal advection, decay, and settling have more significant impacts on the displacement of eDNA in the ocean than mixing. Furthermore, Jeunen et al. (2019) determined the ability of eDNA metabarcoding surveys to distinguish vertically localized community assemblages. To test this, three vertical transects along a steep rock wall at three depths were sampled, covering two distinct communities separated by near-permanent water column stratification in the form of a strong halocline at approximately 3 m. The study found distinct communities among the different localities sampled but also found a vertical structure of the species influenced by the halocline. Nearshore marine habitats are among the most physically dynamic and biologically diverse on earth (HELMUTH et al., 2006). A scenario in which eDNA communities change with each new tide would suggest that exogenous DNA arrives at a site drawn from a pool of organisms existing elsewhere.

To evaluate how marine eDNA community composition changes across tidal cycles Kelly, Gallego e Jacobs-Palmer (2018) analyzed metabarcoding sequencing data of benthic and planktonic taxa from Hood Canal, Washington, USA. Rather than changing with each tidal cycle, the authors found that both taxa tended to be consistent with the site. However, where physical and chemical water characteristics shifts occurred, the eDNA community changed accordingly. Another work held on Palmyra Atoll targeting the fish community from the sand flat zone also found that tides or water depth (or their effects through temperature) did not affect the eDNA richness estimate or the habitat signal (LAFFERTY et al., 2021).

The movement of water associated with the tide is a fundamental property in these habitats, dramatically shaping the life histories and ecology of organisms that

live there (BABSON; KAWASE; MACCREADY, 2006). Surprisingly, studies showed consistent eDNA communities over multiple tidal cycles, strongly endorsing an endogenous source and highly localized signal. Nevertheless, it was revealed that eDNA communities are more closely associated with water mass and the ecological variables associated to it.

1.3.2.3 Sunlight (UV)

It is well known that UVB and UVA can cause lesions on the DNA (CADET; SAGE; DOUKI, 2005). There have been limited experimental studies on the impact of UV radiation on eDNA degradation in marine environments, with the majority focusing on freshwater systems. For this system, Pilliod et al. (2014) simulated environmental conditions of incidence of sun and shade on the eDNA of a stream-dwelling amphibian. eDNA was detected in all samples collected during the first 3 days of each treatment, but detection rates subsequently declined, with more rapid declines under the sun than shade treatment conditions. After 4 days, eDNA was detected in 40% of sun samples and 80% of samples in the shade. No eDNA was detected in sun samples after 8 days, while 20% of shade samples had detectable eDNA even after 11 days. On the other hand, eDNA was detected in 100% of control samples even after 18 days of exposure at 4 ℃ with no light. However, the difference in the degradation could have been due to differences in total temperature since the study aimed at environmental simulated conditions.

Another study by Strickler, Fremier e Goldberg (2015) investigated the impact of UVB radiation intensity, temperature, and pH levels in a full factorial design on bullfrog (*Lithobates catesbeianus*) eDNA. Isolatedly, UVB didn't show an impact on eDNA, however, moderated UV-B, in combination with other factors, produced a faster degradation of eDNA. A study by Mächler, Osathanunkul e Altermatt (2018) tested natural levels of UV radiation for detecting macroinvertebrates. The study found no difference in eDNA detection with the exclusion of either UVB or both UVA and UVB rays, suggesting that eDNA-based species assessments could be relatively robust concerning these types of radiation. When natural sunlight reaches the earth's surface, UVA rays are 10 to 100-fold more prevalent than UVB (MOAN, 2001), penetrating more profoundly in the water column (HÄDER et al., 2007). Considering that, in the sole study conducted for marine systems, Andruszkiewicz, Sassoubre e Boehm (2017) placed dialysis bags under different depths and extracted eDNA from water to be used as a template for *Scomber japonicus* on qPCR. Nonetheless. no effect of sunlight exposure on the fish eDNA decay was observed. It's important, however, to emphasize that temporal effects were not addressed in this study.

1.3.2.4 Temperature

The temperature in the ocean's open waters varies depending on location and depth. The tropical surface ocean can reach about 30 ℃, while the deep sea can approach freezing temperatures (LALI; PARSONS, 1997). Elevated temperatures can speed up processes that cause DNA degradation, such as cell and organelle breakdown, DNA molecule hydrolysis and oxidation, and extracellular enzyme decomposition (MCCARTIN et al., 2022). In the amplification of ancient DNA (aDNA), for instance, the thermal history of the material is more critical than its age in ensuring successful amplification (HARRISON; SUNDAY; ROGERS, 2019).

A study conducted by Toshiaki Jo et al. (2019) utilized microcosm experiments on Japanese Jack Mackerels (*Trachurus japonicus*) to examine the impact of temperature (13 ℃, 18 ℃, 23 ℃, 28 ℃) on eDNA decay rates with a particular emphasis on DNA fragment length. The study findings indicate that the higher temperatures increased the proportion of smaller eDNA fractions (0.8–3 µm and 0.4–0.8 µm), while there were no significant correlations for longer fractions (>10 µm and 3–10 µm). The researchers concluded that different eDNA states might be correlated with varying rates, as demonstrated by the necessity to fit a biphasic decay rate constant to the observed experimental data. In a second study, Andruszkiewicz Allan et al. (2021) explored the impact of temperature on eDNA decay rates on four different organisms: one fish (mummichog, empth *Fundulus heteroclitus*), one crustacean (grass shrimp, *Palaemon spp.*), and two scyphomedusae (moon jelly, *Aurelia aurita* and nettle *Chrysaora spp.*). The results showed that the decay rate constants for mummichog and grass shrimp increased at higher temperatures, whereas scyphomedusae did not display any clear temperature dependence. The authors suggest that the lack of relationship could be attributed to higher variabilities in eDNA concentrations or differences in eDNA forms being shed from fish/shrimp and scyphomedusae, which can directly impact its degradation rates. Due to the diverse eDNA sources and decay mechanisms, the authors highlight the importance of studying temperature influence on the decay processes according to the state and size of eDNA shed from organisms if the intention is to determine eDNA decay.

Furthermore, McCartin et al. (2022) performed degradation experiments in conditions relevant to the open ocean and the deep sea. The process was observed to occur in two phases, each with different decay rate constants. During the initial phase, eDNA degraded rapidly, unaffected by temperature. In the second phase, degradation occurred slower, strongly influenced by temperature. At temperatures below or equal to 10 ℃, marine eDNA was found to persist for over 2 weeks at quantifiable concentrations. However, at 20 ℃ or higher temperatures, the persistence was limited to a week or less. The model predicts that eDNA persistence reduces by approximately 0.74 days

for each increase in degree Celsius. However, differently from Allan, in this study, the authors state that the relationship between temperature and eDNA persistence remains independent of the source species. The authors suggest that the persistence of eDNA in smaller-size fractions may be more affected by temperature than in larger-size fractions. Their results align with the findings by Andruszkiewicz Allan et al. (2021), who also found that higher-order decay models with tails fit the data better than first-order log-linear models and suggest temporal variability in eDNA decay rates. Similarly, Mauvisseau et al. (2022) reviewed marine and freshwater fish data and found a linear relationship between increased temperature and faster decay rate constant in marine and freshwater fish studies. Moreover, a meta-analysis by Lamb et al. (2022) revealed that eDNA breaks down faster in higher temperatures in marine environments than in freshwater. However, further investigations at a broader and higher temperature range should be considered to improve models and contemplate tropical environments, besides studies considering multiple shore conditions and temperatures.

1.3.2.5 Temporal Variation

Seasonal monitoring is crucial for detecting temporal variations in community composition (THOMSEN; KIELGAST; IVERSEN; MØLLER et al., 2012). However, there is currently limited information regarding the efficacy of eDNA metabarcoding in surveying long-term variation in coastal marine biodiversity (PORT et al., 2016; THOMSEN; WILLERSLEV, 2015). To investigate the potential of eDNA metabarcoding of seawater samples to detect seasonal changes in a coastal marine fish community Sigsgaard, Nielsen, Carl et al. (2017) performed a water sampling and snorkeling visual census over one year at a temperate coastal habitat in Denmark. Additionally, long-term data previously gathered over seven years of the visual census were compared to the eDNA data information. The study showed a high level of congruence on fish species recovered by eDNA sequencing and visual census, although the overlap between methods varied widely among sampling events. Moreover, eDNA data information on the seasonality composition of the coastal fish community was congruent to what was observed in the period of visual census (SIGSGAARD; NIELSEN; CARL et al., 2017).

Using an integrated approach Djurhuus et al. (2020) analyzed a time series of eDNA metabarcoding data to assess changes in the proportional abundance of taxa in a marine setting and the linkages between organisms ranging from microorganisms to mammals. eDNA multilocus amplicon metabarcoding sequencing was used in an eighteen-month time series of seawater samples from Monterey Bay, California. Network analysis found that groups of co-occurring organisms spanning different trophic levels correlated to changes in environmental parameters. It provided insights into the underlying response of whole communities to the environment and highlighted co-occurrences and potential trophic interactions. That way, eDNA-based analyses could

contribute with detailed information about marine ecosystem dynamics and identify sensitive biological indicators that can suggest ecosystem changes and inform conservation strategies. Besides community composition changes, the turnover rate of eDNA also varies seasonally, which may impact study outcomes. Nonetheless, it's known that DNA has a substantial temporal variation related to plankton activity (BOEHME et al., 1993; WEINBAUER et al., 1995; BRUM, 2005). For instance, studies in the Aegean Sea showed that seawater DNA concentrations are higher during boreal summer months than winter months, coinciding with periods of increased primary productivity in this area (DELL'ANNO; FABIANO; MEI et al., 1999). In 2018, Salter (2018) investigated the seasonal variability of dissolved eDNA turnover in marine surface waters of the Northwest Mediterranean using a radiotracer bioassay approach to quantify its kinetic parameters. Biological uptake and turnover of D-DNA had significant seasonal variability, ranging from several hours to over one month. Maximum uptake rates of D-DNA occurred in summer during intense phosphate limitation (turnover < 5 hrs) due to microbial utilization of D-DNA as a source of organic phosphorus.

1.3.2.6 pH

A study conducted by Strickler, Fremier e Goldberg (2015) in microcosms tested the degradation rates of eDNA under a range of acidic, neutral, and alkaline water found in temperate freshwater systems. The findings indicated that eDNA degradation rates do not experience acceleration even at pH levels as low as four, which encourages the usage of eDNA from highly acidic aquatic environments. Additionally, the study found that factors promoting microbial growth, such as moderate UV-B, neutral pH, and high temperatures, are associated with higher degradation rates, suggesting biological mediation may be at play. These findings can aid in better understanding the environmental conditions that affect eDNA persistence, improve the design of sampling intervals and inference, and enhance species detection.

1.4 eDNA PERSISTENCE

1.4.1 Sediment Interaction

DNA persistence in sediments relies on its capacity to bind to soil minerals and humic substances (LEVY-BOOTH et al., 2007). Both soil constituents and DNA have a net negative charge above pH 5 [isoelectric point of DNA (THENG, 1979)], and thus require cations to mediate adsorption (GREAVES; WILSON, 1969; GREAVES; WILSON et al., 1970). At the circumneutral pH typically found in marine sediments, the surfaces of clay and sand charged with inorganic cations like Na^{+}, K^{+}, NH^{4+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, $Fe^{2+/3+}$ and Al^{3+} make the bridge for the electrostatic interaction between the minerals and the phosphate groups of DNA (LORENZ; WACKERNAGEL, 1987).

However, below pH 5, the phosphate moieties of DNA become protonated, resulting in a net positive charge, and DNA adsorption may occur without the need for cation bridging (GREAVES; WILSON, 1969).

Soil's clay content is also important for underlying its binding capacity(LORENZ; WACKERNAGEL, 1994). This mineral presents a high number of binding sites per unit volume and thus can adsorb tens of milligrams of DNA per gram of mineral (LORENZ; WACKERNAGEL, 1994; CRECCHIO et al., 2005). The adsorption capacity of clay is typically 100-fold higher when compared to sand, primarily due to a more extensive surface area, and that of silt is expectedly intermediate (LEVY-BOOTH et al., 2007). Moreover, Nielsen, Calamai e Pietramellara (2006) demonstrated that clay minerals efficiently bind DNA or other molecules due to isomorphous substitutions. However, they do it better on the charged edges rather than on the plan surfaces of the grains (NIELSEN; CALAMAI; PIETRAMELLARA, 2006). A neutralizing bridge is formed either by direct or indirect adsorption. Therefore, the sediment proportion of clay, silt, and sand will affect the amount of mineral-adsorbed DNA (BLUM; LORENZ; WACKERNAGEL, 1997).

Lorenz, Aardema e Krumbein (1981), Lorenz e Wackernagel (1987) reported that DNA bound to sea sand is more preserved than DNA in the water column. This suggests marine sediments offer environmental DNA protection against immediate degradation through reduced nuclease accessibility. In this regard, Romanowski, Lorenz e Wackernagel (1991) showed that DNA adsorbed on sand and clay particles becomes 100- to 1,000-fold more resistant to DNase. The binding protects extracellular DNA from hydrolysis due to partial deactivation and physical separation of the enzymes from their substrate (KHANNA; STOTZKY, 1992; DEMANÈCHE et al., 2001). Rather than the often pictured simple monolayer of DNA on top of a mineral grain, evidence has shown that sedimentary organic matter (which includes DNA) can be physically protected within the pores of mineral surfaces resulting from the alternation of organic matter and clay nanolayers (SALMON et al., 2000). DNA can be readily envisioned to participate in the formation of such aggregates (TORTI; LEVER; JØRGENSEN, 2015).

Furthermore, shorter DNA fragments adsorb better than longer fragments due to higher diffusion rates and faster exposition to a limited number of adsorptive mineral pores (OGRAM et al., 1994; OGRAM, 1998). DNA binding to biogenic sediment components, such as humic substances and proteins, is also known to occur (NIELSEN; CALAMAI; PIETRAMELLARA, 2006; LEVY-BOOTH et al., 2007). Nonetheless, if DNAses saturate binding sites of clay particles adjacent to adsorbed DNA, portions of the DNA may also be degraded (DEMANÈCHE et al., 2001). Adsorption of DNA onto minerals is rapid and occurs within minutes on pure sand and clay (LORENZ; WACKERNAGEL, 1994). Once established, the interactions are tight and stable (DANO-

VARO; CORINALDESI et al., 2006; NIELSEN; CALAMAI; PIETRAMELLARA, 2006). The chemical, physical and biological properties of eDNA influences its capacity to bind to marine sediments (TORTI; LEVER; JØRGENSEN, 2015).

Therefore, soil variability of these constituents in the field causes the persistence of DNA challenging to predict (ROMANOWSKI; LORENZ; WACKERNAGEL, 1991; ROMANOWSKI; LORENZ; SAYLER et al., 1992), and should be carefully considered in sampling design. The eDNA entrapped in the oceans hints at the potential information that can be discovered from marine eDNA.

1.4.2 Long Persistence

Recently, Angeles et al. (2023) investigated changes in biodiversity and community composition from prokaryotes to multicellular organisms through a multi-markers metabarcoding approach on eDNA encapsulated in sediments. In the study, eDNA archives reconstructed the two-century natural history of Bagnoli-Coroglio (Gulf of Pozzuoli, Tyrrhenian Sea), one of Europe's most polluted marine-coastal sites. Data analysis revealed a five-phase evolution of the area where changes appear due to a multi-level cascade effect of impacts associated with industrial activities, urbanization, water circulation and land-use changes as supported by the historical archives and geochronology. Seagrass meadows and high eukaryotic diversity characterized the site until the beginning of the 20^{th} century. Then, the ecosystem completely changed, with seagrasses, associated fauna, and diverse groups of planktonic and benthic protists replaced by low-diversity biota dominated by dinophyceans and infaunal metazoan species.

Upon cell death, DNA repair mechanisms cease to be active, and DNA starts accumulating lesions inflicted by several physical and chemical factors (WILLERSLEV; COOPER, 2005). These factors promote DNA decay by acting on the structure of the individual bases and the overall DNA molecule. Under hydrated conditions, DNA is especially prone to hydrolysis, oxidation, and UV-mediated damages (WILLERSLEV; COOPER, 2005). Lack of light and free oxygen probably protects against oxidation and irradiation in most sediments, leaving hydrolysis as the potential main DNA decay route of DNA (WILLERSLEV; COOPER, 2005).

Hydrolysis is responsible for the loss of purine bases (depurination) and cleavage of the phosphodiester bond, resulting in single-strand breaks (TORTI; LEVER; JØRGENSEN, 2015). Approximately, rates decrease by orders of magnitude every 16 ℃ decrease (LEVER et al., 2015), and have been experimentally determined *in vitro* by (LINDAHL; NYBERG, 1972). The survival of DNA fragments of a certain length and initial concentration can be estimated using these rates (LINDAHL; NYBERG, 1972). Hofreiter et al. (2001) predicted that no fragments would survive undamaged after 5,000

years at 15 °C and pH 7, starting with 1012 copies of 800-bp long, fully hydrated DNA molecules. Under the same conditions, depurination of an equal number of 150-bp long molecules would require 26,700 years (GOLENBERG, 1991). The rate of depurination decreases with increasing pH for pH values between approximately 4.5 and 7.5. Additionally, depurination rates decrease with increasing ionic strength. For example, a 10 mM NaCl concentration can produce a seven-fold rate decrease at 45 °C and pH 5 (LINDAHL; NYBERG, 1972).

The deamination of nitrogen bases is another well-known hydrolytic process. Deamination converts adenine and guanine to hypoxanthine and xanthine, respectively. However, cytosine and its naturally- occurring analog 5-methyl-cytosine are the primary targets for this reaction (LINDAHL, 1993; SHAPIRO, 1981). Deamination of cytosine yields uracil and deamination of 5-methyl-cytosine yields thymine. Similarly to depurination, cytosine deamination rates have been measured *in vitro*, and increase by leveraging temperature and under acidic conditions (EHRLICH; ZHANG; INAMDAR, 1990; FREDERICO; KUNKEL; SHAW, 1990; LINDAHL, 1979; LINDAHL; NYBERG, 1974). In single-stranded DNA, cytosine deamination and depurination proceed at similar rates. In such DNA, the half-life of cytosine residues is about 200 years at 37 °C and pH 7.4. However, the double helix seems to protect cytosines from deamination effectively, as in double-stranded DNA, rates drop to 0.5–0.7% of those in single-stranded DNA. Deamination rates of adenine are lower (2–3%) than those of cytosine and similar to those of guanine (KARRAN; LINDAHL, 1980).

In the dark and anoxic conditions prevailing in many sediments, oxidation and irradiation are unlikely to inflict extensive damage to DNA. However, DNA molecules released in oxic or sunlight environments eventually accumulate oxidative and UV-induced lesions before being deposited and buried in the sediments (HANSEN et al., 2006). UV radiation is responsible for the formation of pyrimidine dimers. Oxidative damages include the formation of 8-hydroxylamine from guanine and hydantoins from pyrimidines (HANSEN et al., 2006). Finally, interstrand crosslinks (ICLs), lesions that are a covalent linkage between strands of double-stranded DNA, can form between the two strands of the same double helix or two different helices (HANSEN et al., 2006). In contrast, adducts can form upon the covalent bonding of DNA with other biomolecules, such as proteins. All these types of damages variably affect the ability to study ancient DNA sequences (HANSEN et al., 2006).

1.5 CONCLUSIONS

The study of eDNA in marine environments encompasses complex interactions under the influence of marine dynamics. Much has been clarified on DNA in the environment before the perspective of studying it using next-generation sequencing.

However, the need to unveil this nano universe has scaled up under the perspective of understanding the whole communities' biodiversity and taking a holistic approach. To get back to the literature regarding DNA pool in the environment and recover it to current knowledge on eDNA is essential to the scope of eDNA. However, information on the shed of eDNA from marine organisms in marine environments is still an area to be explored. Yet, the work that has been done on freshwater ecosystems can provide a baseline for studies in marine environments.

It may be hard to untangle biotic from abiotic factors regarding the integrity of eDNA. However, considering the papers analyzed in this review, we can infer that abiotic influence, such as sunlight, probably doesn't affect short-term timing. Rather, temperature effects may influence the degradation of eDNA by creating conditions for microbial-induced degradation. Seasonality also affects either the community recovered by eDNA or its degradation. Moreover, eDNA reflects the spatial distribution of discrete habitats. Signs of eDNA are pretty localized community-wise, and mostly, eDNA is degraded fastly before it reaches other areas. However, when considering certain works as invasive species detection, for example, it's important to consider that an eventual detection of a sign may not reflect reality. Such studies have concluded that there is a negligible impact of horizontal eDNA transport on species detection accuracy. Therefore, eDNA studies show that it work as a community snapshot. Moreover, holistic approaches can rely on eDNA information to make broader assumptions when crossing this data with other abiotic information. On the other hand, it's possible that a detected sign was shed from kilometers away from the sampled site. This has a higher relevance when considering the detection of invasive species (RODRIGUEZ-EZPELETA et al., 2021). Still, eDNA signs on the water are more connected to the water mass than the tides. Interaction with the sediment may cause longer permanence of eDNA in these environments. By protecting eDNA from degradation, these signs can be recovered longer. However, exposition to high temperatures and extreme pH for longer may cause eDNA degradation to a structure level, causing deamination and depurination. These molecules can still be recovered to study paleogenomics.

Finally, untangling many biotic and abiotic interactions in natural environments can be a constraint. Nonetheless, once we unfold and discuss recent works, it is possible to arrange it to make it easier for scientists in the field to consider what may or may not be necessary when designing experiments and interpreting data on the eDNA of marine environments. Using this approach, we critically appraised this topic in-depth and compiled concise information on eDNA from marine environments. We also highlighted knowledge gaps and pointed out future directions in the field, providing a relevant framework to develop standardized and integrated eDNA analysis methods for marine environments. Disentangling these myriad interactions in marine environments is a challenge in the literature. Studying eDNA in a multidisciplinary framework will provide

conditions for testing hypotheses about how the origin and physical-chemical processes interact to control its persistence in the environment.

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2 ENVIRONMENTAL DNA IN WATER AND SEDIMENT: SOURCE COMPARISON IN MARINE ECOSYSTEM

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ABSTRACT

Marine environments are critical to the blue economy and provide essential ecosystem services. Environmental DNA (eDNA) surveys are increasingly being used for biodiversity monitoring in marine environments, principally because they are sensitive and can provide high-resolution community composition data. However, despite considerable progress in recent years, eDNA studies examining how different environmental sample types can affect biotic profiles and species detectability remain rare. Comparisons of environmental samples are especially important for providing best practice guidance on early detection and subsequent mitigation of non-indigenous species. Here we used eDNA metabarcoding of the 18S rRNA encoding gene to compare community composition of eukaryotes between sediment and water samples in the Forno Port, Southwest Brazil. We first detected markedly different communities and a consistently greater number of distinct operational taxonomic units in sediment compared to water. We also compared differences regarding spatial sampling. We then compared haplotypes of the 18S rRNA gene sequences generated for scleractinian corals and found species concordance against previously published genetic data for the *Tubastraea* genus. Finally, our eDNA survey was able to detect the invasive coral *Tubastraea coccinea*, highlighting the applicability of eDNA metabarcoding for both biomonitoring of marine environments and the detection of non-indigenous species. We conclude that careful consideration of environmental sample type is needed when conducting eDNA surveys, especially for studies assessing non-indigenous species.

Keywords: Marine biodiversity, sediment, water, NIS, *Tubastraea coccinea*, invasive species.

2.1 INTRODUCTION

Marine environments provide essential ecosystem services as they are the biggest regulators of the global climate and the world's largest store of carbon (BARBIER, 2017). Additionally, they are critical to the 'blue economy', which delivers substantial benefits from food (e.g., fisheries and aquaculture) to tourism and shipping (BLASIAK et al., 2023). These benefits are critically connected to biodiversity measurements. It is important to note that in some highly disturbed environments due to anthropogenic stress, there is a rapid decline in species resulting in biodiversity loss (OJAVEER et al., 2018). Managing environmental perturbation involves employing techniques to detect community assemblages, which are vital in providing comprehensive baselines for policymaking (ARDURA; PLANES, 2017; ELBRECHT et al., 2017; DARLING; MAHON, 2011). Therefore there is a pressing need to optimize and design more effective biomonitoring techniques in these environments (ELBRECHT et al., 2017; BOHMANN et al., 2014).

Molecular techniques such as using environmental DNA (eDNA) are a newly developed approach to biodiversity assessment. Environmental DNA can be defined as the genetic material obtained directly from environmental samples (sediment, sediment, water, etc.) without any obvious signs of biological source material (THOMSEN; WILLERSLEV, 2015). Initially, studies utilized eDNA for species identification and employed species-specific PCR assays to detect target taxa (FICETOLA et al., 2008). However, in recent years, high-throughput sequencing (HTS) using metabarcoding techniques, in which DNA barcodes from multiple organisms can be sequenced in parallel, have been implemented (HAJIBABAEI et al., 2011; THOMSEN; KIELGAST; IVERSEN; MØLLER et al., 2012; STAT et al., 2017). This approach has allowed the genetic imprinting left by the organisms to be detected in high-resolution community composition data (SHOKRALLA et al., 2012; ZAIKO; SAMUILOVIENE et al., 2015; KOZIOL et al., 2019).

Environmental DNA is particularly amenable to describe ecosystem-level processes such as trophic, energetic and aquatic interactions instead of single species studies (BISHOP et al., 2015; DJURHUUS et al., 2020). Studies encompassing eDNA in marine environments made it possible to sample environments rather than organisms and are transforming accepted ecological and taxonomic paradigms (LERAY; KNOWLTON, 2016; BOHMANN et al., 2014). It also allows for the simultaneous examination of organisms across multiple trophic levels and domains of life, providing insight into complex biotic interactions related to ecosystem change (DJURHUUS et al., 2020). For instance, eDNA can provide information on newly introduced species (FICETOLA et al., 2008; SEYMOUR, 2019). The studies in this field have been enhancing the scope for assessing biodiversity and functional diversity, contributing to our understanding of community and ecosystem processes, invasive species, wildlife and conservation

biology (FICETOLA et al., 2008; BOHMANN et al., 2014). Surveys applying eDNA have provided important insights for assessing biodiversity in marine ecosystems (DEINER et al., 2017).

However, despite recent progress, the eDNA technique still needs optimization to produce knowledge on the state and distribution of biodiversity not yet explored (THOMSEN; WILLERSLEV, 2015; NGUYEN et al., 2020). A meta-analysis of eukaryotic metabarcoding studies found that approximately 96% of these focused on a single biological substrate, primarily water, while only 4% generated data from two or more substrates (KOZIOL et al., 2019). Therefore, a reliable community-wide approach is needed to assess marine biodiversity across different sample sources. This approach would provide a more holistic picture of ecosystem health and function by establishing biodiversity patterns across different environmental sources (BOHMANN et al., 2014; NGUYEN et al., 2020). Moreover, while traditional NIS monitoring studies in marine environments were performed with benthic invertebrates, the environmental DNA research applied to NIS to date has focused on vertebrate organisms (DEINER et al., 2017; ANDRUSZKIEWICZ; SASSOUBRE; BOEHM, 2017; OJAVEER et al., 2018).

Accordingly, in this study, we aimed to fill the gap about the advantages and limitations of selecting two different substrates for eDNA studies verifying how it affects the detectable biotic profiles within the same environment and how it affects the detection of NIS.

2.2 METHODOLOGY

2.2.1 Study Site

Fieldwork was performed at the Forno Port at the Arraial do Cabo Bay (22° 58 09.12S, 42° 01 06.39W), Rio de Janeiro, Brazil (Fig. 3). The inner portion of the bay reaches 49 m (maximum depth) and is bounded by the mainland, Cabo Frio Island and Porcos Island (CANDELLA, 2009). Coastal water is predominant inside the bay, with temperatures above 20 ℃. The rocky shores in the outside portion contain mainly subtropical fauna and flora due to upwelling phenomena, and the inner portion is characterized by tropical reef communities (COELHO-SOUZA, Sérgio A et al., 2013; FERREIRA, 2003). At this point, the Brazilian coast changes north-south to the east-west direction and represents a unique site with the co-occurrence of tropical and subtropical species in proximity (LABOREL, 1969).

2.2.2 Sampling

Water and sediment samples were collected along the line of the breakwater in three spots distant 80 m from each other (Fig. 2.2.2)) The sediment samples were

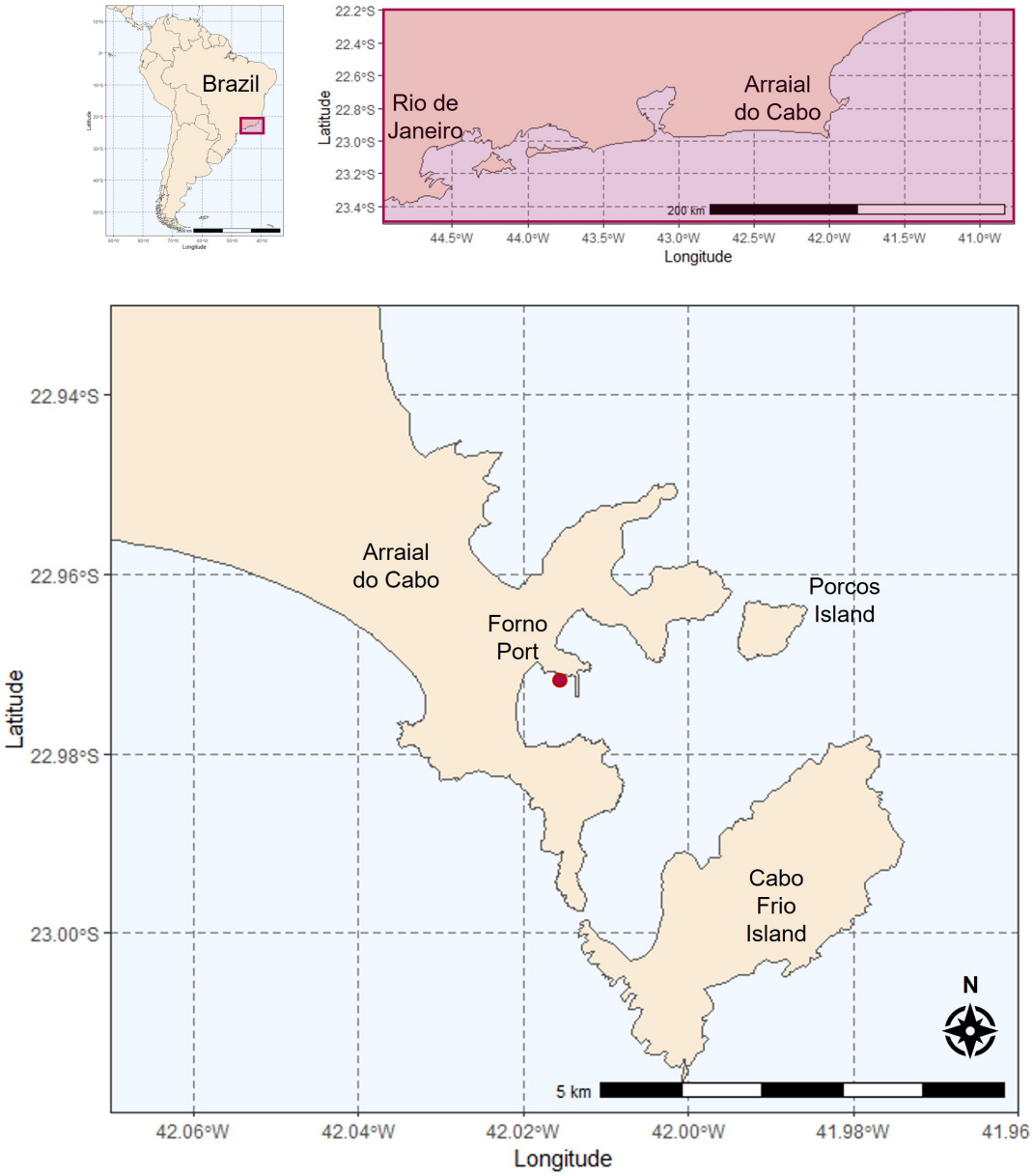
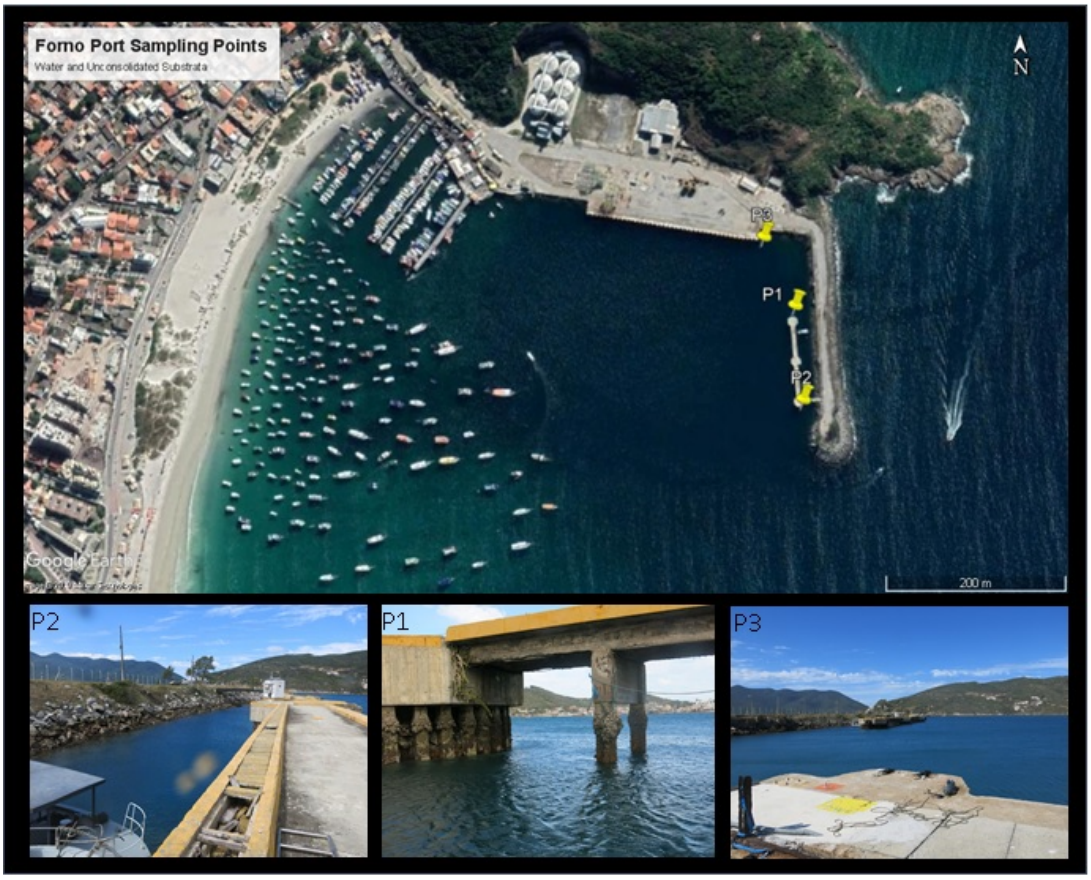


FIGURE 3 – Forno Port location (red dot), Arraial do Cabo, east of Rio de Janeiro City, Brazil.

collected by scuba diving. For each spot, two biological replicates were sampled 1 m apart. Tube cores on PVC 15 cm x 7,5 cm diameter were used for the collection. The tubes were sterilized using 10% hypochlorite solution, 70% alcohol, then put under UV for 20 min, and stored until usage on zip locks equally sterilized. The water samples were collected from the same spots as the sediment. One Niskim bottle of 5 L was used, and the water was collected 1 m above the sediment and transferred to 10 L buckets. Disposable latex gloves were worn during sampling and replaced between each sample, and the bottle was sterilized with sodium chloride and alcohol. All the material was kept cool during transportation to the Marine Genetics Laboratory of IEAPM, which is 2 Km away from the site. For the sediment, 2 cm of the top layer was scraped off and transferred to 15 mL falcon tubes. Everything was stored at four ℃ until extraction.



Photographies of the sampling points at the Forno Port. Sampling points 1, 2 and 3 are approximately 80 m apart.

2.2.3 DNA Extraction

The eDNA extraction of all samples occurred within two days after collection. Each sediment sample was homogenized, and three technical replicates were extracted with Qiagen Power Sediment Kit®according to the manufacturer's instructions. In contrast, the water samples were extracted using the Qiagen Blood and Tissue Kit®(HINLO et al., 2017) with modifications (SIGSGAARD; NIELSEN; BACH et al., 2016; SIGSGAARD; NIELSEN; CARL et al., 2017). For each water point sampled, three 1 L replicates were filtered on a 0.45 µm Cellulose Nitrate filter (Sartorius, Germany) (MAJANEVA et al., 2018). Extractions were confirmed using 1% agarose gel and checked on a NanoDrop™ spectrophotometer for concentration and quality. One blank extraction was added to each of the spots of the sample combination.

2.2.4 PCR

The PCR was carried out with a primer that used targets for the variable V4 region of the eukaryote SSU rRNA gene: TAReukF (5'-CCAGCA(G/C)C(C/T)GCGGTAATTCC-3') and TAReukR (5'-ACTTTCGTTCTTGAT(C/T)(A/G)A-3') (STOECK et al., 2010) (380 bp) with tags of 6 bp. A set of 94 barcodes were used, and the amplifications were

performed using matching nucleotide tags (e.g., forward primer tag 1 – reverse primer tag 1, forward primer tag 2 – reverse primer tag 2, etc.) to account for potential tag jumps and to avoid false assignment of sequences to samples (SCHNELL; BOHMANN; GILBERT, 2015). All PCR's were run in triplicates. The 18S rRNA geneamplification comprised an initial activation step at 95 ℃ for 5 min, followed by ten three-step cycles consisting of 94 ℃ for 30 s, 57 ℃ for 45 s, and 72 ℃ for 1 min, which was followed by 25 further cycles consisting of 94 ℃ for 30 s, annealing at 48 ℃ for 45 s and a final 2 - min extension at 72 ℃. The total PCR reaction was 25 µL and consisted of 1 µL DNA template, 2,5x Gold PCR Buffer (Applied Biosystems), 2.5 mM MgCl2 (Applied Biosystems), 0.2 mM dNTP mix (Invitrogen), 0.2 µL AmpliTaq Gold (Applied Biosystems) and 0.5 mg/ml Bovine Serum Albumin (BSA - Bio Labs) and 1,5 µL of each forward and reverse primers (STOECK et al., 2010). PCR amplifications were confirmed on 2% agarose gel using GelRed against a 50 bp ladder. PCR was run at the Contemporary DNA Lab, red section, at the GLOBE Institute, at the University of Copenhagen, Denmark.

2.2.5 Library Building and Sequencing

Amplicons were combined into three pools to contain one of the three PCR replicates. Amplicon pools were purified using MinElute (Qiagen) and eluted in 25 µL EB buffer. Elution was done at 25 µL with one incubation of 10 min at 37 ℃. Concentration was analyzed on Qubit High Sensitivity (HS-dsDNA) (Thermo Fisher). Then the three libraries plus one library blank were built using TruSeq DNA PCR-free Library Prep Library Build and indexes IDT for Illumina – TruSeq DNA UD Indexes v2. After that, they were purified using MagBio HighPrep beads (LabLife) under 1.5X proportion. The concentration was verified on Qubit HS Kit, and the fragment size distribution of the libraries was checked on an Agilent 2100 Bioanalyzer. Libraries were then pooled on equimolar concentration (nM). Sequencing was performed at the Danish National High-throughput DNA Sequencing Centre at the University of Copenhagen using the Illumina MiSeq v2 platform. Libraries were produced at the Contemporary DNA Lab, yellow section, at the GLOBE Institute, at the University of Copenhagen, Denmark.

2.2.6 Bioinformatic Processing

The raw libraries were analyzed regarding their quality using the program FastQC. Then reads were processed on DADA2 (v. 1.18) under Bioconductor using a customized pipeline written in the R programming language. The DADA2 algorithm makes use of a parametric error model to correct sequencing errors and infers sample sequences exactly, resolving differences of as little as 1 nucleotide (CALLAHAN et al., 2016). The program was used for demultiplexing the barcodes and removing the primer sequences. The reads were truncated to 230 and 200 bp (forward and reverse

reads, respectively) and filtered with a maximum number of “expected errors” (maxEE) threshold of two (forward reads) and six (reverse reads). If reads did not meet this threshold, they were discarded from further analysis. A parametric error matrix within DADA2 was constructed, followed by sequence dereplication of sequence variants for the forward and reverse reads determined. Paired-end reads were merged after singletons were discarded, with a maximum mismatch of 1 bp and a minimum overlap of 10 bp. Chimeric sequences were then removed within DADA2 using the consensus option in the removeBimeraDenovo script. The samples that produced Amplicon Sequence Variant (ASV) counts under the 25% percentile of total ASV distribution were filtered off. Subsequently, extractions replicates were pooled. Further analyses were run using the R base, vegan (OKSANEN et al., 2022) and phyloseq (MCMURDIE; HOLMES, 2013)packages. Alpha diversity measures were run using the Phyloseq package, and subsequently, samples were rarefied under the "rrarefy"function for the dissimilarity and taxonomical analysis.

2.2.7 Taxonomic Assignment

The amplified region was checked against the SILVA Silva SSU taxonomic training database (v. 138) (QUAST et al., 2012), available at https://zenodo.org/record/3731176. After the assignment, the ASVs were indexed into those found in sediment or water to explore taxonomic patterns of detection in these eDNA sources.

2.2.8 Species Identification

The species identification was made using the BLAST tool. All sequences for *Tubastreae* 18S rRNA gene were downloaded along with an outgroup from the same family *Dendrophylliidae* (Fig. 4). The sequences were analyzed on MEGA X (KUMAR et al., 2018). Alignment was done by CLUSTALW, and evolutionary history was inferred using the Neighbor-Joining method (SAITOU; NEI, 1987). The optimal tree is shown. (next to the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method (TAMURA; TAO; KUMAR, 2018) and are in the units of the number of base substitutions per site. This analysis involved 10 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option).

Species	Accession Number	Reference
Tubastraea coccinea	AJ133556.1	(Won, Rho and Song, 2001)
Tubastraea coccinea	JQ688008.1	(Yang *et al* ., 2013)
Tubastraea coccinea	KF733548.1	(Hu et al.. 2014)
Tubastraea coccinea	LT630999.1	*(Arrigoni et al., 2016)*
Tubastraea micranthus	LT631000.1	(Arrigoni *et al* ., 2016)
Tubastraea coccinea	Z92906.1	(Song and Won, 1997)
Rhizopsammia minuta	Z92907.1	(Song and Won, 1997)

FIGURE 4 – 18S rRNA genes for *Tubastraea* and respective accession numbers from Genbank.

2.2.9 Statistical Analyses

All statistical analyses were conducted in R (v. 4.1.0) using the base R and the Vegan (v. 2.6-4) package. The number of ASVs was calculated after the total number of ASVs across the pooled extraction replicates. A Shapiro Test was run prior to a two-way Analysis of Variance (ANOVA) which was then run with the aov function. The test was made to verify if there was a significant difference between the number of ASVs generated by sediment and water eDNA (NumObservations Spot * Source). Non-metric multidimensional scaling (NMDS) ordination plots were generated from Bray-Curtis dissimilarity values derived from the Vegan package. Then a Permutation Analysis of Variance (PERMANOVA) (adonis) was implemented to test if there was a significant difference among sampled spots of sediment under the (Sediment Spot, Metadata) function.

2.3 RESULTS

2.3.1 ASV Richness

The ASV count barplot shows the number of ASVs generated for every sampled source and spot at Forno Port (Fig. 5). The ANOVA test showed a significant main effect of Source ($p<0.05$ - 3e-0,6***) and some interaction between Spot and Source. No difference was found when considering Spot ($p>0.05$ - 0.1372). Finally, for Spot:Source, no difference was found for Spot: Source ($p>0.0507$). The results of the Shapiro-Wilk test to check the normality of the residuals show that they are normally distributed ($p>0.05$ - 0.1218). The number of ASV generated from water was higher than the ones produced from sediment. Subsequently, it's possible to observe that the average ASV count for water on spots two and three are the same. On the other hand, Sediment samples present differences in the average among each other. It's possible to perceive that spot two presented the highest amount of ASV counts while spot three presented the lowest amount, and spot two it's an intermediary between them.

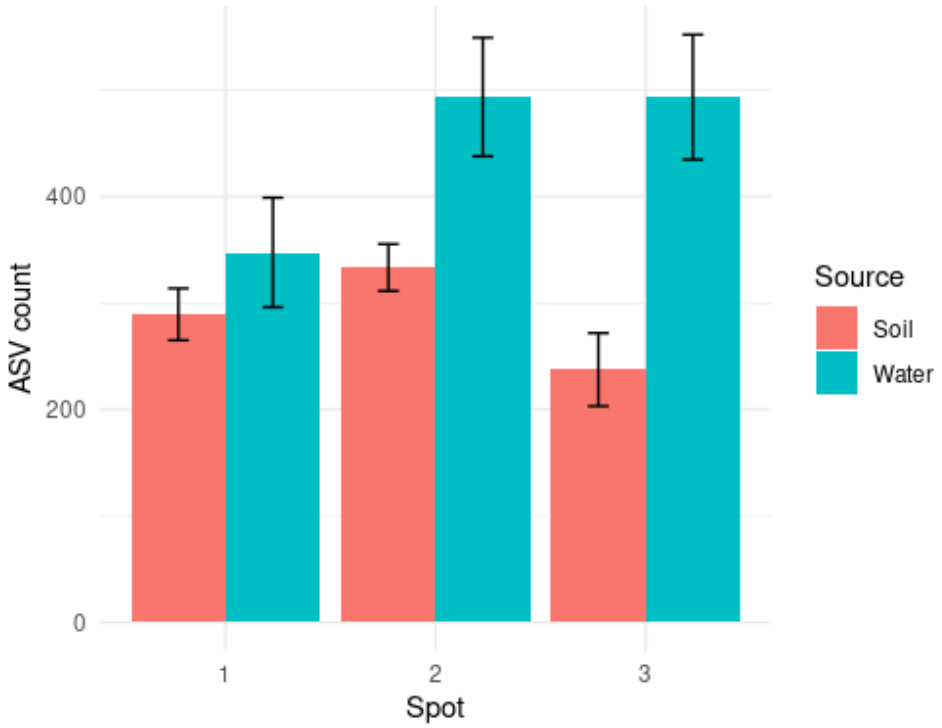


FIGURE 5 – Total ASVs detected across sampling spots and eDNA source sample with standard error.

2.3.2 Alpha Diversity

The species richness was inferred by the alpha diversity using the Chao1 and Shannon index. Indexes were plotted accordingly to non-rarefied data measures for Sediment and Water regarding each of the spots sampled (Fig. 6). In Chao1, it is possible to observe that spot 3 represented the sample with the least diversity among all the studied sources and spots. Meanwhile, water source diversity for this point remarks as very similar to spot 2. On the other hand, despite spot 1 showing a higher alpha diversity measure for this index when considering the sediment source compared to spot 3, the water source represents a lower diversity of individuals sampled compared to the other two. Yet, the Shannon index shows a more diverse community recovered when using the water source for every sampled spot, with similar results, suggesting that the community recovered from each sampled spot when using water is very similar regarding community diversity. The difference tendency observed on the Chao1 index for the source sediment is congruent with the results for Shannon, with spot 2 showing higher diversity than spot 1, followed by spot 3. It is possible to state that for spot 1 on the water source, despite having a similar community index when inferring Shannon analysis, it showed a similar diversity to the soil when Chao1 is inferred. This may represent that because Chao1 is sensitive to rare OTUs, a smaller abundance of these taxa was recovered in these samples.

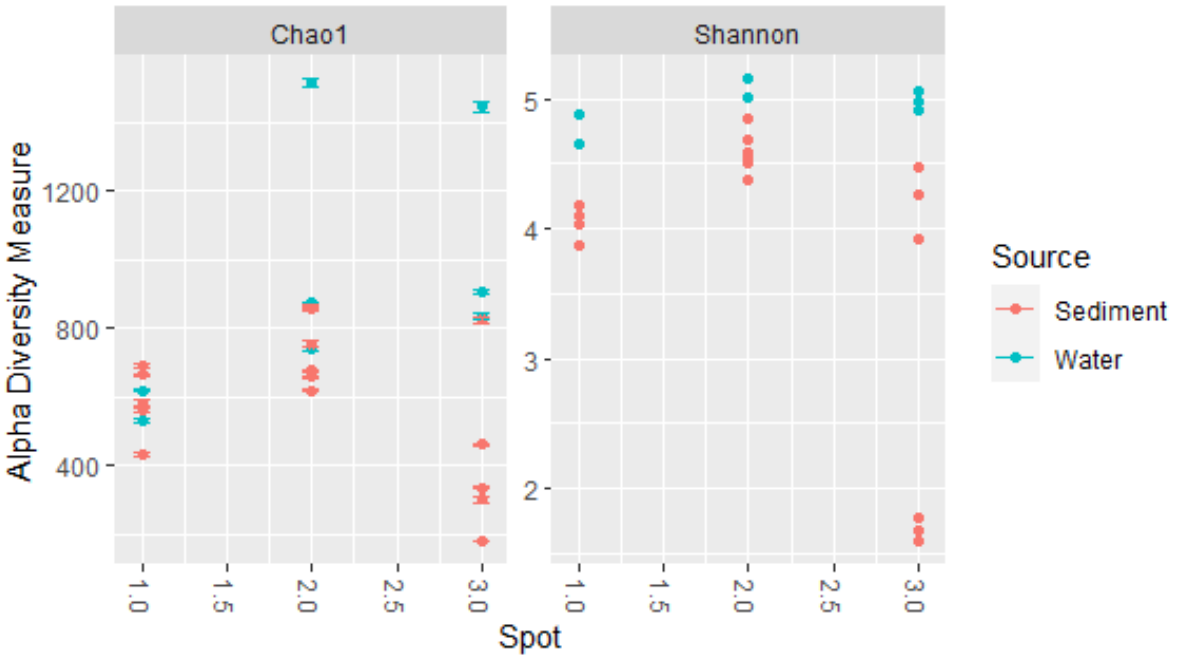
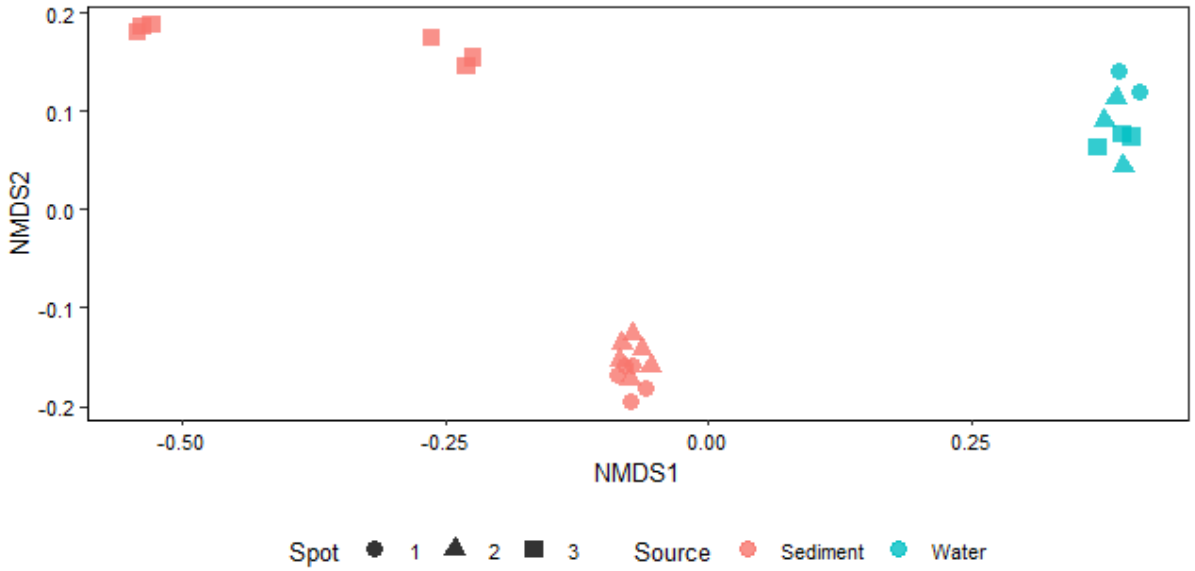


FIGURE 6 – Alpha Diversity based on Chao 1 and Shannon indexes.

2.3.3 Ordination Bray-Curtis Dissimilarity

The NMDS plot based on the ordination of Bray-Curtis dissimilarity shows the grouping of different clusters (Fig. 7) after rarefaction. Spots are acknowledged in different shapes and sources in different colors. The results of the Shapiro-Wilk test to check the normality of the residuals showed that they are not normally distributed ($p<0.05$ - 2.986e-08). The PERMANOVA test showed a significant main effect of Spot ($p<0.05$ - 0.001 ***) when tested for Sediment. It is possible to see that the samples are grouped into three main clusters. One is the aggregation of Water samples, joining the three sampled Spots colored blue. The other two correspond to the Sediment samples, colored brown. It is possible to see that samples from Spots two and three are closely related. These two sample types are distant, approximately 80 m. On the other hand, another cluster containing samples only from Spot 3 is isolated on the plot. Yet, within this cluster, it is possible to see that despite being more closely related to one another, there is a division with two sample subsets likely to result from the biological replicates for this area.



FIGUR E 7 – Non-metric multidimensional scaling ordination plots based on Bray-Curtis dissimilarities of 18S rRNA metabarcoding of Forno Port sediment and water eDNA samples. Stress 0.04380095. Spots have different shapes and sources in different colors.

2.3.4 Taxonomy

2.3.4.1 Assignment Proportions

The taxonomic proportions of classified ASVs per sample type were calculated based on the total ASVs generated (Fig. 8). A total of 7760 ASVs of 18S rRNA were produced among the sediment sources. The total number of different sequences for sediment was 2780 and for water 2532, and the lowest taxa achieved was Genus level. Water was by 4 orders of magnitude more processed than sediment. Yet, the difference between the ASVs recovered was 248 ASVs (3% difference of the total). Despite that, ASVs from water sources presented more ASVs assigned for Phylum (76.9%) compared to sediment (59.50%). Therefore, lower taxons were also affected. Nonetheless, while the difference in Phylum assigned is 17,4% between the sources, to the Genus level this difference decreased to 8,4%.

2.3.5 Taxa occurrence among samples

2.3.5.1 Phyla

The most abundant phyla identified on water, sediment and, or both were analyzed to verify their distribution on the sources used. In the graph (Fig. **??**), columns represent the number of AVS assigned in the Phylum level and are organized from the most to the least frequent based on their occurrence. *Diatomea* was the most abundant Phylum observed in the data, with a count of over 800. Sediment showed higher counts for *Diatomea* than water. Still, part of the ASVs were found in either water and sediment.

TAR (18S)		
Taxonomic Classification	**Sediment** ASV assigned (%)	**Water** ASV assigned (%)
Phylum	59.50	76.9
Class	50.8	68.9
Order	39.1	56.3
Family	31.7	46.6
Genus	24.4	32.8

FIGURE 8 – 18S Taxonomy classification identification rate according to taxon and source (Forno Port).

Cercozoa followed by *Dinoflagellata* accounted for more than 400 counts. *Protalveolata* and *Ciliophora* accounted for between 400 and 200 counts, whilst the following taxa all accounted for less than 200. From the 20 most frequent taxa assigned for Phylum, *Porifera*, MAST-3 and *Incertae Sedis ph* were only found on water. Despite the different frequencies on the sources, all the other taxa were found on water, soil and had ASVs represented in both.

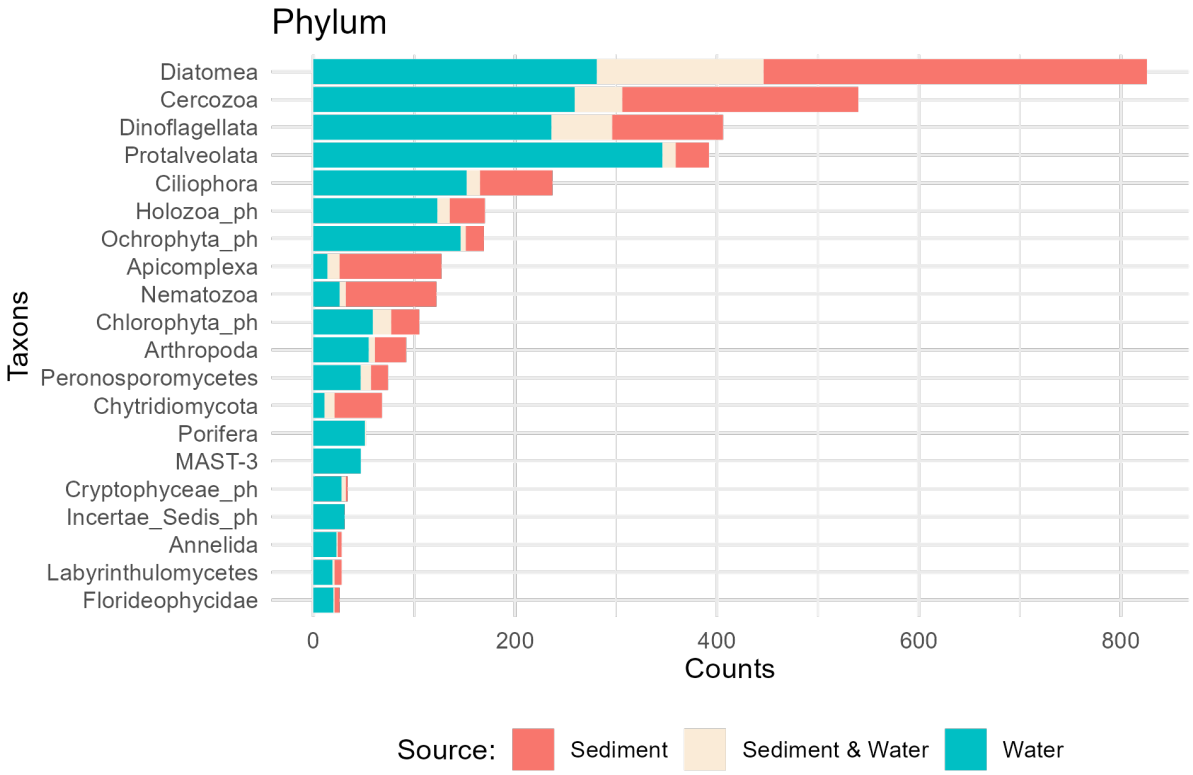


FIGURE 9 – Horizontally stacked bar chart detailing the proportion of Phyla detected in eDNA from sediment, water or both in the 18S rRNA gene metabarcoding survey (Forno Port).

2.3.5.2 Genus

The most abundant genus identified on water, sediment and, or both were analyzed to verify their distribution on the sources used. The frequency of genus was plotted to get a better picture of which is the total identified distribution of this taxon in the sampled area. The most abundant genus identified on water, sediment and, or both were analyzed to verify their distribution on the sources used (Fig. **??**). *Pseudopirsonia*, a *Cercozoa* that infects diatoms, was the most abundant Genus observed in the data, with a count of over 150 ASVs. *Rhogostoma*, another heterotrophic *Cercozoa*, was the second most abundant, with about 100 counts. Following there is *Amoebophyra*, which is a syndinian parasite that infects dinoflagellates, was mostly found on water with a little less than 100 counts. Meanwhile, all other genera taxa were found in less than 75 counts. Besides, all other genera were found in either water and sediment, with the exception of *Telonema*, which was found only in water source. Rarer genera accounting for less than ten AVSs were put in the category "Other"(Anexo, Fig. 20).

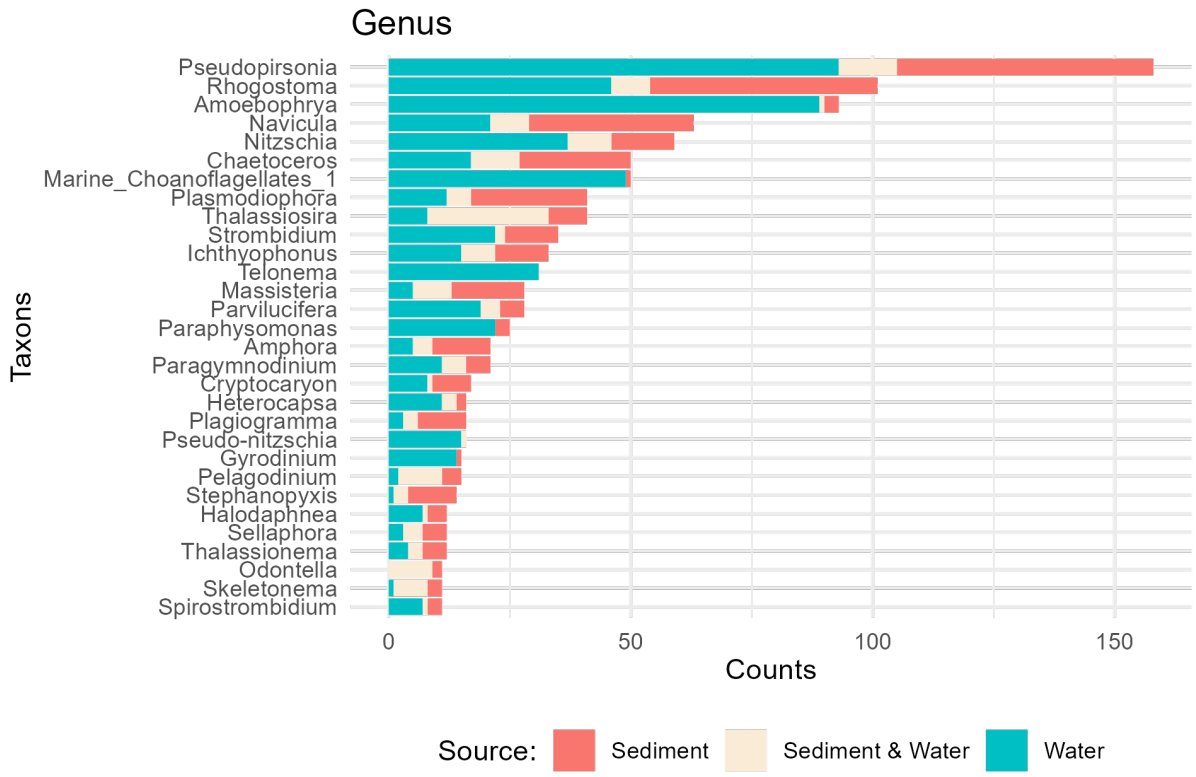
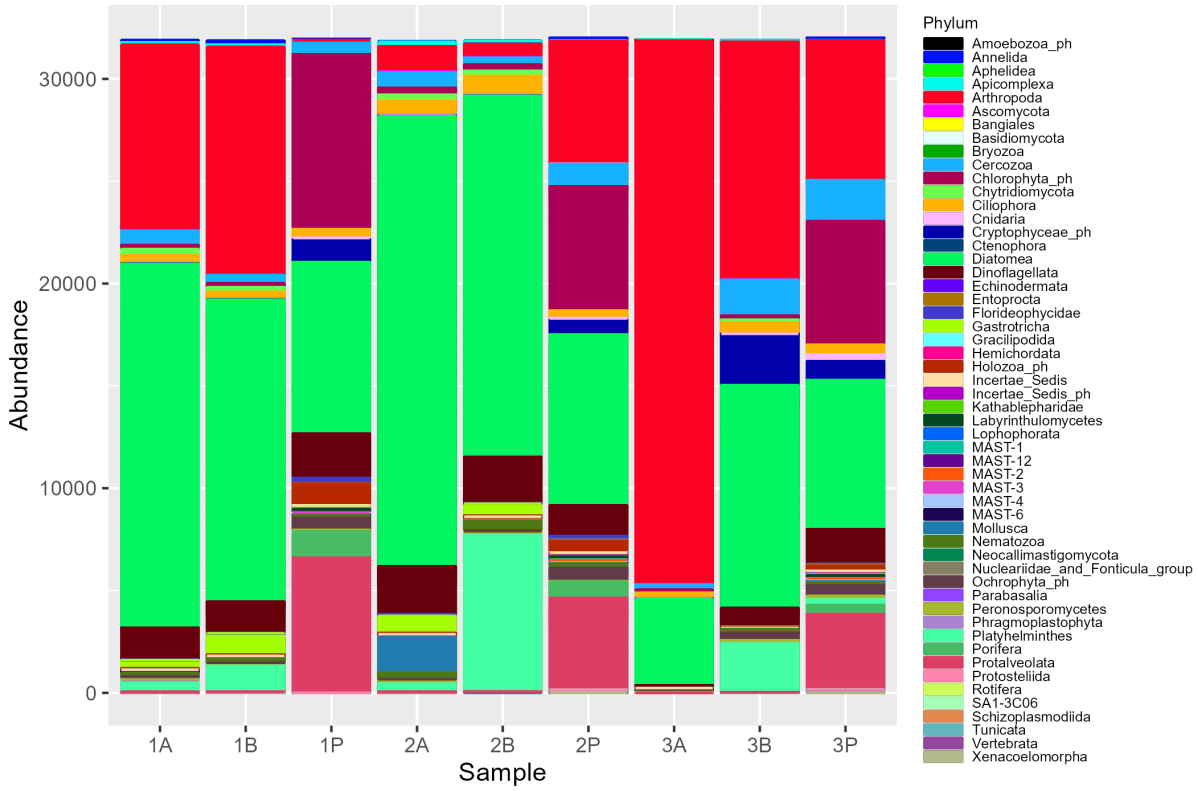


FIGURE 10 – Horizontally stacked bar chart detailing the proportion of Genus detected in eDNA from sediment, water or both for 18S (Forno Port).

2.3.5.3 Phyla Abundance

The biological replicates for sediment and water were verified regarding their abundance. It is possible to see in (Fig. 2.3.5.3) the patterns regarding each of the biological replicates. The most abundant non-metazoan was *Diatomea*, which was present in all samples. Meanwhile, *Arthropoda* was the most abundant metazoan. *Clorophyta* was highly abundant in water samples. Our metabarcoding analysis shows that each source recovered a distinct subset of the port community. Biological duplicates of spot 3 show a marked difference from each other. On the other hand, biological duplicates for spot 1 and 2 are visibly similar in community composition.



Phyla abudance biotic profiles recovered with 18s rRNA from the Forno Port on sediment biological replicates and water. The numbers on the sample names correspond to their counterparts of "spot".

2.3.5.4 Metazoans Phyla

2.3.5.5 Source

The taxonomic proportions were calculated based on the total abundance of ASVs for Metazoan phyla generated and collapsed accordingly to the source. The Metazoa group was subsetted, and the abundance of Phyla in sediment and water was calculated, merging together the samples accordingly to their provenience. It represents the relative abundance of different Phyla taxa on each of the sources (Fig. **??**). The most abundant source was sediment, despite the significant difference in the

amount of ASV generated and the higher alpha diversity indexes for water. In sediment, *Arthropoda* represented the most abundant phyla, followed by *Plathelmintes*, *Mollusca*, *Gastrotricha*, and *Nematozhoa*. On the other hand, in water, the most abundant phyla were *Protalveolata*, followed by *Arthropoda*, *Porifera* and *Annelida*.

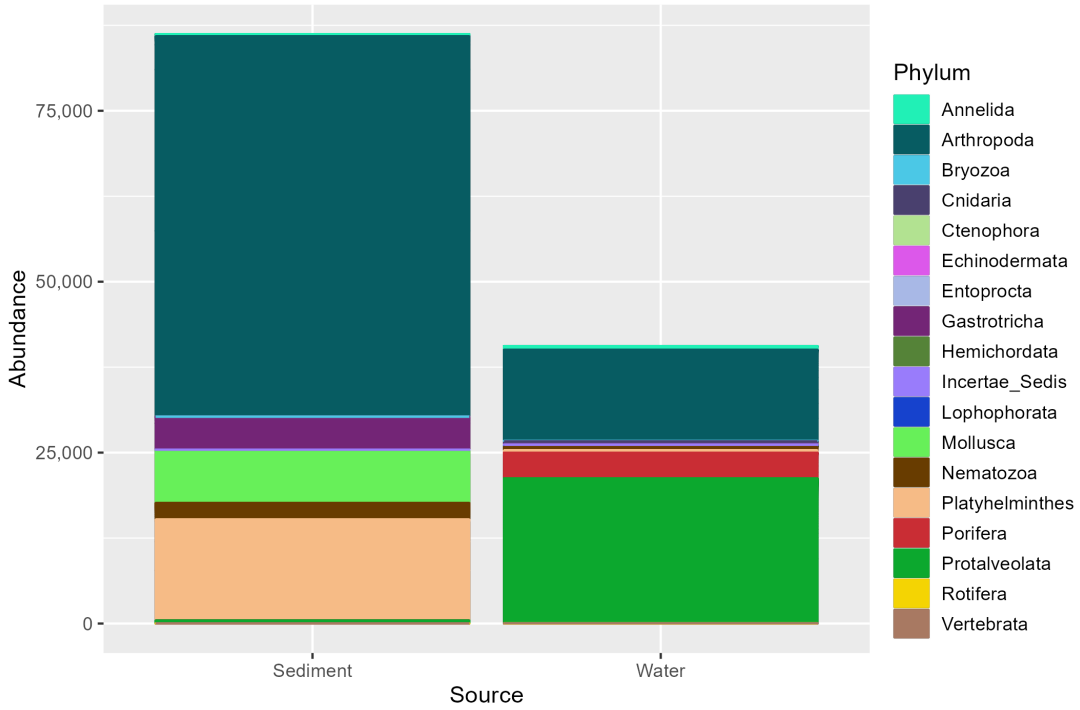


FIGURE 11 – The abundance of metazoan phyla on the sources sampled from the Forno Port.

2.3.5.6 Spot

The taxonomic proportions were calculated based on the total abundance of ASVs for Metazoan phyla generated accordingly to the spot sampled (Fig. 12). *Arthropoda*, *Protoalveolata* and *Platyhelmintes* are markedly detected on the three spots. *Porifera* was also observed in the three places, although more abundant in spots 1 and 2. Spot 1 and 3 also share a bigger abundance of *Gastrotricha*, and spot 2 differs markedly in its biotic profile due to the *Mollusca* abundance.

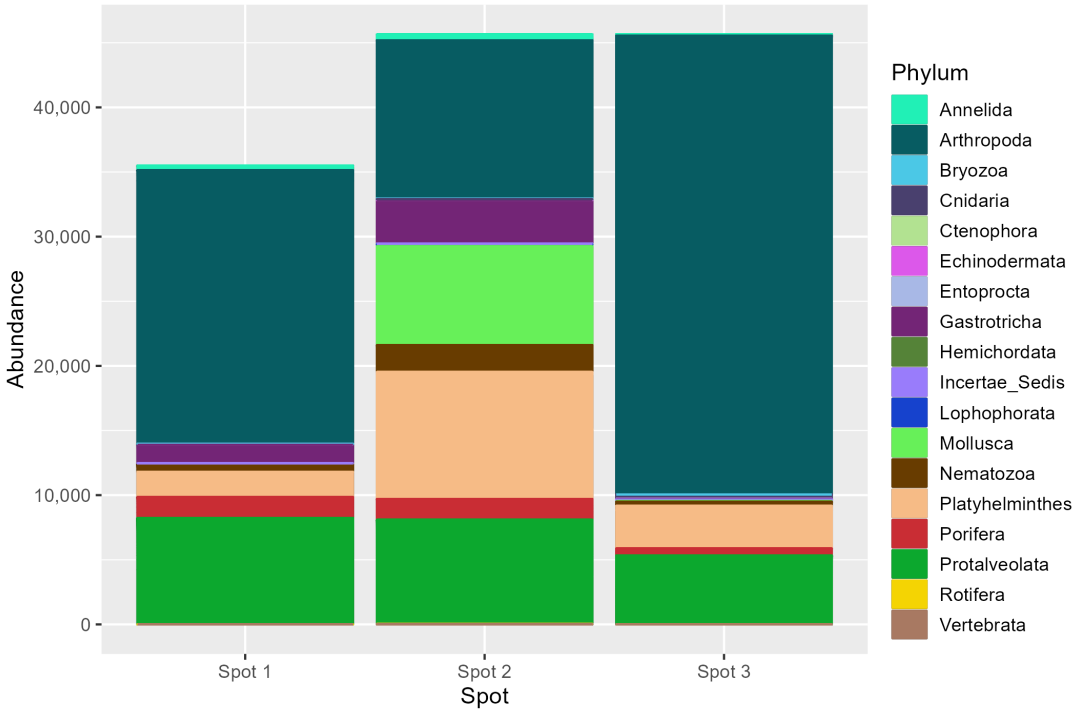


FIGURE 12 – The abundance of metazoan phyla on the three spots sampled from the Forno Port.

2.3.5.7 Phyla ordination

An ordination of the samples was run on phyloseq and plotted for an NMDS graph representing metazoans and their respective class (Fig. 13). In the graph, it is possible to see, according to their grouping position (see 7, the sources from which they were recovered). For instance, *Echinodermata*, *Entoprocta*, *Lophophorata* , *Vertebrata* and *Cnidaria* were only recovered from the sample group of the water source. On the contrary, *Rotifera*, for example, was only recovered from soil samples.

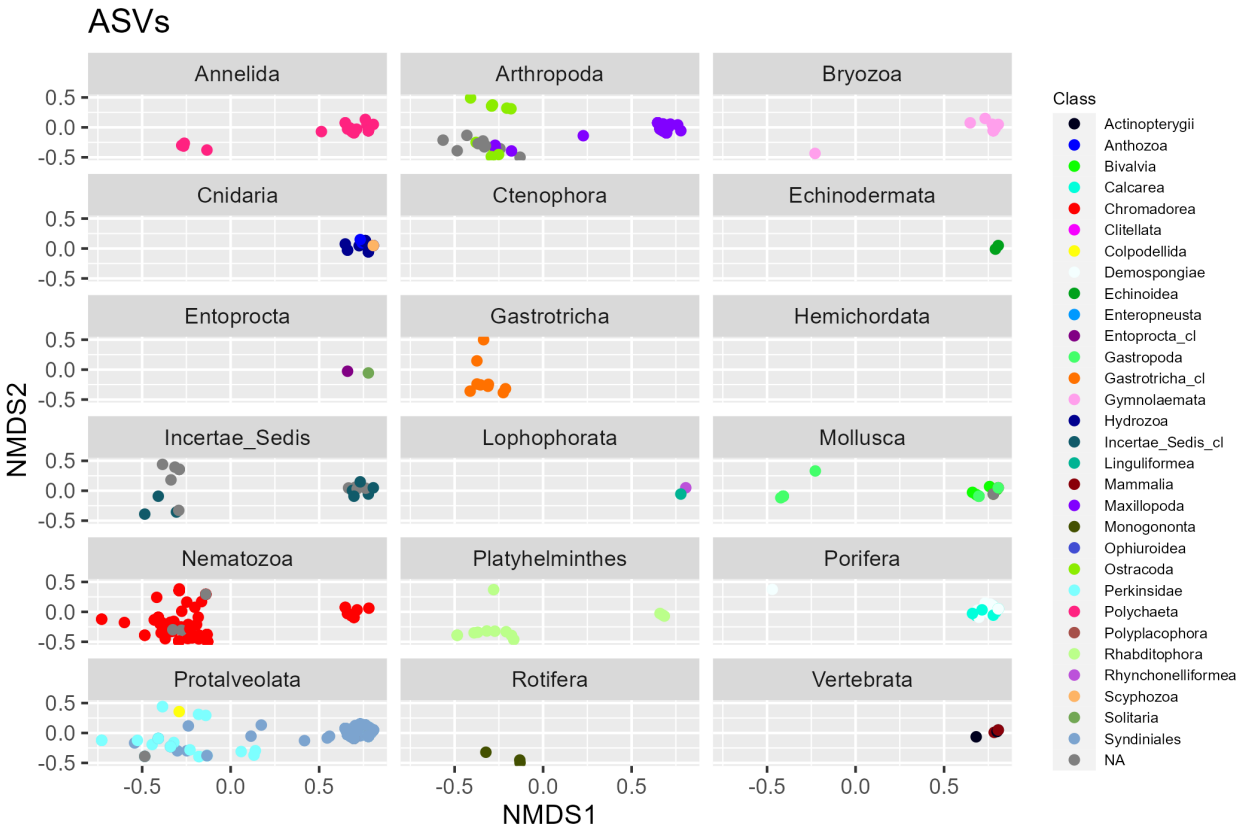


FIGURE 13 – NMDS of Metazoan phyla ordination accordingly to Class distribution.

2.4 DISCUSSION

2.4.0.1 ASV richness, Alpha Diversity and NMDS

In our work, we first detected markedly different communities and a consistently greater number of distinct ASVs in water compared to sediment. In our study, 4000 times more water than sediment was processed, so it is natural that this should have been the result. The same was reported by Shaw et al. (2016), who compared the technique of eDNA metabarcoding with different sources and traditional net trapping in a river system in southern Africa, Australia. In their study, it was shown that the column of water seemed more effective for detecting fish communities by eDNA than the sediment. The authors further argue that although they processed more water, this means of eDNA collection is more suitable to be applied due to the largest volume capacity that can be worked on. The also found that 1L of water per point was insufficient to detect less abundant taxa, but 5 L allowed a rate of 100% detection, which corroborates with the need for replicates and pooled samples when studying communities through the usage of eDNA using water as a source. In a study conducted to survey eukaryotic diversity in surface water samples in the coral reef tract within the Florida Keys National Marine Sanctuary (FKNMS) Sawaya et al. (2019) recovered 16,203 18S OTUS of 18S rRNA. In this work, we recovered 3,294 18S OTUs from water samples, a much lower number compared to what was found for FKNMS. However, the number in Sawaya et al. (2019)

represents the total amount of three different areas of the reef tract of four different year periods put together. Nevertheless Sawaya et al. (2019) had 52.8% taxonomically annotated OTUs for 18S rRNA, while in our data, 76.9% of the ASVs were annotated. In a case study in tropical harbors done in Tahiti (PEARMAN et al., 2021), a total of 4,980 ASVs were detected for the 18S rRNA gene dataset. Four locations were sampled for water eDNA, including two marinas and one port, with varying anthropogenic impacts. The number of ASVs recovered for this study was similar to what was recovered from our samples (3,294 ASVs). One hypothesis is that because of the anthropogenic impacts intrinsic to port areas, the biodiversity richness may decrease, which is reflected by the 18S gene marker. On the other hand, in Pearman et al. (2021) investigation, 68.8% of the ASVs could be assigned to the genus level, while our study could assign 32.8% to the genus. In contrast, however, in our work, the SILVA database was used solely for taxonomy assignment, while in Pearman et al. (2021) the data was classified against a different database, the PR2 (GUILLOU et al., 2012) using a two-step process (bootstrap cutoff of 0.9 and then 0.5 on taxonomic ranks equal and above family). This approach could also be tested on our data. On the other hand, in SILVA, duplicate hits have been removed from the database and only reference sequences with clear taxonomic classifications have been retained, allowing clear assignment.

Furthermore, Holman, Bruyn et al. (2019) found higher OTU richness in sediment community composition by comparing sediment and water samples in artificial coastal sites across the United Kingdom. Yet, in their work, the authors filtered 1,2 L|per site, less than half of the volume processed for Forno Port. Nonetheless, the number of 18S rRNA OTUs in water (aimed at retrieving eDNA from macroorganisms and microbial eukaryotes) in Rey, Basurko e Rodriguez-Ezpeleta (2020) work resulted in higher OTU richness and unique OTUs than other sampling methods, such as settlement plates, pairovet net and sieved sediment. This result is coincident with our work since the same outcome was observed when comparing ASV richness between water and sediment eDNA sources. While richness was similar among sediment samples, the NMDS (7) showed a significant difference in community composition between the spots, driven especially by spot 3, which clustered separately from the other two spots, closer to each other. The most abundant phylum among the non-metazoan eukaryotic group was *Diatomea*. Although among the three most abundant in Holman, Bruyn et al. (2019) (UK), Rey, Basurko e Rodriguez-Ezpeleta (2020) (Spain) and Nascimento et al. (2018) (Sweden), Pearman et al. (2021) the most abundant non-metazoan in their work was *Dinoflagelata*. Meanwhile, *Arthropoda* was the most abundant metazoan in all works. Our metabarcoding analysis shows that each source recovered a distinct subset of the port community as seen in other works (HOLMAN; BRUYN et al., 2019; KOZIOL et al., 2019).

2.5 CONCLUSION

In this work, it was demonstrated that the type of environmental sample in eDNA metabarcoding studies affects the measured community composition. This indicates that careful consideration of environmental sample type is needed when conducting eDNA survey in marine environments. Likewise, scrupulous source choices should be made in studies for assessing non-indigenous species. Moreover, it provides evidence that metabarcoding studies, when coupled with the appropriate gene marker, can provide meaningful genetic data information at the species level. This is important as early detection of NIS can be an important tool for the management and conservation of native species.

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3 NOTE - LOW GENETIC DIVERSITY OF *BALANUS TRIGONUS* IN THE ATLANTIC AND PACIFIC OCEANS

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ABSTRACT

The barnacle *Balanus trigonus* Darwin, 1854, is a prominent fouling organism on vessels with a broad native distribution in the Indo-Pacific region. It was introduced in the Atlantic Ocean Basin, where it was first recorded in the South of Brazil in 1867. *B. trigonus* ubiquitous occurrence makes them a useful model for studying the genetic diversity driven by ship biofouling transport. This work aims to investigate the genetic identity (COI gene) of specimens of *B. trigonus* from southeastern Brazil in comparison to sequences from different regions of the Pacific Ocean. The specimens from Arraial do Cabo, southeastern Brazil are conspecific to the ones from China, Taiwan, the Philippines, Guam, and Alaska. The haplotype network shows no geographic structure indicating that Pacific and western Atlantic populations of B. trigonus are connected and maintain a gene flow.

Keywords: *Balanus trigonus*, Cirripedia, Non-Indigenous Species (NIS), introduced species, genetic diversity, haplotype network.

3.1 INTRODUCTION

Many marine invertebrates have wide geographic distribution, occurring in different oceans, which would not be justified by their natural dispersal capacity (CARLTON; NEWMAN; PITOMBO, 2011). Historically, biogeographical boundaries in the marine environment were largely determined by marine currents, depth distribution, pelagic larval duration and reproduction type (PAPPALARDO et al., 2015; SEIXAS et al., 2017). However, shipping allowed some organisms to evade these natural barriers (ASHTON et al., 2016). Some invertebrates are dispersed around the world attached to hard surfaces like boat hulls or in ballast water in the form of cyprid or larvae (ZENETOS et al., 2005; CHEN; HØEG; CHAN, 2013). These dispersion processes have important evolutionary consequences in the long term, affecting speciation and biodiversity (RONCE et al., 2001; ROBINSON et al., 2011).

Cirripedes are some of the most recognizable and well-known fouling organisms in the world (CARLTON; NEWMAN; PITOMBO, 2011). *Balanus trigonus* Darwin, 1854, also known as the triangle barnacle, is a prominent fouling organism on vessels (ASHTON et al., 2016). It is a medium-sized barnacle, ranging from conical to nearly cylindrical in shape and reaching up to 25 mm in diameter (BUCKUP; BOND-BUCKUP, 1999). *B. trigonus* has a broad native distribution in the Indo-Pacific region (CARLTON; NEWMAN; PITOMBO, 2011). Currently, it occurs from southern tip of Africa, Red Sea, Japan, Australia and from California to Peru. It was introduced in the Atlantic Basin, where it was first recorded in South Brazil (Florianópolis, former Desterro Island) in 1867 (MÜLLER, 1868; ZULLO, 1992). Currently, the barnacle is recorded on the entire Brazilian coast (DE JANEIRO-BRASIL, 1994). It may have been introduced into the South Atlantic by ships from the Pacific and Indian Oceans sailing via Cape Horn and the Cape of Good Hope (ZULLO, 1992). Later, it may have been dispersed into central and northern parts of the Atlantic basin by whaling ships working South Atlantic and Antarctic waters in the late 1800s (ZULLO, 1992). Nowadays, it is recorded on both sides of the Atlantic and in the Mediterranean Sea, where it is also considered an introduced species.

Even though the historical retrospect record about *B. trigonus* is reasonably clear, there is little genetic information about this species. The few sequences available came from specimens from the species' native distribution. This work aims to investigate the genetic identity (COI gene) of specimens of *Balanus trigonus* from southeastern Brazil in comparison to sequences from different regions of the Pacific Ocean.

3.2 MATERIAL AND METHODS

Specimens of *B. trigonus* were collected in July 2017 at Cabo Frio Island, Arraial do Cabo, Rio de Janeiro, southeastern Brazil. They were collected manually through free autonomous dives with the help of a palette knife and tweezers and put into flasks with seawater. Then the individuals were photographed (Fig. 14), fixed and preserved on ethanol 96%.

The DNA of specimens was extracted using the DNeasy-Kit (Qiagen) following the protocol of the manufacturer. The cytochrome oxidase I (COI) gene was amplified through PCR using universal primers LCO1490: 5-GGTCAACAAATCATAAAGATATTGG-3 and HCO2198: 5-TAAACTTCAGGGTGACCAAAAAATCA-3 (FOLMER et al., 1994). Amplification was made in reaction with 2 µL of template DNA, 12 µL of master mix (1.5 mM $MgCl_2$), 1 µM of each primer, and ddH_2O to a total volume of 20 µL. PCR conditions were set as follows: 2 min. and 30 s. at 94 ℃ for initial denaturation, then 30 cycles of 30 s. at 95 ℃, 30 s. at 48 ℃, and 1 min. at 72 ℃, with final extension for 5 min. at 72 ℃. The amplification was then checked by electrophoresis on 2% agarose gel.

FIGURE 14 – Specimens of *Balanus trigonus* collected at the Cabo Frio Island, Arraial do Cabo, RJ, Brazil.

The DNA sequences were purified and then sequenced on Genetic Analyzer 3500 ABI (Applied Biosystems, California, USA) at the Central Lab of the Federal University of Pernambuco. Chromatograms were edited, and consensus sequences were generated in Geneious R6 (6.1.5 version) (http://www.geneious.com) (KEARSE et al., 2012) . The sequences were then analyzed on BLAST to check possible contamination.

COI sequences from China, Taiwan, the Philippines, Guam and Alaska were downloaded from GenBank (Fig. 15). A haplotype net was built using parsimony statistics (TEMPLETON; CRANDALL; SING, 1992) implemented on the program TCS (v. 1.21) (CLEMENT; POSADA; CRANDALL et al., 2000) with a ligation limit of 95%.

Species	Location	COI GenBank accession	Reference
B. trigonus	China	JQ035523	Yuan Shuai et al. (2012)
B. trigonus	China	JQ035524	Yuan Shuai et al. (2012)
B. trigonus	Philippines, Puerto Galera	KC138451	Chen et al. (2013)
B. trigonus	Taiwan, Matsu Island	KC138452	Chen et al. (2013)
B. trigonus	Guam, Para	KU204377	Ashton et al. (2016)
B. trigonus	Guam, Para	KU204332	Ashton et al. (2016)
B. trigonus	Alaska, Ketchikan	KU204234	Ashton et al. (2016)
B. trigonus	Alaska, Ketchikan	KU204228	Ashton et al. (2016)
B. trigonus	Alaska, Ketchikan	KU204222	Ashton et al. (2016)

FIGURE 15 – Individuals of *B. trigonus* included in the present study.

3.3 RESULTS

The dataset included a total of 12 specimens/sequences: three from Arraial do Cabo, Brazil; two from the Chinese coast; one from Taiwan (Matsu Islands); one from the Philippines; two from Guam and three from Alaska. Among the sequences examined (12) there is an overall high diversity (11) of haplotypes. The haplotypes are not distributed geographically, indicating that there is no regional population structure. Brazilian samples are divided into three different haplotypes, one of them shared with a sample from China (Fig. 16).

In this study we found that *Balanus trigonus* has a relative diversity of haplotypes, with 11 COI haplotypes among the 12 analyzed organisms (Fig. 16). The haplotypes are not distributed geographically, suggesting that there is no regional population structure. In Alaska, for example, 3 haplotypes were identified, all of them far apart. Our samples got also divided into 3 different haplotypes. While Brazil shares one haplotype with the Chinese Coast, the third is closer to one found in Guam.

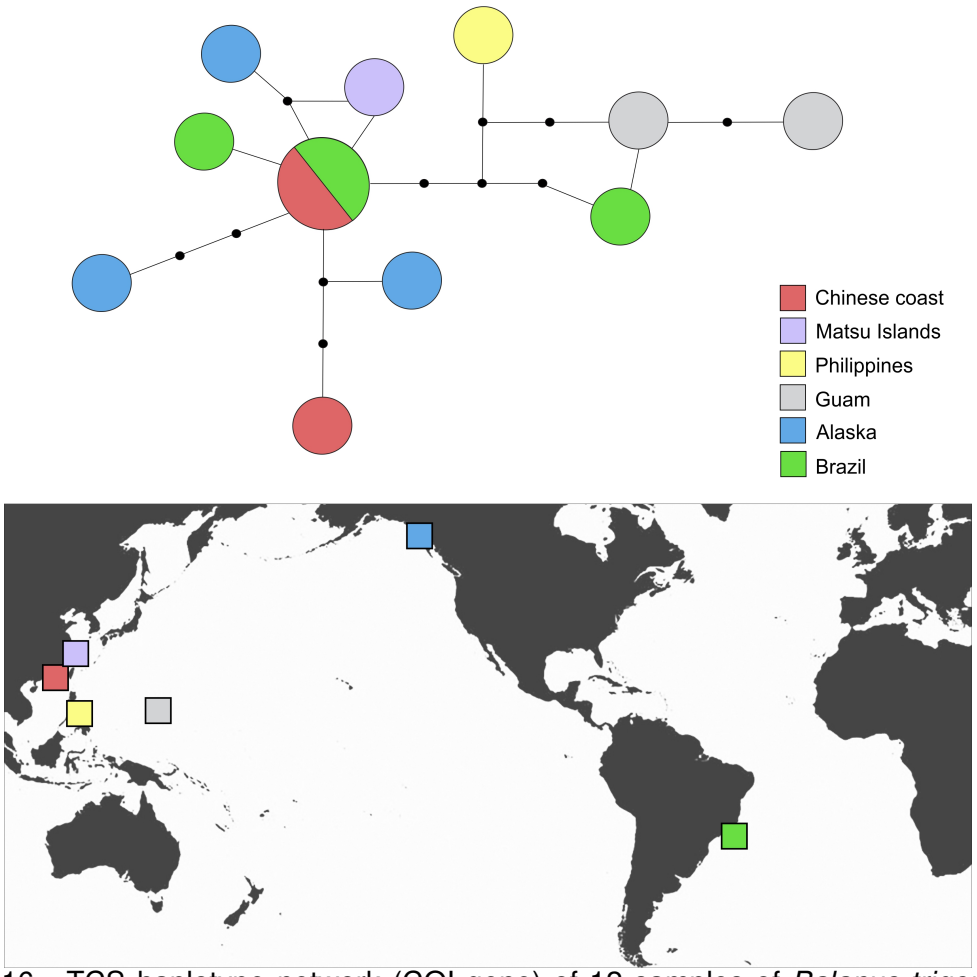

FIGURE 16 – TCS haplotype network (COI gene) of 12 samples of *Balanus trigonus*. Map indicates the procedence of the specimens.

3.4 CONCLUSION

In this work, we provide the first COI data for *Balanus trigonus* out of the Pacific Ocean. The specimens from Arraial do Cabo, southeastern Brazil, are conspecific to the ones from China, Taiwan, the Philippines, Guam, and Alaska. The haplotype network shows no geographic structure, and although the number of haplotypes is high, the number of mutations between them is small. Based on this preliminary data, the Pacific and western Atlantic populations of *B. trigonus* are connected and maintain a gene flow. Besides that, our data corroborate (CARLTON; NEWMAN; PITOMBO, 2011), who suggested that *B. trigonus* spread in the Atlantic Ocean is the result of multiple introductions.

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4 FINAL CONSIDERATIONS

The usage of DNA to decode patterns encrypted in nature sheds light on uncountable discoveries. Multiple fields within natural sciences have benefited from the information hidden in such molecules. In Smith (1937), the author underlies the nature of incremental advances in science and society. From studying evolutionary history, and passing through improved crops to elucidating climate tendencies and ecological trends by using eDNA, studying the DNA molecule brings many answers to our questions and necessities (TABERLET et al., 2012).

In this work, we acknowledge the capacity of this molecule to bring answers to marine ecosystem dynamics. We proposed to make a biodiversity assessment via environmental DNA in a region under the influence of the Cabo Frio upwelling phenomenon. We first accessed and compiled information on which factors module the eDNA dynamics in marine ecosystems. It was seen that its entrance, integrity and permanence go through myriad interactions that affect eDNA detection in different marine eDNA sources. Works that had been done prior to the advent of HTS sequencing must be considered under the scope of environmental DNA since they add valuable information about how DNA behaves once released into the environment. They answer questions that have been made under a limited scope of eDNA studies.

It is a difficult task to untangle biotic from abiotic factors and their influence on eDNA dynamics. However, once making premises clear and acknowledging possible interactions between factors, it is possible to organize them. For example, in the chapter "Environmental DNA (eDNA) in Marine Ecosystems – Entrance, Integrity and Persistence", we could infer that sunlight (UV) itself doesn't have an effect on short-term timing degradation. Rather, temperature effects may influence the degradation of eDNA by creating conditions for microbial-induced degradation. Furthermore, it was observed that seasonality also has an effect on the community recovered by eDNA. On the other hand, despite time-wise fluctuations, we concluded that eDNA signs are pretty localized community-wise, and mostly, eDNA is degraded fastly before it reaches other areas.

Therefore, eDNA is suitable for studies on the detection of invasive species detection, for example. In this regard, however, it is important to be aware of false positive detections and be critical of them prior to sparkling an alert to authorities about a specific detection. Moreover, it is possible to rely on eDNA information to make broader assumptions about communities of marine environments. Yet, adding abiotic information enhances the accuracy and insights in these type of studies and provide the possibility to expand studies on communities to a holistic level. Additionally, because eDNA interaction with the sediment cause longer permanence of eDNA in these environments, this eDNA

source holds a potential not yet explored. Investigations on ancient eDNA can be held in these environments and for investigations that encompass shorter periods. For instance, the time of the introduction of a certain species could be investigated and potentially traced back by using sedimentary eDNA. Still, the change in communities due to climate variation could also be accessed. Besides that, we compared eDNA sediment and water sources regarding their identification potential for a Eukaryotic genetic marker.

Additionally, we verified if an introduced species (*Balanus trigonus*) in Southeastern Brazil represents a distinct genetic lineage or population from its native distribution. In this work, we provide the first genetic data for *B. trigonus* collected from the Atlantic Ocean. The specimens obtained from Arraial do Cabo are similar to those from the Pacific Ocean. We also present the first results of the COI haplotype diversity of *B. trigonus* samples between the Indo-Pacific and Brazil and suggest that this species may have been introduced multiple times in the area. Besides that, in a holistic view, studying cryptic species is important as it serves as a bridge connecting taxonomy studies and genetic studies and provides data on evolutionary processes and macroevolutionary trends, including speciation, parallelism, convergence and stasis.

5 FINAL CONCLUSIONS

In this study, eDNA metabarcoding in an area of the South Atlantic Ocean was performed and methodological information regarding the usage of this technique was produced. We concluded that it was possible to unfold and organize information about the factors that module the eDNA dynamics in marine ecosystems, despite its myriad interactions. We also managed to compare eDNA sediment and water sources regarding their identification potential for the 18S rRNA genetic marker and how spatiality may affect the detection of biotic profiles. Moreover, by using as a model the invasive species *Tubastraea coccinea*, we determined that it is possible to detect non-indigenous species using eDNA and metabarcoding. We show that using environmental DNA may provide information as a biomonitoring tool with biotechnological applications. Additionally, we verified that the introduced species (*Balanus trigonus*) in Southeastern Brazil represent distinct genetic lineages and found evidence that it may have been introduced multiple times in the area. To our knowledge, this is the first eDNA study on marine environments on the Brazilian Coast. Thus, this study also contributes to advancing the eDNA field in the country.

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ANNEX

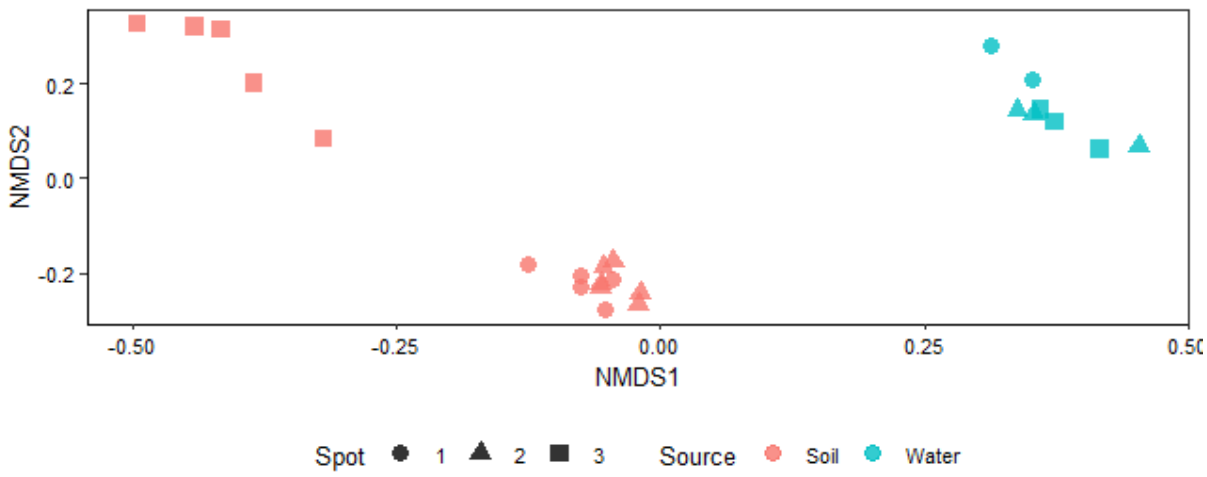

FIGURE 17 – NMDS no rarefection.

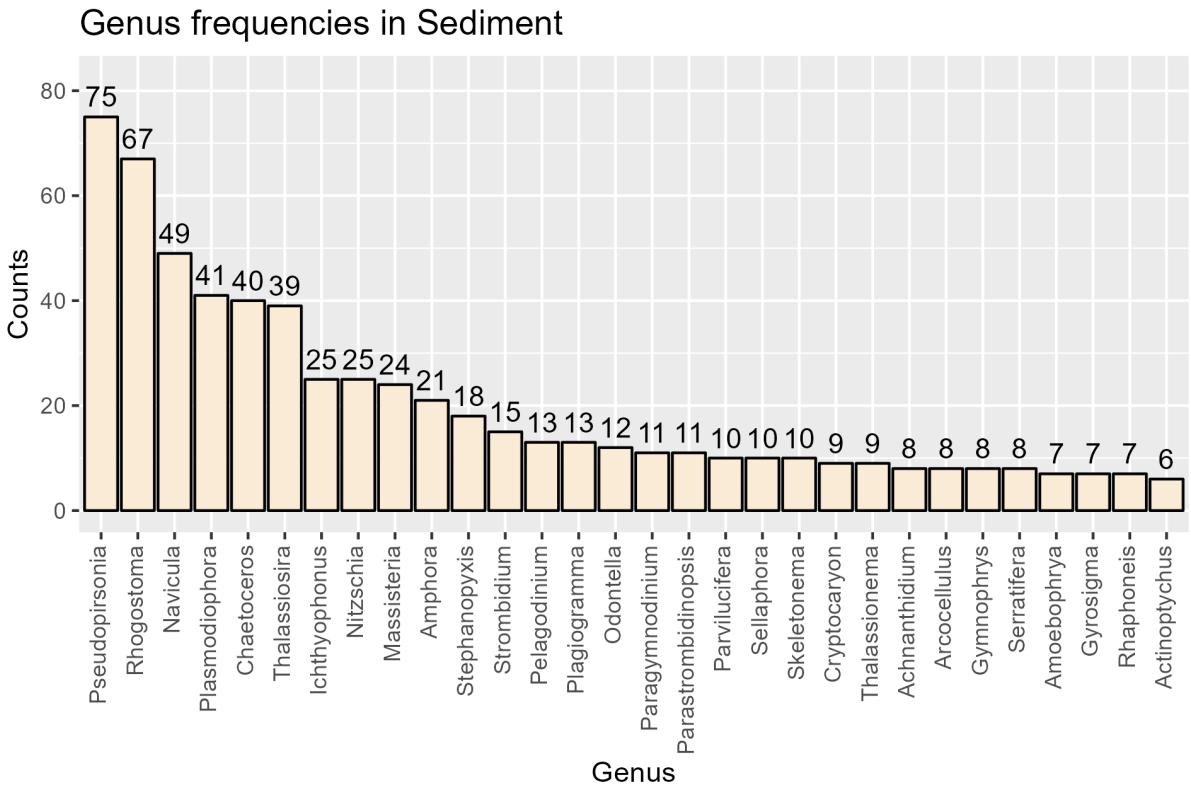

FIGURE 18 – Most frequent Genus in Sediment.

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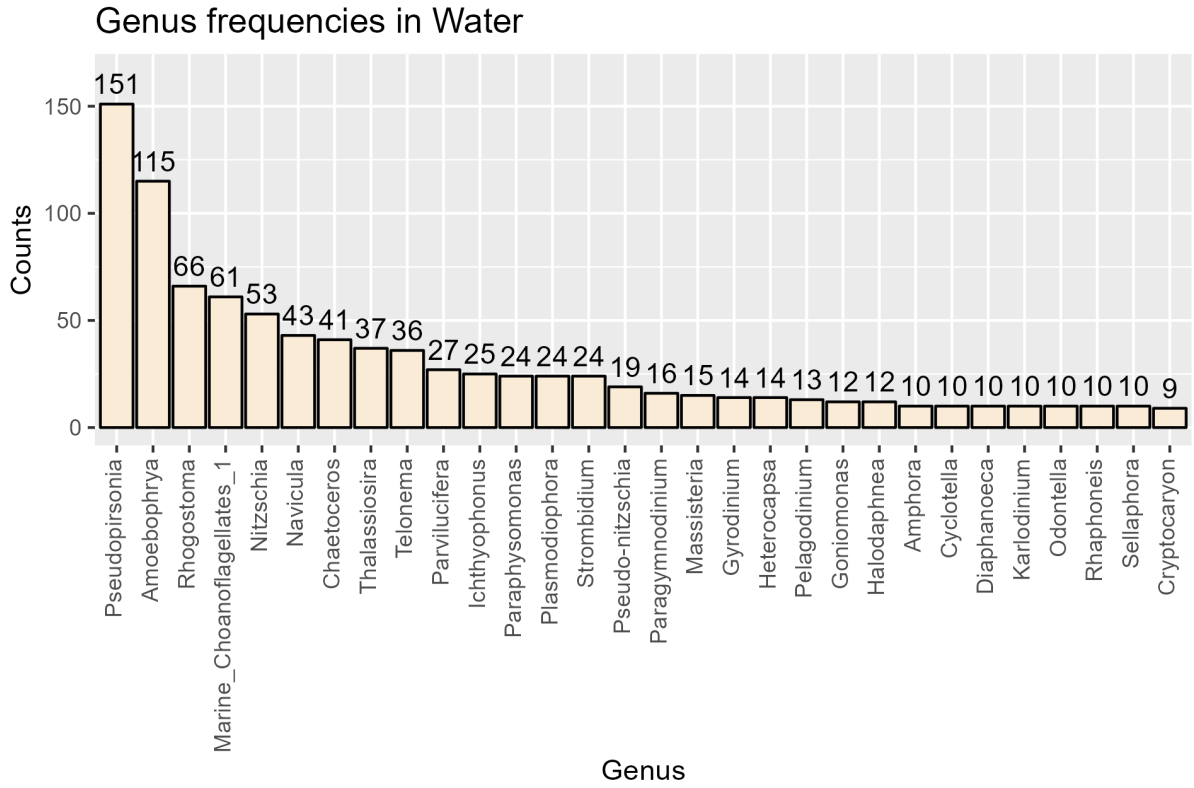

FIGURE 19 – Most frequent Genus in Water.

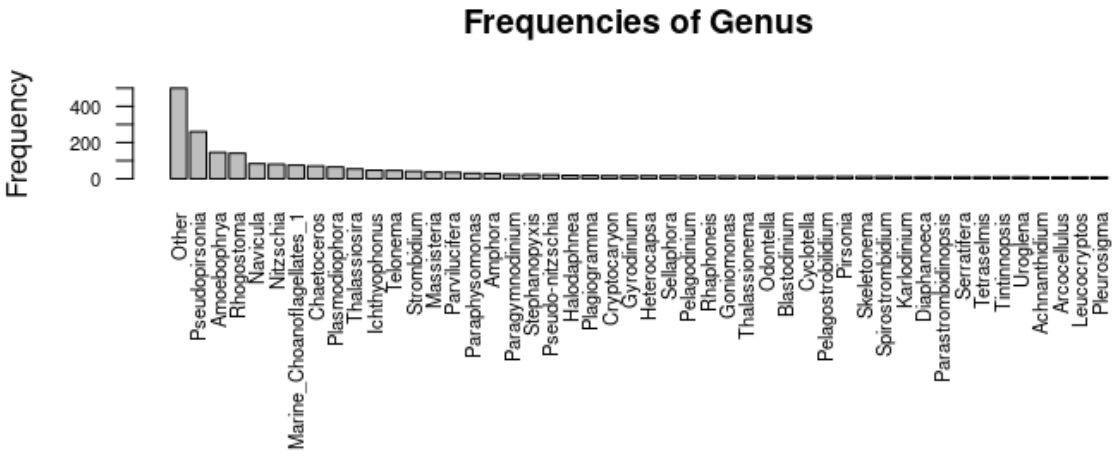

FIGURE 20 – Most frequent Genus, both in Sediment and Water.

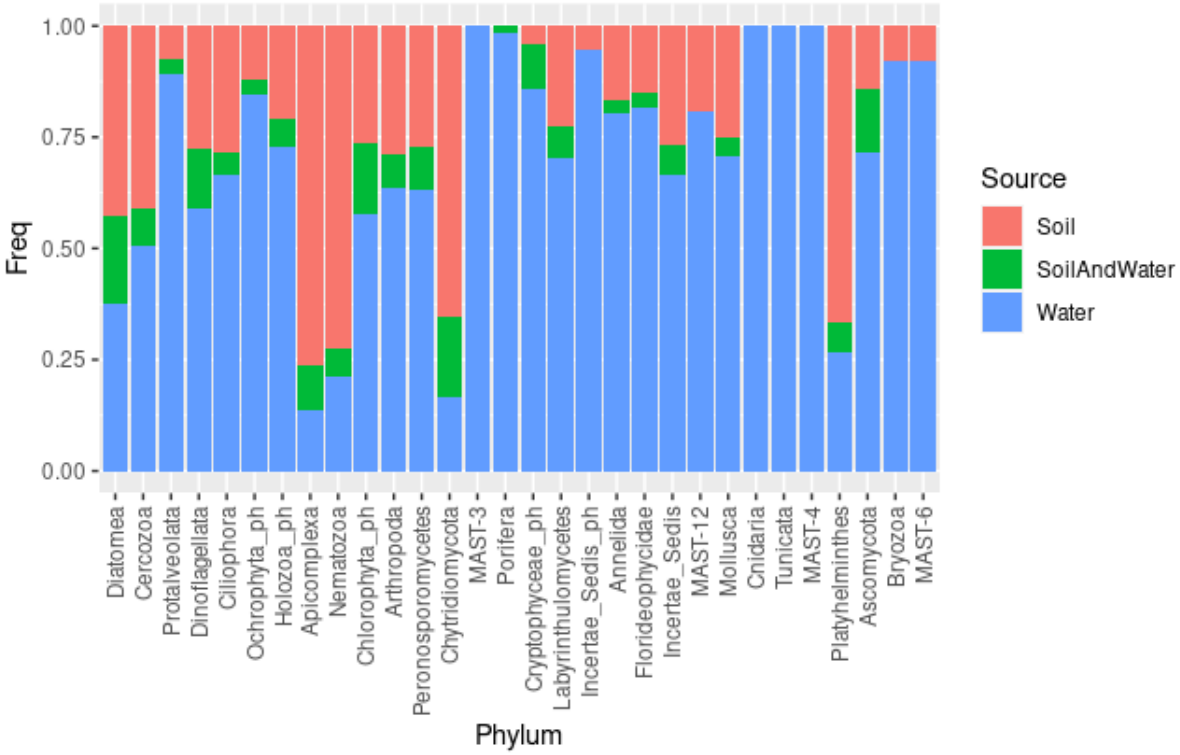

FIGURE 21 – Phylum Frequency in Soil, Water and both (normalized).

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