UNIVERSITÀ POLITECNICA DELLE MARCHE

DIPARTIMENTO SCIENZE DELLA VITA E DELL'AMBIENTE

Corso di Laurea Magistrale

Biologia Marina

STRUTTURA DI COMUNITÀ E ANDAMENTO TEMPORALE DEL FITOPLANCTON: UN CONFRONTO TRA MICROSCOPIA E METABARCODING

PHYTOPLANKTON COMMUNITY STRUCTURE AND TEMPORAL PATTERNS: A COMPARISON BETWEEN MICROSCOPY AND METABARCODING APPROACHES

Tesi di Laurea Magistrale
di:
Montali Giorgia

Relatore:
Chiar.mo Prof. *Accoroni Stefano*

Correlatore:
Chiar.ma Prof.ssa *Totti Cecilia Maria*
Dott. Neri Francesca

Sessione – Luglio 2024

Anno Accademico 2023 / 2024

ITALIAN ABSTRACT

Il monitoraggio della biodiversità della comunità fitoplanctonica è essenziale per rilevare la salute degli ecosistemi marini, anche in relazione ai cambiamenti climatici. In questo studio è stata analizzata la comunità fitoplanctonica della stazione costiera del sito eLTER Senigallia-Susak transect nel mare Adriatico settentrionale, combinando microscopia ottica e metabarcoding (considerando le regioni V4 e V9 del gene 18S rRNA e utilizzando SILVA e PR2 come database di riferimento), al fine di evidenziare i vantaggi e gli svantaggi di ciascun metodo.

Il metabarcoding è uno strumento straordinario per rilevare la composizione tassonomica del popolamento fitoplanctonico ma permette di ottenere solo la sua composizione in termini percentuali (abbondanza e biomassa relativa). D'altra parte, il metodo classico (microscopio ottico rovesciato) fornisce i valori di abbondanza e biomassa assoluti, ma ha un potere risolutivo molto inferiore nell'identificazione dei taxa.

Il metabarcoding ha rilevato 99 generi e 151 specie mai registrate nell'area di studio; d'altra parte, l'analisi al microscopio ha identificato 14 generi e 44 specie non trovate mediante analisi tramite metabarcoding.

Considerando che il metabarcoding misura la quantità di determinate sequenze di DNA presenti in un campione e non il numero delle singole cellule presenti, gruppi fitoplanctonici caratterizzati da nuclei più grandi (es. dinoflagellate), che generalmente possiedono più copie del gene 18S rRNA, risultano sovrastimati. Pertanto, in questo studio sono stati applicati fattori di conversione (CF) sia ai valori di abbondanza sia a quelli di biomassa, e i risultati ottenuti sono stati paragonati a quelli forniti dalla microscopia.

La composizione del popolamento in termini di abbondanza, ottenuta tramite analisi con il metebarcoding, dopo l'applicazione del CF è risultata comparabile con quella ottenuta attraverso il conteggio al microscopio: le fitoflagellate erano il gruppo dominante in tutto il periodo di studio, seguite dalle diatomee e dalle dinoflagellate. Al contrario, senza l'applicazione del CF, il

metabarcoding sovrastimava le dinoflagellate che risultavano dominanti in quasi tutto il periodo di studio, anche quando il gruppo dominante era quello delle diatomee.

Per quanto riguarda la biomassa, l'applicazione del CF ai valori ottenuti tramite il metabarcoding non ha prodotto risultati soddisfacenti quando confrontati con i valori ottenuti al microscopio tramite la stima dei biovolumi cellulari.

In conclusione, il metabarcoding è in grado di identificare un numero di taxa ben superiore a quello ottenuto tramite microscopia, ma richiede l'implementazione dei database di riferimento e controlli regolari nelle assegnazioni tassonomiche e nella nomenclatura, date le numerose lacune ancora presenti in alcuni dataset relativi a determinati taxa (in particolare i coccolitofori). Tuttavia, il metodo molecolare dovrebbe essere combinato con l'analisi al microscopio che fornisce la stima dell'abbondanza assoluta. In questo contesto, i fattori di conversione si sono rivelati strumenti preziosi che potenzialmente possono migliorare il confronto dei dati ottenuti tramite i due metodi, ma sono necessari ulteriori esperimenti per identificare i CF più adatti, specialmente per i dati di biomassa.

ABSTRACT

Monitoring the structure and composition of phytoplankton communities is essential to detect changes in the marine ecosystems, also related to climate changes. In this study, the phytoplankton community of an eLTER station in the northern Adriatic Sea was analyzed combining microscopy and metabarcoding (targeting the V4 and V9 regions of the 18S rRNA gene and using SILVA and PR2 as reference databases), to highlight the advantages and disadvantages in their use.

Metabarcoding revealed an undetected biodiversity, identifying 99 genera and 151 species that were never recorded in the study area but failed in revealing most coccolithophores. Microscopy analysis found 14 genera and 44 species that were not detected by metabarcoding.

Considering that metabarcoding measures the quantity of sequences present in a sample, larger cells (e.g., dinoflagellates), with more copies of the 18S rRNA gene, could be overestimated. Therefore, we applied conversion factors both for the relative abundances and biomasses. Our results showed that the application of conversion factor narrows the gap between the microscopy and metabarcoding methods in the estimation of the relative abundances. On the contrary, the use of CF for biomass decreases the similarity between metabarcoding and microscopy, compared to the data without the use of CF. In conclusion, metabarcoding is a powerful tool to improve the assessment of phytoplankton diversity, although it requires improvements, such as the implementation of reference databases, and regular checks in the taxonomy assignments and nomenclature. However, it should still be combined with microscopy to have a more comprehensive view of phytoplankton communities. In this context, CF are valuable tools that potentially enhance data comparison between the two methods, but further testing and refinement are necessary to identify the most suitable ones, especially for biomass data.

CONTENTS

INTRODUCTION

Adriatic Sea

The Adriatic Sea, an elongated basin in the northern Mediterranean, spans about 800 km in length and 150 km in width. Its bathymetry varies significantly, starting shallow at around 30 meters in the northernmost region, then deepening with a gentle gradient to 280 meters in the central Jabuka Pit, and reaching depths of approximately 1,270 meters in the Southern Adriatic Pit (Artegiani et al., 1997b; Mantziafou & Lascaratos, 2008; Oddo et al., 2005; Ovchinnikov IM, 1985). The western coasts are mostly sandy and with a regular shoreline, in contrast to the eastern rocky coast characterized by numerous islands (Artegiani et al., 1997a).

The circulation in the basin is primarily influenced by surface buoyancy flux, wind stress, and the exchange of energy and mass through the Strait of Otranto, which connects the Adriatic to the Northern Ionian Sea (Cushman-Roisin, 2013; Oddo et al., 2005). This exchange involves the inflow of warm, salty waters of Levantine origin at intermediate depths, known as Modified Levantine Intermediate Waters (MLIW), which are crucial for the formation of dense local waters (Cushman-Roisin, 2013). Levantine Intermediate Water and Ionian Surface Water enter the Southern Adriatic Pit, while a mix of Adriatic Dense Water exits through the Strait of Otranto, contributing to the Eastern Mediterranean Deep Water (Bensi et al., 2013; Poulain and Cushman-Roisin, 2001; Robinson et al., 2001; Rubino & Hainbucher, 2007). The formation and outflow of water masses in the Adriatic Sea vary annually, influenced by long-term thermohaline changes, local weather conditions, and broader climatic oscillations in the eastern Mediterranean (Grbec et al., 2002; Manca et al., 2003; Ursella et al., 2011; Vilibić and Orlić, 2002). The Adriatic Sea is highly influenced by riverine inputs, affecting nutrient balances, but also thermohaline circulation and dynamics, with consequences on surface buoyancy flux or local dense water formation processes (Cushman-Roisin, 2013; Verri et al., 2024; Vilibić et al.,

2016). For example, Verri et al. (2024) has shown that the decrease of river runoff, along with the enhancement of evaporation, is a major mechanism for the projected salinity increase in the Adriatic basin till 2050.

The Adriatic Sea consists of three distinct regional subbasins, each varying in latitude, bathymetry, physiographic characteristics, and physical, chemical, and biological features (Poulain and Cushman-Roisin, 2001).

The Northern Adriatic extends from the Gulf of Trieste until the 100-meter isobath, aligned with the Giulianova-Sibenik transect (Artegiani et al., 1997b), while initially the southern boundary was defined as the Ancona-Zadar transect (Buljan, 1976). It is a shallow sub-basin with an average depth of 30 m (Artegiani et al., 1997a).

The middle Adriatic extends from the 100-meter isobath until the Pelagosa sill. Its average depth is 140m. Here lies the Pomo Pit, which reaches a depth of 270 m, making it the deepest point of this sub-basin (Artegiani et al., 1997a).

The southern sub-basin extends from the Pelagosa sill to the Otranto strait and is characterized by a maximum depth of 1270 m in the southern Adriatic Pit (Artegiani et al., 1997a). This sub-basin is connected to the northernmost part of the Ionian Sea via the Otranto Channel, which represents the southern boundary of the Adriatic Sea. Compared to the other two basins, this one has a water volume four times larger.

Northern Adriatic Sea

The Northern Adriatic Sea (NAS) is shallow and characterized by a dominant cyclonic circulation (Artegiani et al., 1997b), with the Western Adriatic Current (WAC) flowing southward along the western coast and, carrying fresher, nutrient-rich waters from northern rivers (Campanelli et al., 2011; Marini et al., 2008), and the Eastern Adriatic Current (EAC) flowing northward along the eastern coast, bringing warmer, more oligotrophic waters from the southern sub-basin (Poulain & Cushman-Roisin, 2001). Nevertheless, this circulation shows high spatial and temporal variability, as demonstrated by

many studies (Artegiani et al., 1997b; Poulain & Cushman-Roisin, 2001; Rizzoli and Bergamasco, 1983) due to meteorological phenomena such as regional winds and surface heat, in addition to water exchanges, freshwater contributions especially from the Po River and their interplay with the overall Adriatic circulation at the southern edge (Artegiani et al., 1997b; Poulain & Cushman-Roisin, 2001; Rizzoli and Bergamasco, 1983).

The main winds impacting the NAS are the Bora (NE), Scirocco (SE), and Mistral (NW) (Artegiani et al., 1997b; Poulain & Raicich, 2001). The Bora is a cold, dry northeasterly wind known for its strong gusts, especially common in winter (Pasarić et al., 2007). The Scirocco is a warm, humid wind from the southeast and primarily affects the Adriatic in autumn and winter (Gačić et al., 2009; Grisogono & Belušić, 2009) . Both winds induce evaporation in the NAS, influencing the formation of dense water and impacting surface circulation (Hopkins, 1999; Orlic & Gacic, 1992; Poulain & Cushman-Roisin, 2001).

In the NAS, the contribution of the Po River is very important, as it supplies nutrient-rich waters with an average flow rate of 1500 m3 s−1; thereby creating a semi-enclosed circulation system (Grilli et al., 2005; Supić et al., 2000), reducing water exchanges with other Mediterranean regions. The Northern Adriatic Sea (NAS) is one of the most productive areas of the Mediterranean Sea. Due to the high riverine inputs, the NAS appears particularly sensitive to seasonal and long-term variations of nutrient loads, with effects on plankton communities. In the last decades, several changes in the trophic status of the Adriatic Sea have been highlighted. In the last decades, several changes in the trophic status of the NAS have been highlighted. From the 1970s to the mid- 1980s an increase in terms of productivity and a tendency to eutrophication was reported, followed by a reversal of the trend during the 1988-2000s period, by a return to higher productivity and a tendency to eutrophication in recent year (2007-2016), and by a return to oligotrophication in the more recent years (Giani et al., 2012; Mozetič et al., 2010; Totti et al., 2019) . These changes denote that the nutrient concentration in the NAS is largely

influenced both by the meteorological conditions and the environmental management practices (Grilli et al., 2020).

These projected tendencies underscore the urgent need for comprehensive monitoring of this ecologically and economically significant marine area.

MARINE PHYTOPLANKTON

Marine phytoplankton (from the Greek terms “phyton” or plant and “planktos” or wanderer), the autotrophic part of plankton, derive energy from photosynthesis and inhabit the well-lit surface layers of the ocean, extending to depths of up to 200 meters in clear waters. They include prokaryotes and eukaryotes encompassing various clades of life that acquired photosynthesis through primary or secondary endosymbiosis. Despite being mostly microscopic unicellular organisms ranging from 0.4 to 200 μm, phytoplankton contribute over 45% to the planet's annual net primary production (Field et al., 1998).

Apart from being fundamental to the aquatic food chain, phytoplankton are crucial to the biogeochemical cycles and the biological pump, with the latter involving the uptake of carbon dioxide from the atmosphere and its conversion into organic matter via photosynthesis, and ultimately depositing it into marine sediments (Giering et al., 2014; Longhurst and Glen Harrison, 1989) . Consequently, marine phytoplankton significantly influence Earth's climate through carbon fixation (Beardall et al., 2009; Raven, 1994).

Phytoplankton is considered a good indicator of marine ecosystem status and tendencies due to its ability to respond quickly and sensitively to changes in the aquatic environment (Castellani and Edwards, 2017; European Parliament, 2008), and the monitoring of the structure and composition of phytoplankton communities is essential for the assessment of water quality, the understanding of changes in trophic states, and the responses of marine ecosystem to climate and environmental shifts (Desrosiers et al., 2013; Machado et al., 2023).

When optimal conditions are present (i.e., nutrient concentrations, radiation and oceanographic conditions), phytoplankton blooms can develop. These processes are often visible as a discoloration of the water and can have significant effects on marine ecosystems, influencing water quality, oxygen availability, and the food chain. These blooms, in particular conditions, can be harmful (Harmful Algal Blooms, HABs) with negative consequences and risks to human health, marine ecosystems, aquaculture and economic/touristic activities, as, for example, they can cause water discolorations, mucilage aggregates, bottom anoxia or being characterized by toxin producing species (Berdalet, 2016; Glibert et al., 2018; Hallegraeff, 2004).

Hence, the development and adoption of precise, standardized, and cost-effective methods for monitoring spatial and temporal trends in marine phytoplankton communities are crucial and pressing concerns across various contexts (Campbell and Thompson, 2023).

Within eukaryotic phytoplankton, diatoms and dinoflagellates stand out as some of the most plentiful, diverse, and ecologically impactful groups (Simon et al., 2009). They show seasonal blooms in temperate and polar waters and are pivotal in primary production (Castellani & Edwards, 2017).

Other important groups of microalgae include haptophytes, cryptophytes, raphidophytes, and prasinophytes.

Diatoms

Diatoms belong to the Stramenopili group, included in the TSAR mega-assemblage (Strassert et al., 2019).Marine diatoms are unicellular organisms, living as solitary cells or in colonies with a range size between 2 μm and 2 mm (Halse & Syvertsen, 1996). The cell is encased in a rigid and transparent silica structure called the frustule, made by silica and organic components. Indeed, the frustule for a box structure, with a larger epitheca and a smaller hypotheca. Their cell contains a variable number of chloroplasts, with a slightly darker pyrenoid, derived for secondary endosymbiosis with a red alga (Graham et al., 1991). Main pigments are chlorophyll a, c1, c2, fucoxanthin, diadinoxanthin, diatoxanthin, beta-carotene.

Diatoms often exhibit one large vacuole (Hansen and Visser, 2019) aid in buoyancy regulation and nutrients storage. They are primarily photoautotrophic, with some facultative heterotrophs and fewer than ten obligate heterotroph species.

Modern classification divided diatoms into two clades, the first including radial centrics and the second polar centrics and pennates. The diatoms identified as radial centrics appear to be the oldest group (Kooistra et al., 2007). They exhibit radial symmetry with a central annulus and ribs radiating from the center. Their outline is circular or nearly circular and lacks paired pore fields (Theriot, 2012). Bi- or multipolar centric diatoms are slightly younger (Kooistra et al., 2007). Their external morphology appears elongated, triangular, or star-shaped, with pore fields and setae toward the valve edge, thus imparting various symmetries to the cell (Theriot, 2012). Finally, pennate diatoms, with elongated morphology, constitute a relatively younger group (Kooistra et al., 2007). They are divided into monoraphid, biraphid, and araphid types. The raphe represents a structural weakness in the valve, creating a longitudinal slit that is useful in preventing longitudinal valve splitting under turgor pressure and other stresses and for movement through mucilage expulsion (Round, 1990).

Diatoms have a diplontic life cycle with diploid vegetative cells and haploid gametes (Chepurnov et al., 2004). Agamic reproduction occurs through mitosis, generating two daughter cells. This process is semi-conservative because each daughter cell receives one of the thecae of the mother cell and autonomously produces the missing one, which is placed internally to that of the mother (representing the new hypotheca). Therefore, one of the daughter cells will be smaller. As the agamic reproduction goes on, the size of diatom population decreases. When the cell reaches a size between 42% and 59% of the maximum cell size (Sarno et al., 2010), sexual reproduction restores the maximum cell size. Sexual reproduction requires two gametes (haploid), produced by meiosis, fuse to form a zygote which can then develop into a new diploid cell. This fusion can be oogamous (male gamete is small and motile while the female gamete is large and non-motile; typical of radial and multipolar Centrics),

isogamous (gametes are morphologically similar; typical of raphid pennates) and anisogamous (gametes are morphologically different).

The main environmental factors enhancing diatom growth are nutrient availability (particularly DIN) and mixing conditions. Under specific conditions (seasonal changes, nutrient scarcity, temperature, light, pH), they can produce resting spores.

Dinoflagellates

Dinoflagellates belong to the Alveolata group, included in the TSAR mega-assemblage (Strassert et al., 2019). They are unicellular organisms living as single cells or colonies. Dinoflagellates acquired their plastid mainly through a secondary endosymbiosis with a red alga. Indeed, roughly half of dinoflagellate species loss secondarily their plastids becoming heterotrophic. Typical dinoflagellate plastids contain chlorophyll a and c, beta-carotene, and peridinin (Hackett et al., 2004; Wang & Lan, 2018). Dinoflagellates have a cell envelop called amphiesma, made of alveoli immediately under the plasmalemma. Alveoli can contain cellulosic plates (thecate dinoflagellates), or alveoli can be almost free of cellulose (naked dinoflagellates). Their cell body can have various shapes: rounded, ovoid, or angular and irregular. Thecate species often have horns, spines, or other appendages. The typical dinoflagellate morphotype (dinokont) shows median groove called the cingulum encircles the cell body, dividing it into an epitheca and a hypotheca, and a longitudinal groove called the sulcus runs perpendicular to the cingulum, in the hypotheca. Two heterodynamic flagella emerge from the ventral part of the cell, with one anchored to the cingulum (transverse flagellum) and the other in the sulcus (longitudinal flagellum) (Graham et al., 1991).

Dinoflagellates are mostly haploid and reproduce vegetatively through mitosis. Sexual reproduction occurs through both isogamy and anisogamy, resulting in the formation of a zygote that, in certain species, develops into resting or temporary cysts. Meiosis occurs at zygote (or cyst) germination.

The main environmental factors enhancing dinoflagellate growth are nutrient availability, particularly dissolved inorganic nitrogen and phosphorus (DIN and DIP) (Dagenais-Bellefeuille & Morse, 2013).

Some species of dinoflagellates can produce bioluminescence during the night (Marcinko et al., 2013). They can flash when stimulated mechanically, chemically, or electrically, and they can also glow dimly spontaneously late at night. *Pyrocystis noctiluca* is one of the most bioluminescent dinoflagellates, and it can cause this phenomenon in equatorial regions.

In addition, a few of dinoflagellates can produce biotoxins implicated in human poisoning such as Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP), and Ciguatera Fish Poisoning (CFP). These toxins can be transferred to humans through the food chain, direct contact, or aerosols, causing biointoxications (Gopalakrishnakone et al., 2016).

Haptophytes

Haptophytes belong to the Haptista supergroup. Most haptophytes are unicellular, photosynthetic flagellates, although some have various other stages such as coccoid, colonial, amoeboid, or filamentous. Most haptophytes possess a distinct filamentous appendage called the haptonema, located between the two flagella, that is useful for obstacle avoidance, food capture, and attachment. Species with longer haptonema are more often phagotrophic.

They typically contain one or two chloroplasts, each with an immersed or bulging pyrenoid (Sáez, 2004). The main pigments present are chlorophyll a, chlorophyll b, chlorophyll c2 and/or c3, and the most important accessory pigment is 19'-hexanoyloxyfucoxanthin.

Phylogenetically, Haptophyta constitute a well-defined group, divided into two classes: Pavlovophyceae and Prymnesiophyceae (Coccolithophyceae) (Eikrem et al., 2016). The Prymnesiophyceae (Coccolithophyceae) are characterized by two equal smooth flagella and organic scales that may or may not be calcified (coccoliths). Coccoliths, flagella and haptonema can be absent or present depending on the life stage. Instead, the Pavlovophyceae have two unequal flagella with mastigonemes, a haptonema, organic scales and do not possess coccoliths.

Coccoliths can be complex crystal units (heterococcolith) or minute euhedral crystallites (holococcoliths). Coccolith layers continuously detach from the cell and new coccoliths are produced and coccoliths are useful for cell protection, buoyancy regulation and have biochemical convenience. Cells with coccoliths are called coccosphere (Heimdal, 1997) and they can be monomorphic (one type of coccoliths), dimorphic (two types of coccoliths), monothecate (one layer of coccoliths) and dithecate (two layers of different coccoliths).

Haptophytes can reproduce both asexually and sexually. Asexual reproduction can occur through a form of open mitosis (where the nuclear membrane temporarily disintegrates), typically taking place at night. In sexual reproduction, haplodiplontic cycles are common, and the two generations (haploid and diploid) can differ in morphology and type of coccoliths.

Coccolithophores are common in tropical seas as warm waters are supersaturated of CaCO3, necessary for coccolith formation.

Cryptophytes

Cryptophytes (Cryptista supergroup), are unicellular and flagellate microalgae common in marine and freshwater. They contain a plastid derived from secondary endosymbiosis with a red alga. Some species are autotrophic, thanks to the presence of chlorophylls a and c2, carotenoids, phycobilins (phycoerythrin, or phycocyanin), while others are heterotrophic (Pereira & Neto, 2014), mixotrophic, and auxotrophic.

Externally, they possess a proteinaceous envelope called periplast, composed of polygonal plates arranged above and below the plasmalemma. Morphologically, the cell is asymmetric with a convex dorsal part and a flat ventral part. The upper anterior part is obliquely truncated and has an anterior depression (vestibule) from which two flagella emerge. These flagella consist of one longer (pleuronematic) and one shorter (stichonematic), both covered with stiff flagellar hairs and rosette scales, enabling movement through a reversal of thrust (Hibberd et al., 1971).

Typically, reproduction is agamic, and cytokinesis occurs through an infolding in the posterior part. Only some species have sexual reproduction, sometimes with a dimorphic life cycle that differs in terms of ploidy (haploid or diploid), size, periplast structure and flagella.

Growth is better at relatively low temperature values, but they can live also in extreme environments such as soil, snow, underground waters.

They do not represent a homogeneous functional group because in the same area different species occur in different periods e some could make vertical migrations (within 5 m) to avoid grazing.

Raphidophytes

Raphidophytes belong to the Stramenopiles group, included in the TSAR mega-assemblage (Strassert et al., 2019).

They are unicellular and flagellate microalgae with a big size (30–80 μm), their morphology can be ovoid to pyriform, and some are dorsoventrally compressed. Raphidophytes do not have a cell wall but they are characterized by the presence of two flagella. Both originate from a shallow ventral groove at the cell apex (gullet); the longer flagellum possesses mastigonemes (pleuronematic) and is pointed forward during swimming, while the shorter one is smooth, pointed backward and less mobile (Horiguchi, 2016).

Raphidophytes have numerous ellipsoidal, peripheral plastids that are green, yellow-green, or yellow-brown, and the main pigments present are chlorophyll a, chlorophyll c1 and c2, beta-carotene, fucoxanthin, and violaxanthin. They are characterized by large and conspicuous nucleus with high chromosome number (up to 97 couples).

Their reproduction occurs through closed mitosis and some species can form resting cysts. Sexual reproduction has only been described in freshwater species (Horiguchi, 2016).

In some genera (e.g*., Chattonella, Fibrocapsa*), massive blooms with water discoloration can occur (Throndsen, 1997). In addition among them some species are toxic. Generally, blooms especially in

semi-enclosed bays during the summer when waters are stratified. They may form cysts that rest in sediments (Cucchiari et al., 2010).

Prasinophytes

Prasinophytes belong to the Chlorophyta group (Archaeplastida supergroup) and are among the oldest microalgae. They exhibit diverse cellular morphologies, including pyramid-shaped, spherical, or ovoidal forms, which can be rounded or bilaterally compressed.

Typically, their cells are small with a diameter of less than 3 μm, classifying them as picoplankton (Stockner and Antia, 1986). They contain one or two chloroplasts (primary origin) that house chlorophylls a and b, along with accessory pigments such as beta-carotene and uriolide (Guillou et al., 2004). Most species have cells and flagella covered by one or more layers of intricate organic scales (Throndsen, 1997). The flagella, located at the cell apex or laterally, can vary in number from one to sixteen.

Taxonomically, they are divided into nine clades, identified through studies of evolutionary relationships based on the 18S ribosomal RNA gene sequence (Guillou et al., 2004; Viprey et al., 2008).

Prasinophytes are primarily autotrophic and undergo a complex life cycle involving alternation between a flagellated phase and an immobile phase known as phycoma (Graham et al., 2010). Reproduction is generally asexual, with sexual reproduction rarely observed (De Clerck et al., 2012).

Phytoplankton communities in the northern Adriatic Sea

Over the past decade, several long-term studies have been conducted on phytoplankton communities in the Adriatic Sea highlighting significant interdecadal shifts in phytoplankton communities, attributed to changes in climate and meteorological conditions (Bernardi Aubry et al., 2021; Cabrini et al., 2012; Marić et al., 2012; Neri et al., 2022; Totti et al., 2019).

The NW coastal areas have been characterized by high phytoplankton biomass due to high concentrations of inorganic nutrients (particularly nitrogen and phosphorus) along with the presence of particulate organic matter derived from the Po River and carried by the Western Adriatic Current. Typically, the northwestern coastal waters experience a prominent diatom winter bloom dominated by *Skeletonema marinoi*, resulting in annual maximum of abundance and biomass, followed by traditional spring and autumn blooms, while in the eastern part of the NAS, spring and autumn blooms are more common (Aubry et al., 2004; Cabrini et al., 2012; Cerino et al., 2019; Marić et al., 2012; Totti et al., 2019, 2005). The winter bloom is supported by a transient limitation of the mixed layer depth primarily due to sunny weather and the presence of thin freshwater surface layers (Fischer et al., 2014; Townsend et al., 1994). A reduction in the mixed layer depth can enhance phytoplankton growth by increasing the availability of light and nutrients in surface waters, thereby promoting their development and proliferation. However, the relevance of these effects in the coastal areas of the Adriatic Sea is supposed not to be so strong, considering the shallowness of the water column (12 m) and the homogeneity of the vertical distribution of phytoplankton throughout the year. Whereas in spring and autumn phytoplankton blooms are primarily influenced by rainfall and river runoff (Totti et al., 2019), which carry nutrients such as nitrates, phosphates, and other compounds from land to the sea, enabling the growth of microalgae as essential elements. In contrast, offshore, nutrient and organic matter concentrations are lower (Boldrin et al., 2005), and the highest phytoplankton abundances are observed in the summer season, due to the spreading eastward of riverine waters in stratified conditions (Neri et al., 2022).

Over the past few decades, the phytoplankton communities in the NAS have undergone notable changes, with shifts in seasonal patterns and species compositions, as changes in the physico-chemical parameters are reflected in new tendencies in the phytoplankton communities.

From 1970 to the mid-1980s, eutrophication-related phenomena (HABs, bottom anoxia) were associated with increased productivity (Boni et al., 1983; Degobbis et al., 2000; Honsell et al., 1992),

and until 1991, many phytoplankton blooms, especially of dinoflagellates, occurred in spring and summer. For these reasons, in 1982, a law approved the restriction of phosphorus in household detergents to reduce the environmental impact of phosphates that contribute to the eutrophication of seas and water bodies (Law No. 62 of March 5, 1982). As a result of this decision, the dinoflagellate blooms have almost disappeared. Indeed, between the late 1980s and early 2000s, large mucilage aggregates appeared in the northern Adriatic Sea, associated with a combination of factors such as an unbalanced nutrient ratio (high N and low P) and reduced circulation (Degobbis, 1999; Giani et al., 2005; Russo et al., 2005; Totti et al., 2005; Turk et al., 2010). The nutrient imbalance was caused by reduced phosphorus inputs following the European directive, creating a phosphorus-limited environment. The decrease in phosphorus stressed the phytoplankton, inducing them to secrete photosynthetic products (PER), leading to marine snow formation.

Furthermore, in the years 2000-2007, prolonged droughts, which led to a strong decrease of the riverine inputs caused a general decreasing trend in chlorophyll-a and changes in the seasonality of the blooms (Bernardi Aubry et al., 2021; Cabrini et al., 2012; Cozzi et al., 2020; Djakovac et al., 2012; Giani et al., 2012; Marić et al., 2012).

Recent extreme events, marked by increased temperatures and reduced rainfall, have significantly decreased riverine inputs, leading to a shortage of nutrients crucial for phytoplankton growth; this has created a general oligotrophication of the Northern Adriatic coastal waters (Cerino et al., 2019; Cozzi et al., 2020; Totti et al., 2019). Consequently, it has been observed a decline in the typical winter bloom of *Skeletonema* (Cerino et al., 2019), and a decrease of phytoplankton biomass.

Phytoplankton growth became mainly driven by recycled nutrients instead of continental sources, particularly for nitrogen, while dissolved inorganic phosphorus remains relatively scarce due to limited availability in the basin and the recycling processes playing a crucial role in its regulation (Cozzi et al., 2002).

Phytoplankton plays a central role in the health and productivity of marine ecosystems, in addition to being considered a sensitive indicator of speed and severity of global climate change (Widdicombe et al., 2010). Cloern & Jassby, (2010) proposed the hypothesis that year-to-year variability of phytoplankton is a response to anthropogenic activities or shifts in the climate system.

Extensive research over extended periods is crucial for understanding the dynamics and cycles of phytoplankton communities, given their potential to undergo rapid transformations within mere weeks (Smetacek & Cloern, 2008).

Microscopy method for the study of phytoplankton

Traditional phytoplankton surveys have relied mainly on microscopic observations and morphological diagnostic features using light microscopes (Edler et al., 2010). The Utermöhl method represents the most common technique for phytoplankton enumeration. This method involves the sedimentation of a water sample aliquot (5 to 100 ml) in a chamber, allowing phytoplankton cells to settle at the bottom, ensuring that cells uniformly distribute in the counting chamber. Then a known area of the counting chamber (transects, casual fields) is analyzed under the inverted microscope to identify and count cells. Finally, the abundances are expressed as cells per liter (Edler et al., 2010).

However, an accurate identification using light microscopy is time consuming and relies on highly trained taxonomists. Moreover, the identification of taxa could be affected by different aspects: (i) the operator itself, (ii) the undetectability of many diagnostic morphological features or their variations in different environmental conditions, (iii) the presence of cryptic species (i.e., species that appear morphologically similar are actually genetically distinct) (Bernardi Aubry et al., 2017; Roselli and Basset, 2015; Segura et al., 2013).

Cryptic species, although morphologically indistinguishable, show significant differences in habitat, physiology, and life cycle, with important ecological implications (Amato et al., 2007). For example, among the *Pseudo-nitzschia delicatissima* group, 36 cryptic and pseudo-cryptic species have been

described (Amato and Montresor, 2008; Gai et al., 2018; Lundholm et al., 2012, 2006, 2003; Quijano-Scheggia et al., 2009). The importance of knowing the existence of cryptic species is also significant from an applied perspective because some of them can be toxic.

For these reasons in the last decade, the new field of environmental DNA (eDNA) metabarcoding (Didaskalou et al., 2022; Marinchel et al., 2023) has emerged as a revolutionary approach to biodiversity research (Marinchel et al., 2023; Taberlet et al., 2018).

Metabarcoding method for the study of phytoplankton

eDNA metabarcoding represents a cutting-edge approach for biodiversity assessment. It involves extracting DNA from environmental samples followed by amplification using general or universal primers in polymerase chain reaction and sequencing using next-generation sequencing technology, which generates thousands to millions of reads (Barnes and Turner, 2016; Deiner et al., 2015; Ficetola et al., 2008). This monitoring method blends advanced molecular techniques with traditional field ecology, enabling the determination of species presence and assessment of overall biodiversity (Ruppert et al., 2019). Although still in its early stages, it holds significant potential to revolutionize biodiversity surveys in the molecular era, despite the challenges and obstacles it faces (Andersson et al., 2013; Ruppert et al., 2019; Xiao et al., 2014).

Plankton sample community assessment using DNA "barcodes" began in the 1990s, but experienced significant growth with the introduction of high-throughput sequencing in the 2000s (Fuhrman et al., 1993; Giovannoni et al., 1990; López-García et al., 2001). Metabarcoding has revolutionized the study of plankton diversity, offering an increasingly cheaper and time-efficient approach, (i) enabling the simultaneous analysis of numerous samples, (ii) providing the capability to detect even the most uncommon members of a community and (iii) identifying diverse lineages in a single endeavor (Needham, 2016; Sogin et al., 2006; Wurzbacher et al., 2017). Its effectiveness has been demonstrated by numerous studies conducted across different temporal and spatial scales in both freshwater and

marine environments (Caporaso et al., 2012; de Vargas et al., 2015; Debroas et al., 2017; Ruiz-González et al., 2019).

To entail taxonomic informativeness, metabarcoding requires the establishment of a "good barcode", where the DNA region should mutate at an appropriate rate to distinguish between species, as many biomonitoring programs require species-level identification (van der Loos & Nijland, 2021). However, it also necessitates conserved primer binding areas for the detection of all organisms in the sample (Carugati et al., 2015). Primer choice significantly influences biodiversity assessment results, as complete universality may compromise resolution and limit assessment depth, while limited universality could exclude vital groups and introduce biases (Hadziavdic et al., 2014).Another aspect that could influence metabarcoding results is the choice of the most efficient bioinformatic pipeline. One common approach involves grouping reads into clusters known as Operational Taxonomic Units (OTUs) based on a certain level of genetic variation. One of the common algorithms that group reads in OTU (97% similarity) is USEARCH-UPARSE (Edgar, 2016; Bukin et al., 2023). Another algorithm implemented in DADA2 (Bukin et al., 2023; Callahan et al., 2016) and USEARCH-UNOISE3 (Bukin et al., 2023; Edgar, 2016) identifies unique genotypes (ASV - amplicon sequence variant, ZOTU - zero-radius OTU) with sequencing error correction in amplicons. Both approaches are widely used in the analysis of sequencing results (Bukin et al., 2023).

Metabarcoding studies require a comprehensive and well-maintained database of reference DNA sequences for identified organisms, which is currently a major challenge due to the limited availability of sequences for certain groups (Kembel et al., 2012). Additionally, variations in copy number associated with ribosomal DNA genes can impact abundance estimates, which could contribute to discrepancies between metabarcoding and microscopy results in some cases (Stoeck et al., 2014). For example, in many studies over-representation of dinoflagellates has been related to large genome rather than real abundance values (Piredda et al., 2017; Prokopowich et al., 2003). Technical biases

introduced during DNA extraction (Roh et al., 2006) or PCR amplification could further influence these estimates (Gonzalez et al., 2012).

Eukaryotic cells can have many copies of the 18S rRNA gene, and this number can vary significantly between species and groups. During the DNA or RNA sequencing process, the number of reads obtained may reflect the number of gene copies present in the original sample. Considering that the capability of metabarcoding to quantify the relative abundance of species is not yet clear, using a conversion factor can better align microscopic analysis with metabarcoding. In this way, the data are normalized, and the number of reads obtained during sequencing can be considered proportional to the number of 18S rRNA gene copies present in the sample cells. The gene copy number (GCN) can be correlated with cell biovolume (Vasselon et al., 2018), relative abundance, and biomass (Lapeyra Martin et al., 2022).

Additionally, the development of DNA metabarcoding, which utilizes high-throughput sequencing and high quality sequence databases like PR2 (Guillou et al., 2013), has enabled rapid characterization of eukaryotic communities (Deiner et al., 2021; Fonseca et al., 2022; Pearman et al., 2020; Smith et al., 2017; Valentini et al., 2016) and the establishment of baseline datasets for marine eukaryotic microalgal communities at a large scale (Le Bescot et al., 2016; Piredda et al., 2018; Wang et al., 2022) Among the regions used for metabarcoding, the plastid marker rbcL is commonly used in diatom metabarcoding due to its ability to distinguish between taxa at both the species and intraspecific levels (Turk Dermastia et al., 2023).

The 18S rRNA gene is commonly used to investigate eukaryotic diversity and community structures (López-García et al., 2001; Moon-van der Staay et al., 2001) in particular small hypervariable regions like V9 (150 bp) or V4 (450 bp) (Amaral-Zettler et al., 2009).

In the study of Stuart et al. (2024), V4 and V9 generated ASV abundances were compared: the V9 region generated a higher number of raw reads, captured more diversity from all high level taxonomic

groups and was more closely aligned with the community composition determined using light microscopy, while the V4 region did resolve more ASVs to a deeper taxonomic resolution within the dinoflagellates, but did not effectively resolve other major taxonomic divisions.

The use of metabarcoding is expanding but still requires clarification and the enrichment of databases with taxonomic lists. Despite the challenges, this technique offers significant potential to improve our understanding of marine phytoplankton. With further methodological developments and increased collaboration among researchers and institutions, metabarcoding could become a valuable tool for marine ecological research, contributing to more effective preservation and management of ecosystems.

AMIS OF THE THESIS

The objective of this thesis was to compare the diversity, relative biomass, and abundances of the phytoplankton community obtained by microscopy and metabarcoding at the eLTER Senigallia coastal station in the northern Adriatic Sea. DNA metabarcoding analysis was performed by combining different 18S regions (V4 and V9) and reference databases (PR2 and SILVA) to evaluate the results obtained from different combinations.

MATERIALS AND METHODS

Sampling

The samples used in this study were collected along at the Senigallia station (SG01, 43.755 °N, 13,2105 °E), which represents the coastal station of the Long-Term Ecological Research (LTER) Senigallia-Susak site (DEIMS.iD: https://deims.org/be8971c2-c708-4d6e-a4c7-f49fcf1623c1) (Fig. 1). Sampling was carried out on board of the Actea oceanographic vessel in 2019 in the months of February, March, April, May, June, July, August, September, and October. For both microscopy and metabarcoding, samples were collected at the surface (0.5 meters) using Niskin bottles.

For microscopy analysis, phytoplankton samples were stored in 250 mL dark glass bottles and preserved by adding 0.8% pre-filtered and neutralized formaldehyde (Throndsen, 1978), while for metabarcoding, 2 L were filtered through cellulose nitrate filters (47 mm diameter, 1.2 µm pore size, Sartorius) and stored at -20°C for subsequent metabarcoding analysis.

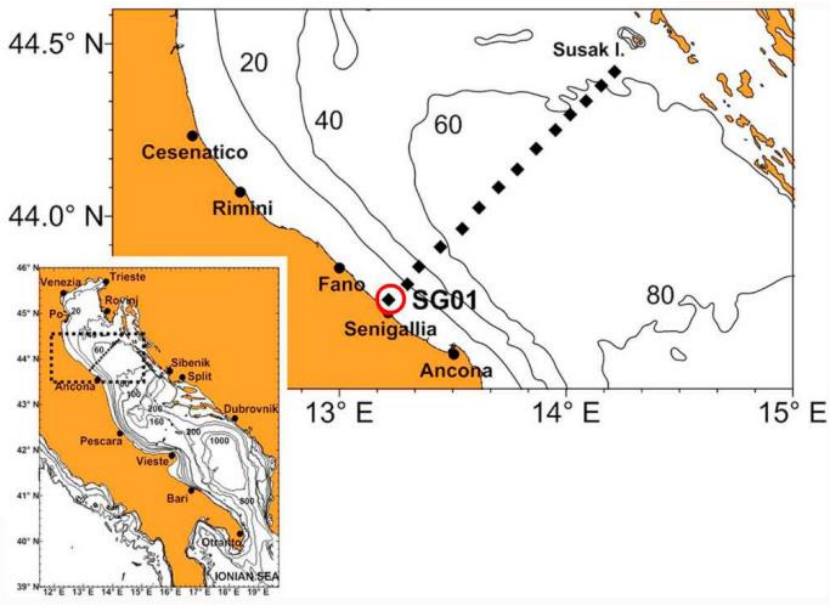

Fig1: Map of the study area. The red circle represents the study station (SG01), while the dashed line indicates the eLTER Senigallia-Susak transect.

DNA extraction, sequencing and sequence analysis

The DNeasy PowerWater Kit (QIAGEN, Germany) was used for DNA extraction, following the manufacturer's instructions. The filters were cut in half, each half was extracted separately and then pooled after elution. Qubit Fluorimeter (Thermo Fisher Scientific) was used to assess the quantity of extracted DNA. The V4 and V9 regions of the 18S rRNA were amplified following the Illumina Sequencing Library Preparation protocol (with 30 PCR cycles) in the amplicon PCR. According to Piredda et al. (2017), the following primers were used for amplification:

V4 18S Next, Forward: CCAGCASCYGCGGTAATTCC,

Reverse: ACTTTCGTTCTTGATYRATGA

V9 18S Next, Forward: TTGTACACACCGCCCGTCGC,

Reverse: CCTTCYGCAGGTTCACCTAC.

The PCR products were 470 bp for V4 and 270 bp for V9. Unfortunately, the V4 primers failed to amplify the May sample, which was not sequenced.

Library preparation (including IDT for Illumina UD index set D (Illumina, San Diego, CA, USA), primers with sequence complementary to overhang adapter and sample specific barcodes), 2 × 250 bp paired end sequencing of equimolar ratios of the purified amplicon libraries on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) and demultiplexing were performed at the Department of Bioscience, Biotechnology and Biopharmaceutics at Università degli Studi di Bari.

The quality control and adapter presence, check for each 18S region, were checked using FASTQC (Andrews and Braham Bioinformatics, 2010), while cutadapt was used to trim the primers from the demultiplexed reads (Martin, 2011).

Subsequently, the sequences were processed and analyzed using the open-source software QIIME2 (v. 2023.2) (Bolyen et al., 2019).

Quality filtering, denoising and pairing of the reads were performed with DADA2 (Callahan et al., 2016), using a --p-trunc-len based on quality scores.

Finally, the Naive Bayes classifier was used for taxonomy assignment of amplicon sequence variants (ASVs). The SILVA 138 99 % OTU reference database (Quast et al., 2012) and PR2 (Guillou et al., 2013) (v.5.0.1) were trained for the specific target regions of the 18S rRNA gene (V4, V9).

Phytoplankton analysis

The Utermöhl method (Edler et al., 2010) was followed for the identification and counting of phytoplankton, using an inverted microscope (ZEISS Axiovert 135) equipped with phase contrast. Cell counting (minimum 200 cells) was performed at 400x and 200x magnification along transects or in random visual fields, based on the cell concentration in the sample. The 200x magnification was used for half of the Utermöhl chamber to obtain a better estimate of the larger and rarer species. Diatoms, dinoflagellates, coccolithophores, and phytoflagellates are the groups into which the phytoplankton were categorized. When possible, identification was made at the genus and species levels. Both autotrophic and heterotrophic species were included in the dinoflagellates. Groups not identifiable by light microscopy were classified as phytoflagellates, that include haptophytes (except coccolithophores), cryptophytes, chrysophytes, dictyochophytes, raphidophytes, chlorophytes, and euglenophytes.

Data and statistical analyses

From the original dataset, organelles sequences (mitochondrial, nucleomorph) and all phyla or classes not belonging to phytoplankton (e.g., proteobacteria, ascomycota, tintinnids, metazoans) were removed using the phyloseq package (McMurdie and Holmes, 2013). The taxonomic database Algaebase (Guiry & Guiry, 2024) was used to verify genus and species names obtained, ensuring any updated names were appropriately substituted. Further verification was conducted on NCBI in cases of uncertain taxonomy, using ASVs, and correcting or removing errors.

As in microscopy phytoflagellates are considered a unique artificial group (e.g., Neri et al. 2023), for the comparison between methods, we maintained the same group also in the metabarcoding analysis.

The phytoplankton community in terms of relative abundances and biomasses of the different phytoplankton groups as obtained by the five approaches used to assess the biodiversity i.e., microscopy, V4-PR2, V9-PR2, V4-SILVA, V9-SILVA were compared. In this case, undetermined taxa were also considered (i.e., taxa whose taxonomy is known at the upper classification or class levels), as normally included in routine phytoplankton monitoring.

Due to the importance of removing the bias related to number of copies of the 18S rRNA gene present in the sample cells, metabarcoding data were converted using the conversion factors by Lapeyra Martin et al. (2022). This study starts from the theoretical assumption that "1 read" corresponds to 1 copy number of the gene (GCN) 18S rRNA. In this way the number of reads obtained during sequencing may be proportional to the number of copies of the 18S rRNA gene present in the sample. So, two conversion factors (CF) were developed, to normalize the relative cells abundance and biomass within each phytoplankton group: the metabarcoding read counts of the different phytoplankton groups were corrected dividing the number of gene copies per cell (GCN $cells^{-1}$) and per C content (GCN pgC^{-1}) for abundance and biomass, respectively.

The CF used to convert GCN to abundance (cells/liter) varies among groups. Specifically, a CF of 166 has been used for diatoms, 4919 for dinoflagellates and 5.23 for phytoflagellates and coccolithophores. Instead, CFs values for estimating the corrected relative biomass were 4.41 for diatoms, 27.17 for dinoflagellates and 0.94 for phytoflagellates and coccolithophores.

This approach aimed to facilitate a robust comparison between data obtained from microscope readings and those derived from metabarcoding. We decided to compare the results obtained at the genus and species levels, both excluding and including undetermined taxa. These were included in the barplots while they were not considered in the pie charts.

Pie charts were used to compare the number of identified genera and species in each phytoplankton groups obtained by the five approaches, using presence-absence data. Pie charts were generated using the ggplot2 package (Wickham, 2016), with the number of genera and species that were identified by

microscopy and metabarcoding, using the V4 and V9 regions of the 18S rRNA gene, and SILVA and PR2 as reference databases for taxonomic assignment.

Barplots were generated using the ggplot2 package (Wickham, 2016), to compare the phytoplanktonic community at genus and species level, present each month, with and without CF of relative abundance and biomass.

A Non-Metric Multidimensional Scaling (NMDS) was performed on the relative abundances and biomasses of the different phytoplankton groups obtained from microscopy and metabarcoding (including all the determination up to the group level), both with and without the use of the respective CF, to evaluate the capability of the chosen CFs in increasing the comparability of metabarcoding and microscopy data. The metaMDS function from the vegan package (Oksanen et al., 2022) was used, setting the distance as bray. Permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences among the groups that were highlighted by the NMDS, using the adonis function in the vegan package (Oksanen et al., 2022).

RESULTS

Taxa composition

Trought the study period, the percentages of presence of genera and species belonging to the main phytoplankton groups (diatoms, dinoflagellates, coccolithophores and phytoflagellates) obtained using the five applied approaches (microscopy, V4-PR2, V4-SILVA, V9-PR2 and V9- SILVA) are shown in Fig. 2 and Fig. 3 for genera and species, respectively, without considering undermined taxa.

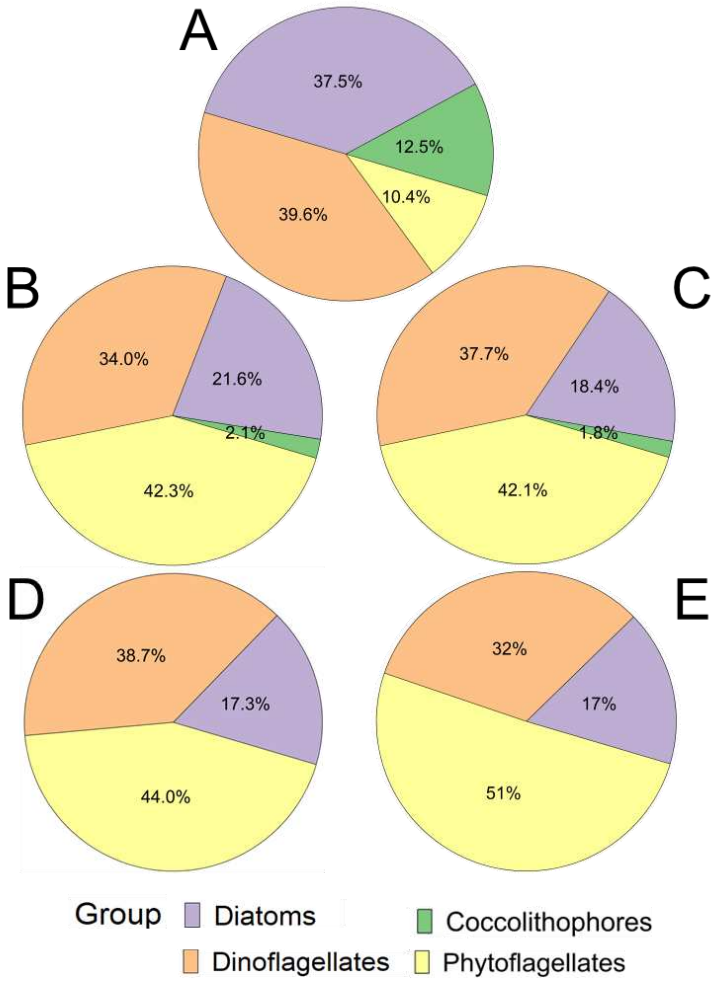

Fig.2: Pie charts diagrams representing the percentage of identified genera, for each phytoplankton group: diatoms (violet), dinoflagellates (orange), coccolithophores (green), phytoflagellates (yellow) obtained using the five applied approaches: microscopy (A), V4-SILVA (B), V4-PR2 (C), V9-SILVA (D) and V9-PR2 (E).

As regards genera, using microscopy (Fig.2A), dinoflagellates and diatoms showed the highest percentages (39.6% and 37.5%, respectively), followed by coccolithophores (12.5%) and phytoflagellates (10.4%). Using metabarcoding (Fig. 2B,C,D,E), phytoflagellates had higher percentages in each methods used (51% with V9-PR2, 44% with V9-SILVA, 42.3% with V4-SILVA and 42% with V4-PR2), followed by dinoflagellates (32% with V9-PR2, 38.7% with V9-SILVA, 34% with V4-SILVA and 37.7% with V4-PR2), while lower percentages were found for diatoms (17% with V9-PR2, 17.3% with V9-SILVA, 21.6% with V4-SILVA and 18.4% with V4-PR2). Coccolithophores has been recognised only by V4-PR2 and V4-SILVA (1.8% and 2%, respectively).

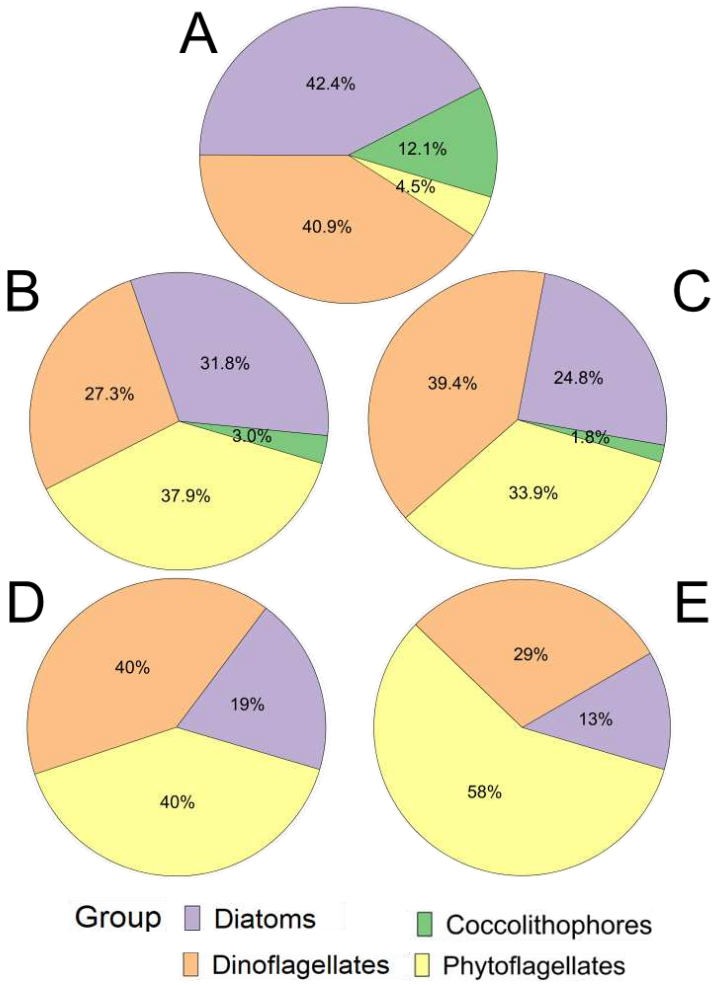

Fig.3: Pie charts diagrams representing the percentage of identified species, for each phytoplankton groups: diatoms (violet), dinoflagellates (orange), coccolithophores (green), phytoflagellates (yellow) obtained using the five applied approaches: microscopy (A), V4-SILVA (B), V4-PR2 (C), V9-SILVA (D) and V9-PR2 (E).

Regarding identified species, using metabarcoding analysis (Fig.3B,C,D,E), the dominant group were the phytoflagellates with V9-PR2 (58%), V9-SILVA (40%) and V4-SILVA (37.9%), while with V4-PR2 dinoflagellates were dominant (39.4%), followed by phytoflagellates and diatoms (33.9 and 24.8%, respectively). Dinoflagellate species represented 40% of total species with V9-SILVA, 29% with V9-PR2 and 27.3% with V4-SILVA. Diatom species were present with percentages of 31.9% with V4-SILVA, 18% with V9-SILVA and 13% with V9-PR2. Coccolithophore species occurred with percentages of 1.8% and 3% for using V4-PR2 and V4-SILVA, respectively.
Using microscopy (Fig.3A) dinoflagellates and diatoms showed the highest percentages (40.9% and 42.4%, respectively), followed by coccolithophores (12%) and phytoflagellates (4.5%).

Going into detail, at genus level, all approaches together identified 178 genera: microscopy found 48 genera (Table 1) while metabarcoding identified 114 genera with VA-PR2, 77 with V9-PR2, 97 with V4-SILVA and 75 with V9-SILVA. Among the genera identified by microscopy 18 were diatoms, 19 were dinoflagellates, 6 were coccolithophores, and 5 were phytoflagellates. While, among the total genera found by metabarcoding (162), 32 were diatoms, 56 were dinoflagellates, 3 were coccolithophores, and 71 were phytoflagellates. The total number of genera identified by metabarcoding and not by microscopy were 130, while the total number of genera identified by microscopy and not by metabarcoding were 10. We verified that, out of the total 10 genera detected only microscopy, six were absent from the PR2 and SILVA databases (*Oxyphysis, Oxytoxum, Calciosolenia, Michaelsarsia, Rhabdosphaera, Ollicola*).

35 diatoms genera were identified by all approaches together. Among 18 identified through microscopy, only 3 were not detected by metabarcoding (*Bacteriastrum*, *Dactyliosolen*, *Synedra*). Metabarcoding analysis identified a total of 32 diatoms genera, among which 17 were not revealed by microscopy.

The total number of dinoflagellate genera identified by all approaches combined was 61. Microscopy revealed 19 genera, all identified also by metabarcoding except *Diplopsalis*, *Oxyphysis* and *Oxytoxum* that were not detected by any metabarcoding approach. A high number of dinoflagellate genera (41) were identified only by metabarcoding, both naked (e.g. *Grammatodinium, Gyrodiniellum, Karlodinium, Lebouridinium, Lepidodinium, Levanderina, Margalefidinium, Paragymnodinium, Proterythropsis, Polykrikos, Syltodinium, Torodinium*) and thecate (e.g. *Archaeperidinium, Fragilidium, Heterodinium, Triadinium*).

Coccolithophore genera identified by all approaches were 7. Microscopy identified 6 genera, of which only 2 reported also by metabarcoding, while *Calciosolenia*, *Calyptrosphaera*, *Michaelsarsia*, *Rhabdosphaera* were not detected by any metabarcoding approach. Metabarcoding detected *Algirosphaera* that was not detected by microscopy.

Among the entirety of genera included in the phytoflagellate group (75), only 5 were detected by microscopy, while the majority were found only from the metabarcoding, especially chlorarachniophytes (e.g. *Chlorarachnion, Minorisa*), chlorophytes (e.g. *Desmodesmus, Mychonastes*), chrysophytes (e.g. *Paraphysomonas, Poterioochromonas*), haptophytes (e.g *Chrysochromulina, Phaeocystis*), cryptophytes (e.g. *Hemiselmis, Proteomonas*), dictyochophyceans (e.g. *Pedinella, Pseudochattonella*) and mamiellophyceans (e.g. *Dolichomastix, Micromonas*), and choanoflagellates (*Helgoeca, Calliacantha*).

At species level (Table 2) all approaches together identified 240 species: microscope analysis identified 66 species while metabarcoding found 109 species with V4-PR2, 85 with V9-PR2, 66 with V4-SILVA and 62 with V9-SILVA.

Among the total species identified by microscopy 28 were diatoms, 27 were dinoflagellates, 8 were coccolithophores and 3 were phytoflagellates. While, among the total genera found by metabarcoding, 47 were diatoms, 65 were dinoflagellates, 3 were coccolithophores, and 81 were phytoflagellates.

The total number of genera identified by metabarcoding and not by microscopy were 174, while the total number of genera identified by microscopy and not by metabarcoding were 44. We verified that out of the 44 species identified only by microscopy, 22 were absent from both databases (e.g., *Chaetoceros compressus, Nitzschia gobbii, Dinophysis sacculus, Oxytoxum caudatum, Calciosolenia murrayi, Michaelsarsia adriatica, Rhabdosphaera clavigera, Dinobryon porrectum*), while 2 were absent only from the SILVA database (C*haetoceros lorenzianus and Chaetoceros tortissimus*), and 1 was absent only in PR2 database (*Dictyocha fibula*).

64 diatom species have been identified considering all approaches, among them 17 were found only by microscopy (e.g. *Asteromphalus hookeri, Chaetoceros tenuissimus, Dactyliosolen fragilissimus, Pseudo-nitzschia delicatissima*), while metabarcoding found 36 species of diatoms (e.g. *Amphora ovalis, Chaetoceros debilis, Cyclotella litoralis, Pseudo-nitzschia pungens*). Only 11 species of diatoms have been identified by both approaches.

A high number of dinoflagellate species (83) were found by both metabarcoding and microscopy. 56 of them have been found only by metabarcoding, both naked (e.g. *Gymnodinium impudicum, Gyrodiniellum shiwhaense, Lebouridinium glaucum, Lepidodinium viride, Levanderina fissa, Margalefidinium fulvescens, Paragymnodinium shiwhaense, Polykrikos geminatus, Syltodinium listii*) and thecate (e.g. *Archaeperidinium saanichi, Fragilidium duplocampaniforme, Triadinium*

polyedricum). While 18 species were found only by microscopy (e.g. *Dinophysis sacculus, Karenia papilionacea, Oxytoxum elongatum, Prorocentrum cordatum, Protoperidinium diabolus*).

9 genera of coccolithophores were identified by all approaches, 6 of them only from the microscopy (*Calciosolenia brasiliensis*, *Calciosolenia murrayi*, *Calyptrosphaera oblonga*, *Michaelsarsia adriatica, Michaelsarsia elegans, Rhabdosphaera clavigera*), whereas metabarcoding found only 1 species, *Algirosphaera robusta*, not detected by microscopy.

All the approaches together detected 84 species included into phytoflagellates. Among them, 81 were found only from the metabarcoding: chlorarachniophytes (e.g. *Chlorarachnion reptans, Minorisa minuta*), chlorophytes (e.g. *Chlorochytrium lemnae*, *Desmodesmus abundans*), chrysophytes (e.g. *Paraphysomonas foraminifera, Poterioochromonas malhamensis*), haptophytes (e.g *Chrysochromulina leadbeateri, Phaeocystis cordata*), cryptophytes (e.g. *Hemiselmis rufescens, Teleaulax amphioxeia*), dictyochophyceans (e.g. *Pedinella elastica, Pseudochattonella verruculosa*) and mamiellophyceans (e.g. *Dolichomastix tenuilepis, Micromonas bravo*) and choanoflagellates (*Helgoeca nana*). Only, 3 species (*Dinobryon faculiferum, Dinobryon porrectum, Dictyocha fibula*) were identified by microscopy, while the same were not revealed by metabarcoding.

Groups' relative abundances

The relative abundances of the four groups (diatoms, coccolithophores, dinoflagellates, and phytoflagellates), including the undetermined taxa, in the different sampling months, without and with the use of the conversion factors, are shown in Fig. 4A and 4B, respectively.

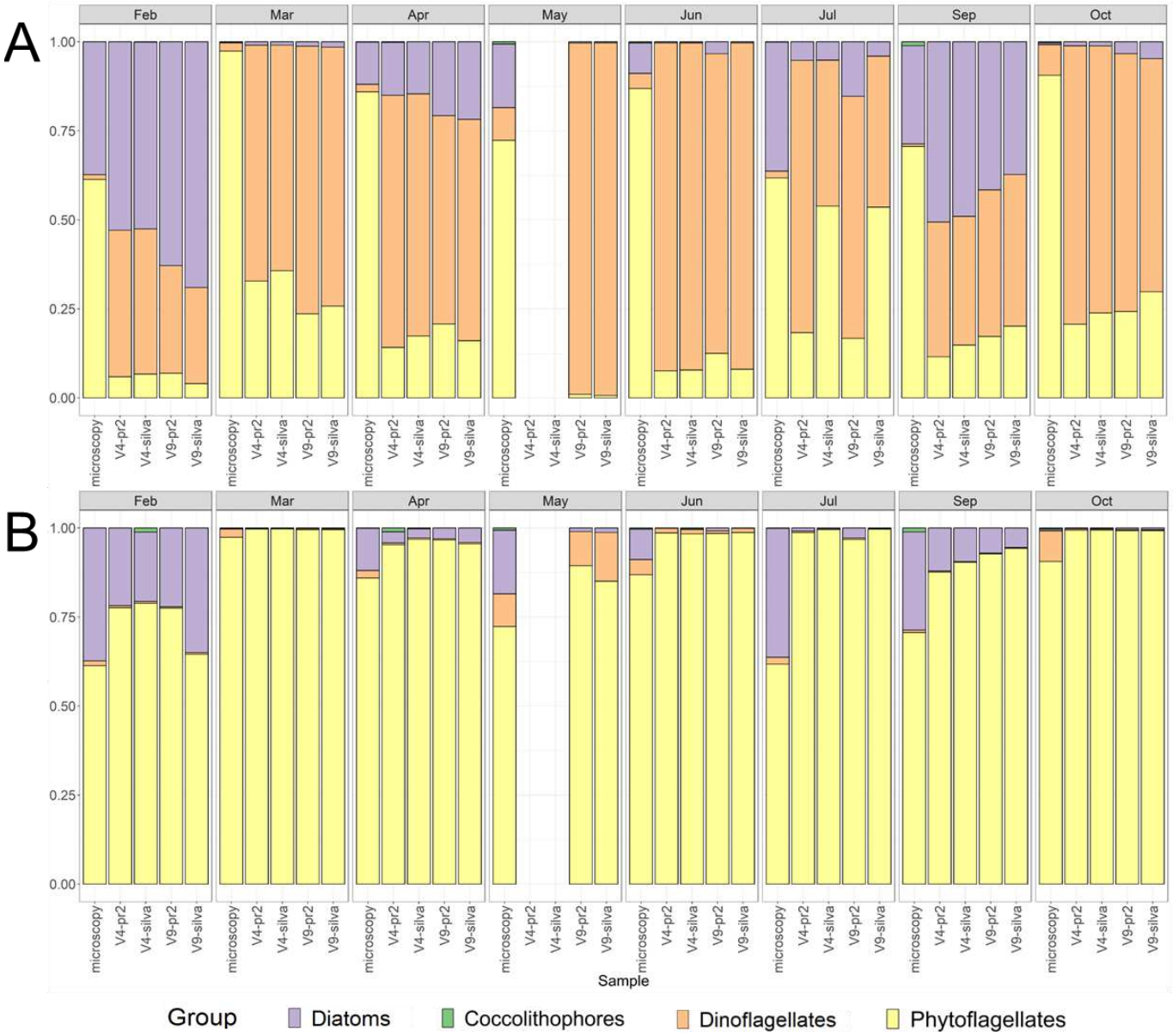

Fig. 4: Bar plots representing the percentage relative abundances of genera belonging to the main phytoplankton groups (diatoms (violet), coccolithophores (green), dinoflagellates (orange), phytoflagellates (yellow)) obtained using the five applied approaches (microscopy, V4-PR2, V4-SILVA, V9-PR2 and V9-SILVA), without (A) and with (B) the use of conversion factors.

As regards microscopy (Fig.4A), the highest percentages were represented by phytoflagellates throughout the year (among 61% in February and 97% in March), followed by diatoms (37% in February, 12% in April, 9% in June, 36% in July and 28% in September) or dinoflagellates (2% in

March, 9% in May and October), while coccolithophores represented the lowest percentages (<1%). Using metabarcoding (Fig.4A), without applying the conversion factor, the community was dominated by diatoms in February (69% with V9-SILVA, 53% in V4-PR2, V4-SILVA, and V9-PR2) and September (51% V4-PR2 and V4-SILVA, 42% with V9-PR2, 37% with V9-SILVA), followed by dinoflagellates (41% with V4-PR2 and V4-SILVA in February, 41% with V9-PR2 and 43% with V9-SILVA in September). Dinoflagellates were the most represented in March (63 - 75%), April (59 - 71%), May (99% with V9-PR2 and V9-SILVA), June (84 - 92%) and October (65 – 78%), and were followed by phytoflagellates in March (24 – 36%), in April (14 – 21%), in May (1%), in June (8 – 12%) and October (21 – 30%). In July, the highest percentage of phytoplankton was represented by dinoflagellates (42% with SILVA, 68 and 76% with PR2) and phytoflagellates (18% with PR2 and 54% with SILVA). Coccolithophores showed the lowest relative abundances in each month, and in February, March, April no taxa were detected.

When the conversion factor was applied, the relative abundances showed a strong decrease of dinoflagellate contribution (Fig.4B): phytoflagellates were the dominant group in each month (61 - 99.7%), especially in March (99.4 - 99.7%), in June (98 - 99%) and October (99.2 - 99.4%). Phytoflagellate were followed by either diatoms (20 - 35% in February, 3 - 4% in April, <1% in March and June, <3% in July and 5 - 12% in September) or dinoflagellates (10 - 13% in May and <1% in October), while coccolithophores represented the lowest percentages.

The biomass of the four groups (diatoms, coccolithophores, dinoflagellates and phytoflagellates,), including the undetermined taxa, in the different sampling months, without the conversion and with the use of the conversion factors, are shown in Fig. 5A and 5B, respectively.

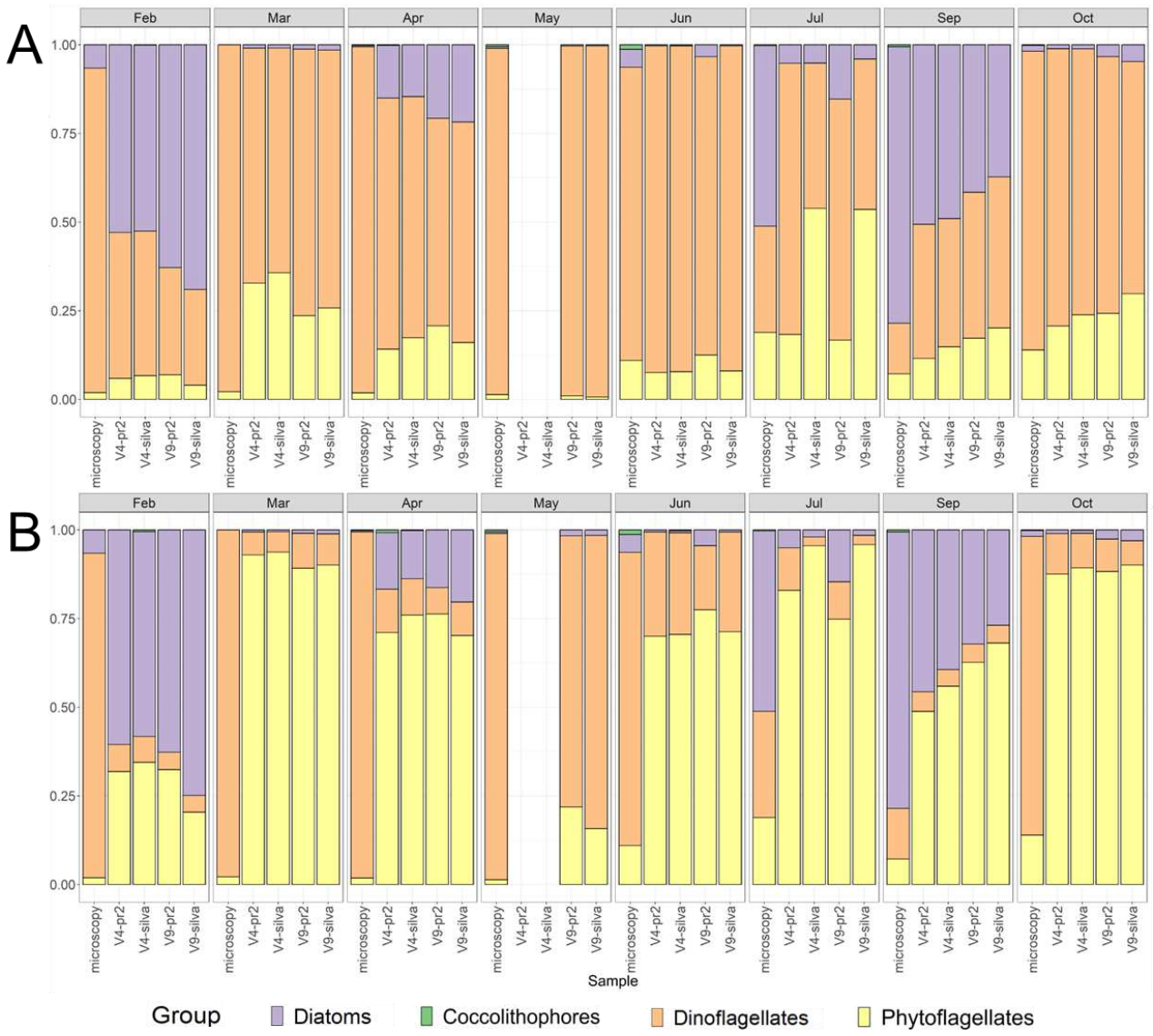

Fig.5: Bar plots representing the biomass percentage of genera belonging to the main phytoplankton groups (diatoms (violet), coccolithophores (green), dinoflagellates (orange), phytoflagellates (yellow)) obtained using the five applied approaches (microscopy, V4-PR2, V4-SILVA, V9-PR2 and V9-SILVA), without (A) and with (B) the use of conversion factors.

Microscopy analysis revealed a high contribution of dinoflagellates to the total biomass in February (91%), March (98%), April (98%), May (98%), June (83%) and October (84%) (Fig.5A). The second group were the phytoflagellates, that in March, April, June and October with a percentage between 2 and 14%. In July and September diatoms were the most represented (51 and 78%, respectively),

followed by dinoflagellates (30 and 14%, respectively) and phytoflagellates (19 and 7%, respectively). While, throughout the study period, coccolithophores were present with a percentage <1%, or absent (April, February, June, May, October).

As regards metabarcoding (Fig. 5B,C,D,E), without the application of conversion factors, diatoms showed high percentages in February (52 - 69%) and September (37 – 51%), followed by dinoflagellates (27 - 41% in February, 36 - 43% in September) and phytoflagellates (4 – 7% in February 12 - 20% in September). Very low percentages (<1%) of coccolithophores were found.

In other months (March, April, May, Jun, October), dinoflagellates were dominant with a percentage between 59 (April with V9-PR2) and 99% (May with V9-SILVA). Followed by phytoflagellates in March (24 - 36%), April (14 - 21%), Jun (8 - 12%) and October (21-30%). In July V4-SILVA and V9-SILVA showed a high percentage of phytoflagellates (54%), while V4-PR2 and V9-PR2 showed a high percentage of dinoflagellates (76 and 68%, respectively). Diatoms were identified at a lower percentage (4 – 15%). In each month coccolithophores showed the lowest percentages (<1%).

Using the conversion biomass factors (Fig. 5B) in metabarcoding analysis, phytoflagellates were the dominant group in March (89 – 94%), April (70 - 76%), Jun (70 – 77%), July (83 – 96%), September (49 - 68%) and October (88 - 90%). They were followed by dinoflagellates in March (6 - 10%), June (18 - 29%) and October (7 - 11%) and by diatoms in April (14 - 20%) and September (27 - 46%).

Only in February diatoms were the dominant group in terms of biomass (between 58% with V4-SILVA and 75% with V9-SILVA), followed by phytoflagellates (20 – 32%) and dinoflagellates (5 – 8%). Dinoflagellates were the most contributors to biomass in May (76% with V9-PR2 and 83% with V9-SILVA) followed by phytoflagellates (22% with V9-PR2 and 16% with V9-SILVA) and diatoms (2%). Coccolithophores were present with a percentage between 0,5 and 1% in April (with V4-PR2 and V4-SILVA), in February and June with V4-SILVA.

The results of the Non-Metric Multidimensional Scaling (NMDS), performed on the phytoplankton group relative abundances (A) and biomasses (B), with and without the use of converting factors, are shown in Fig. 6.

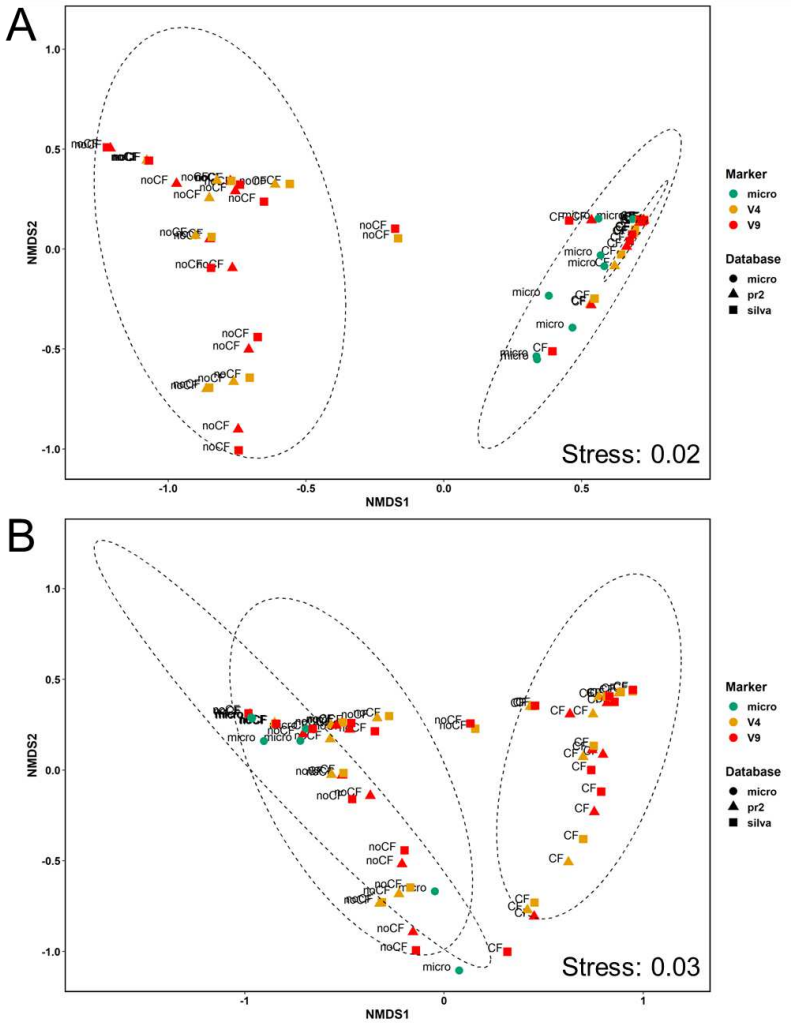

Fig. 6: NMDS performed on the relative abundances (A) and biomasses (B) of the different phytoplankton groups obtained from microscopy (green, ■) and metabarcoding (V4-SILVA (yellow, ■), V4-PR2 (yellow, ▲), V9-SILVA (red, ■) and V9-PR2 (red, ▲)), with (CF) and without (noCF) the use of converting factors (CF).

As regards the relative abundances (Fig.6A), microscopy data were grouped together with the metabarcoding ones with the used of CFs, while a clear divergence between the two and metabarcoding data without the use of CFs was observed.

On the contrary, considering relative biomasses (Fig.6B), microscopy data grouped with metabarcoding data without the use of CF, while a divergence is evident between the two and metabarcoding data with the use of CFs.

With both abundances and biomasses, no clear difference was observed among approaches based on metabarcoding (V4-SILVA, V4-PR2, V9-SILVA and V9-PR2).

The PERMANOVA analysis highlighted significant differences between microscopy and metabarcoding with and without CFs, with both relative abundances and biomasses ($p < 0.001$).

The percentages of genera (without undetermined taxa) found with the five approaches, are reported in Fig. 7 without (B) and with (C) the application of the CF for the abundances. The proportions of the dominant genera were strongly affected by the application of the CF.

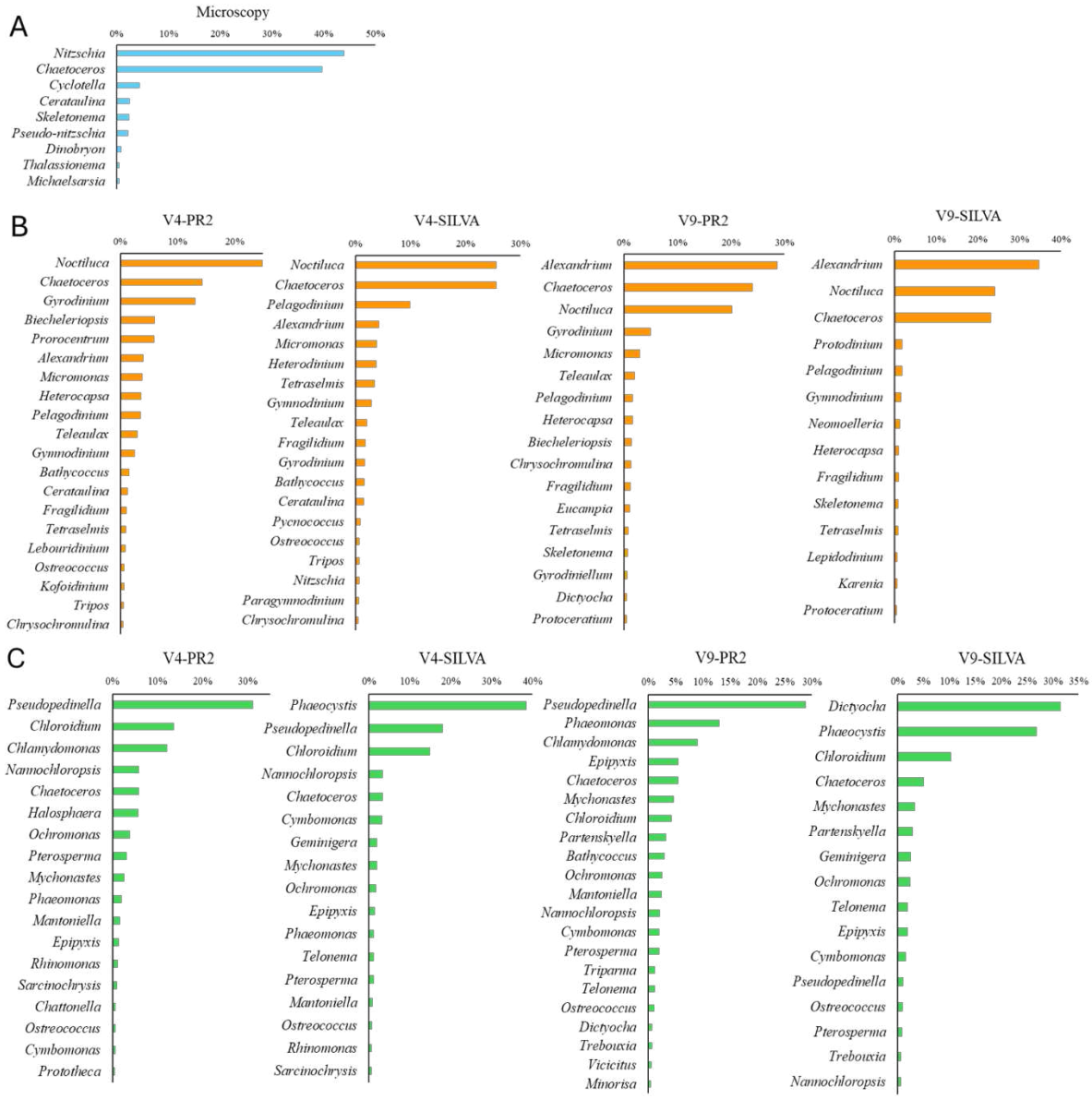

Fig. 7: Percentages of dominant genera in microscopy (A-light blue bars) and metabarcoding approaches without (B-orange bars) and with (C-green bars) the conversion factor.

As regards microscopy (Fig.7A) the dominant group were the diatoms, with high percentages of *Nitzschia* (44%) and *Chaetoceros* (40%), followed by *Cycletella, Cerataulina, Skeletonema* and *Pseudo-nitzschia* (4,3,2,2%, respectively). 1% was represented by *Dinobryon* and *Thalassionema*. Using metabarcoding, without the conversion factor (Fig.7B), in all four approaches the dominant group were the dinoflagellates. *Noctiluca* (25% with V4-PR2 and V4-SILVA) and *Alexandrium* (29%

with V9-PR2, 35% with V9-SILVA) were the most represented, followed by *Chaetoceros* (14 – 26%). *Cerataulina* and *Skeletonema* were present at a percentage of 1% both with microscopy and metabarcoding (V4 region and V9-SILVA, respectively).

By applying the conversion factor (Fig.7C), in all four approaches the dominant group were the phytoflagellates. *Pseudopedinella* was one of the most represented in V4-PR2 (31%), V9-PR2 (29%) and V4-SILVA (18%). In V4-SILVA the genus with the highest percentage was *Phaeocystis* (39%) whereas in V9-SILVA was *Dictyocha* (32%). In each approach, the genus *Chaetoceros* was present, as also identified through microscopy, although with lower percentages (3 – 6%).

Table 1: List of phytoplankton genera identified by microscopy and metabarcoding (V4-PR2, V9-PR2, V4-SILVA, V9-SILVA).

Group	Class	Genus	V4-PR2	V9-PR2	V4-SILVA	V9-SILVA	microscopy
Coccolithophores	Coccolithophyceae	*Algirosphaera*	X		X		
Coccolithophores	Coccolithophyceae	*Calciosolenia*					X
Coccolithophores	Coccolithophyceae	*Calyptrosphaera*					X
Coccolithophores	Coccolithophyceae	*Emiliania*			X		X
Coccolithophores	Coccolithophyceae	*Michaelsarsia*					X
Coccolithophores	Coccolithophyceae	*Rhabdosphaera*					X
Coccolithophores	Coccolithophyceae	*Syracosphaera*	X				X
Diatoms	Bacillariophyceae	*Amphora*	X		X		X
Diatoms	Bacillariophyceae	*Arcocellulus*			X		
Diatoms	Bacillariophyceae	*Asteromphalus*	X		X		X
Diatoms	Bacillariophyceae	*Bacteriastrum*					X
Diatoms	Bacillariophyceae	*Ceratanaulus*		X			
Diatoms	Bacillariophyceae	*Cerataulina*	X		X		X
Diatoms	Bacillariophyceae	*Chaetoceros*	X	X	X	X	X
Diatoms	Bacillariophyceae	*Cyclotella*	X	X	X	X	X
Diatoms	Bacillariophyceae	*Cylindrotheca*			X		X
Diatoms	Bacillariophyceae	*Dactyliosolen*					X
Diatoms	Bacillariophyceae	*Eucampia*		X			
Diatoms	Bacillariophyceae	*Eupyxidicula*			X	X	
Diatoms	Bacillariophyceae	*Fistulifera*	X		X	X	
Diatoms	Bacillariophyceae	*Fragilariopsis*		X			
Diatoms	Bacillariophyceae	*Guinardia*	X				X
Diatoms	Bacillariophyceae	*Hyalosira*			X		
Diatoms	Bacillariophyceae	*Leptocylindrus*	X	X	X	X	X
Diatoms	Bacillariophyceae	*Mediolabrus*	X				
Diatoms	Bacillariophyceae	*Melosira*		X		X	
Diatoms	Bacillariophyceae	*Minutocellus*	X	X		X	
Diatoms	Bacillariophyceae	*Navicula*	X			X	
Diatoms	Bacillariophyceae	*Neomoelleria*				X	
Diatoms	Bacillariophyceae	*Nitzschia*	X		X		X
Diatoms	Bacillariophyceae	*Papiliocellulus*	X				
Diatoms	Bacillariophyceae	*Pleurosigma*	X	X	X	X	X
Diatoms	Bacillariophyceae	*Proboscia*	X		X		X
Diatoms	Bacillariophyceae	*Pseudogomphonema*		X			
Diatoms	Bacillariophyceae	*Pseudo-nitzschia*	X	X	X	X	X
Diatoms	Bacillariophyceae	*Pseudosolenia*	X		X		
Diatoms	Bacillariophyceae	*Rhizosolenia*	X		X		
Diatoms	Bacillariophyceae	*Skeletonema*	X	X	X	X	X
Diatoms	Bacillariophyceae	*Sundstroemia*			X		
Diatoms	Bacillariophyceae	*Synedra*					X
Diatoms	Bacillariophyceae	*Thalassionema*	X		X		X
Diatoms	Bacillariophyceae	*Thalassiosira*	X	X	X	X	X

Dinoflagellates	Dinophyceae	*Akashiwo*	X		X		X
Dinoflagellates	Dinophyceae	*Alexandrium*	X	X	X	X	X
Dinoflagellates	Dinophyceae	*Amphidinium*			X		
Dinoflagellates	Dinophyceae	*Ansanella*	X	X			
Dinoflagellates	Dinophyceae	*Archaeperidinium*	X	X	X	X	
Dinoflagellates	Dinophyceae	*Azadinium*			X		
Dinoflagellates	Dinophyceae	*Biecheleria*	X	X	X	X	
Dinoflagellates	Dinophyceae	*Biecheleriopsis*	X	X			
Dinoflagellates	Dinophyceae	*Blastodinium*	X	X			
Dinoflagellates	Dinophyceae	*Blixaea*	X		X		
Dinoflagellates	Dinophyceae	*Cochlodinium*				X	X
Dinoflagellates	Dinophyceae	*Dinophysis*	X				X
Dinoflagellates	Dinophyceae	*Diplopsalis*					X
Dinoflagellates	Dinophyceae	*Erythropsidinium*			X		
Dinoflagellates	Dinophyceae	*Fragilidium*	X	X	X	X	
Dinoflagellates	Dinophyceae	*Gonyaulax*	X	X	X	X	X
Dinoflagellates	Dinophyceae	*Grammatodinium*	X		X		
Dinoflagellates	Dinophyceae	*Gymnodinium*	X	X	X	X	X
Dinoflagellates	Dinophyceae	*Gyrodiniellum*		X			
Dinoflagellates	Dinophyceae	*Gyrodinium*	X	X	X		
Dinoflagellates	Dinophyceae	*Heterocapsa*	X	X	X	X	
Dinoflagellates	Dinophyceae	*Heterodinium*			X		
Dinoflagellates	Dinophyceae	*Islandinium*	X		X		
Dinoflagellates	Dinophyceae	*Karenia*	X	X		X	X
Dinoflagellates	Dinophyceae	*Karlodinium*	X				
Dinoflagellates	Dinophyceae	*Kofoidinium*	X				X
Dinoflagellates	Dinophyceae	*Lebouridinium*	X				
Dinoflagellates	Dinophyceae	*Leiocephalium*				X	
Dinoflagellates	Dinophyceae	*Lepidodinium*	X			X	
Dinoflagellates	Dinophyceae	*Lessardia*	X		X		
Dinoflagellates	Dinophyceae	*Levanderina*	X	X	X	X	
Dinoflagellates	Dinophyceae	*Lingulodinium*				X	X
Dinoflagellates	Dinophyceae	*Margalefidinium*	X		X		
Dinoflagellates	Dinophyceae	*Noctiluca*	X	X	X	X	X
Dinoflagellates	Dinophyceae	*Oxyphysis*					X
Dinoflagellates	Dinophyceae	*Oxytoxum*					X
Dinoflagellates	Dinophyceae	*Paragymnodinium*		X	X	X	
Dinoflagellates	Dinophyceae	*Pelagodinium*	X	X	X	X	
Dinoflagellates	Dinophyceae	*Phalacroma*	X		X	X	X
Dinoflagellates	Dinophyceae	*Polykrikos*	X	X	X	X	
Dinoflagellates	Dinophyceae	*Prorocentrum*	X	X		X	X
Dinoflagellates	Dinophyceae	*Proterythropsis*	X		X		
Dinoflagellates	Dinophyceae	*Protoceratium*	X	X	X	X	X
Dinoflagellates	Dinophyceae	*Protodinium*	X		X	X	
Dinoflagellates	Dinophyceae	*Protoperidinium*	X	X	X	X	X
Dinoflagellates	Dinophyceae	*Pseliodinium*	X				

Dinoflagellates	Dinophyceae	*Pyrophacus*	X		X		
Dinoflagellates	Dinophyceae	*Scrippsiella*	X		X	X	X
Dinoflagellates	Dinophyceae	*Sinophysis*			X	X	
Dinoflagellates	Dinophyceae	*Spatulodinium*			X		
Dinoflagellates	Dinophyceae	*Spiniferodinium*	X				
Dinoflagellates	Dinophyceae	*Stoeckeria*				X	
Dinoflagellates	Dinophyceae	*Syltodinium*	X				
Dinoflagellates	Dinophyceae	*Torodinium*	X				
Dinoflagellates	Dinophyceae	*Triadinium*		X		X	
Dinoflagellates	Dinophyceae	*Tripos*	X	X	X	X	X
Dinoflagellates	Dinophyceae	*Wangodinium*	X				
Dinoflagellates	Dinophyceae	*Warnowia*	X	X		X	
Dinoflagellates	Dinophyceae	*Yihiella*	X	X			
Dinoflagellates	Dinophyceae	*Zooxanthella*				X	
Phytoflagellates	Acanthoecidae	*Helgoeca*		X			
Phytoflagellates	Bicosoecophyceae	*Bicosoeca*		X			
Phytoflagellates	Bolidophyceae	*Triparma*	X	X	X	X	
Phytoflagellates	Chlorarachniophyceae	*Bigelowiella*				X	
Phytoflagellates	Chlorarachniophyceae	*Chlorarachnion*	X	X		X	
Phytoflagellates	Chlorarachniophyceae	*Lotharella*				X	
Phytoflagellates	Chlorarachniophyceae	*Minorisa*	X	X		X	
Phytoflagellates	Chlorarachniophyceae	*Partenskyella*		X			
Phytoflagellates	Chlorodendrophyceae	*Tetraselmis*	X	X	X	X	
Phytoflagellates	Chlorophyceae	*Chlamydomonas*	X		X		
Phytoflagellates	Chlorophyceae	*Chlorochytrium*				X	
Phytoflagellates	Chlorophyceae	*Chlorococcum*	X				
Phytoflagellates	Chlorophyceae	*Desmodesmus*	X	X		X	
Phytoflagellates	Chlorophyceae	*Monoraphidium*	X				
Phytoflagellates	Chlorophyceae	*Mychonastes*	X		X		
Phytoflagellates	Choanoflagellatea	*Calliacantha*		X			
Phytoflagellates	Chrysophyceae	*Chrysolepidomonas*	X		X		
Phytoflagellates	Chrysophyceae	*Chrysoxys*			X		
Phytoflagellates	Chrysophyceae	*Dinobryon*					X
Phytoflagellates	Chrysophyceae	*Epipyxis*			X		
Phytoflagellates	Chrysophyceae	*Ochromonas*			X		
Phytoflagellates	Chrysophyceae	*Paraphysomonas*	X	X	X	X	
Phytoflagellates	Chrysophyceae	*Poterioochromonas*	X	X	X	X	
Phytoflagellates	Chrysophyceae	*Poteriospumella*		X			
Phytoflagellates	Coccolithophyceae	*Chrysochromulina*	X	X	X		
Phytoflagellates	Coccolithophyceae	*Chrysoculter*			X		
Phytoflagellates	Coccolithophyceae	*Haptolina*	X	X	X		
Phytoflagellates	Coccolithophyceae	*Phaeocystis*	X	X	X		
Phytoflagellates	Coccolithophyceae	*Prymnesium*	X		X		
Phytoflagellates	Cryptophyceae	*Geminigera*			X	X	
Phytoflagellates	Cryptophyceae	*Hemiselmis*	X	X	X	X	
Phytoflagellates	Cryptophyceae	*Proteomonas*	X	X	X	X	

Phytoflagellates	Cryptophyceae	*Rhinomonas*			X		
Phytoflagellates	Cryptophyceae	*Rhodomonas*	X			X	
Phytoflagellates	Cryptophyceae	*Teleaulax*	X	X	X	X	
Phytoflagellates	Crysophyceae	*Melkoniania*	X				
Phytoflagellates	Crysophyceae	*Ostreococcus*	X	X	X	X	
Phytoflagellates	Dictyochophyceae	*Dictyocha*	X	X	X	X	X
Phytoflagellates	Dictyochophyceae	*Florenciella*	X		X		
Phytoflagellates	Dictyochophyceae	*Pedinella*	X	X	X		
Phytoflagellates	Dictyochophyceae	*Pseudochattonella*	X	X	X	X	
Phytoflagellates	Dictyochophyceae	*Pseudopedinella*	X		X		
Phytoflagellates	Dictyochophyceae	*Rhizochromulina*		X	X		
Phytoflagellates	Dictyochophyceae	*Vicicitus*	X				
Phytoflagellates	Euglenophyceae	*Euglena*					X
Phytoflagellates	Eustigmatophyceae	*Microchloropsis*			X	X	
Phytoflagellates	Eustigmatophyceae	*Nannochloropsis*	X				
Phytoflagellates	Goniomonadophyceae	*Goniomonas*	X		X		
Phytoflagellates	Imbricatea	*Ollicola*					X
Phytoflagellates	Imbricatea	*Paulinella*		X			
Phytoflagellates	Mamiellophyceae	*Bathycoccus*	X		X		
Phytoflagellates	Mamiellophyceae	*Dolichomastix*	X	X	X	X	
Phytoflagellates	Mamiellophyceae	*Mamiella*	X	X	X	X	
Phytoflagellates	Mamiellophyceae	*Mantoniella*		X			
Phytoflagellates	Mamiellophyceae	*Micromonas*	X	X	X	X	
Phytoflagellates	Mamiellophyceae	*Microrhizoidea*		X			
Phytoflagellates	Palpitea	*Palpitomonas*				X	
Phytoflagellates	Pedinophyceae	*Marsupiomonas*	X		X		
Phytoflagellates	Pelagophyceae	*Aureococcus*	X				
Phytoflagellates	Pelagophyceae	*Pelagomonas*	X				
Phytoflagellates	Pelagophyceae	*Sarcinochrysis*	X				
Phytoflagellates	Pinguiophyceae	*Phaeomonas*	X	X	X	X	
Phytoflagellates	Pirsoniphyceae	*Pirsonia*				X	
Phytoflagellates	Pyramimonadophyceae	*Cymbomonas*	X	X		X	
Phytoflagellates	Pyramimonadophyceae	*Halosphaera*					X
Phytoflagellates	Pyramimonadophyceae	*Pterosperma*	X				
Phytoflagellates	Pyramimonadophyceae	*Pycnococcus*	X	X	X	X	
Phytoflagellates	Pyramimonadophyceae	*Pyramimonas*	X	X	X	X	
Phytoflagellates	Raphidophyceae	*Chattonella*	X		X	X	
Phytoflagellates	Raphidophyceae	*Fibrocapsa*	X	X	X	X	
Phytoflagellates	Telonemea	*Telonema*	X	X	X	X	
Phytoflagellates	Trebouxiophyceae	*Chloroidium*	X	X		X	
Phytoflagellates	Trebouxiophyceae	*Picochlorum*	X	X	X	X	
Phytoflagellates	Trebouxiophyceae	*Prototheca*		X			
Phytoflagellates	Trebouxiophyceae	*Trebouxia*		X			

Table 2: List of phytoplankton species identified by microscopy and metabarcoding (V4-PR2, V9-PR2, V4-SILVA, V9-SILVA).

Group	Class	Species	V4 PR2	V9 PR2	V4 SILVA	V9 SILVA	microscopy
Coccolithophores	Coccolithophyceae	*Algirosphaera robusta*	X		X		
Coccolithophores	Coccolithophyceae	*Calciosolenia brasiliensis*					X
Coccolithophores	Coccolithophyceae	*Calciosolenia murrayi*					X
Coccolithophores	Coccolithophyceae	*Calyptrosphaera oblonga*					X
Coccolithophores	Coccolithophyceae	*Emiliania huxleyi*			X		X
Coccolithophores	Coccolithophyceae	*Michaelsarsia adriatica*					X
Coccolithophores	Coccolithophyceae	*Michaelsarsia elegans*					X
Coccolithophores	Coccolithophyceae	*Rhabdosphaera clavigera*					X
Coccolithophores	Coccolithophyceae	*Syracosphaera pulchra*	X				X
Diatoms	Bacillariophyceae	*Amphora ovalis*	X		X		
Diatoms	Bacillariophyceae	*Arcocellulus cornucervis*			X		
Diatoms	Bacillariophyceae	*Asteromphalus hookeri*					X
Diatoms	Bacillariophyceae	*Ceratanaulus creticus*		X			
Diatoms	Bacillariophyceae	*Cerataulina pelagica*	X		X		X
Diatoms	Bacillariophyceae	*Chaetoceros affinis*	X	X	X	X	X
Diatoms	Bacillariophyceae	*Chaetoceros anastomosans*	X				
Diatoms	Bacillariophyceae	*Chaetoceros brevis*	X	X			
Diatoms	Bacillariophyceae	*Chaetoceros compressus*					X
Diatoms	Bacillariophyceae	*Chaetoceros curvisetus*	X				X
Diatoms	Bacillariophyceae	*Chaetoceros danicus*	X				X
Diatoms	Bacillariophyceae	*Chaetoceros debilis*		X			
Diatoms	Bacillariophyceae	*Chaetoceros decipiens*	X				
Diatoms	Bacillariophyceae	*Chaetoceros didymus*			X	X	X
Diatoms	Bacillariophyceae	*Chaetoceros diversus*	X				X
Diatoms	Bacillariophyceae	*Chaetoceros lauderi*	X	X			
Diatoms	Bacillariophyceae	*Chaetoceros lorenzianus*					X
Diatoms	Bacillariophyceae	*Chaetoceros minimus*	X				
Diatoms	Bacillariophyceae	*Chaetoceros muellerii*	X		X		
Diatoms	Bacillariophyceae	*Chaetoceros protuberans*	X	X			
Diatoms	Bacillariophyceae	*Chaetoceros socialis*			X	X	
Diatoms	Bacillariophyceae	*Chaetoceros tenuissimus*					X
Diatoms	Bacillariophyceae	*Chaetoceros tetrastichon*					X
Diatoms	Bacillariophyceae	*Chaetoceros throndsenii*	X	X			X
Diatoms	Bacillariophyceae	*Chaetoceros tortissimus*					X
Diatoms	Bacillariophyceae	*Cyclotella choctawhatcheeana*			X		
Diatoms	Bacillariophyceae	*Cyclotella litoralis*		X			
Diatoms	Bacillariophyceae	*Cylindrotheca closterium*					X
Diatoms	Bacillariophyceae	*Dactyliosolen blavyanus*					X
Diatoms	Bacillariophyceae	*Dactyliosolen fragilissimus*					X
Diatoms	Bacillariophyceae	*Eupyxidicula turris*			X	X	
Diatoms	Bacillariophyceae	*Fistulifera pelliculosa*				X	
Diatoms	Bacillariophyceae	*Fistulifera saprophila*	X		X		

Diatoms	Bacillariophyceae	*Guinardia delicatula*	X				
Diatoms	Bacillariophyceae	*Guinardia striata*					X
Diatoms	Bacillariophyceae	*Leptocylindrus aporus*	X				
Diatoms	Bacillariophyceae	*Leptocylindrus convexus*					X
Diatoms	Bacillariophyceae	*Leptocylindrus danicus*			X	X	X
Diatoms	Bacillariophyceae	*Mediolabrus comicus*	X				
Diatoms	Bacillariophyceae	*Melosira varians*		X		X	
Diatoms	Bacillariophyceae	*Navicula phyllepta*	X				
Diatoms	Bacillariophyceae	*Neomoelleria antarctica*				X	
Diatoms	Bacillariophyceae	*Nitzschia gobbii*					X
Diatoms	Bacillariophyceae	*Nitzschia longissima*					X
Diatoms	Bacillariophyceae	*Nitzschia microcephala*	X		X		
Diatoms	Bacillariophyceae	*Nitzschia sigma*					X
Diatoms	Bacillariophyceae	*Papiliocellulus elegans*	X				
Diatoms	Bacillariophyceae	*Pleurosigma intermedium*		X			
Diatoms	Bacillariophyceae	*Pleurosigma planktonicum*			X		
Diatoms	Bacillariophyceae	*Proboscia alata*					X
Diatoms	Bacillariophyceae	*Pseudo-nitzschia australis*				X	
Diatoms	Bacillariophyceae	*Pseudo-nitzschia delicatissima*					X
Diatoms	Bacillariophyceae	*Pseudo-nitzschia pseudodelicatissima*	X		X		X
Diatoms	Bacillariophyceae	*Pseudo-nitzschia pungens*	X		X	X	
Diatoms	Bacillariophyceae	*Pseudosolenia calcar-avis*	X		X		
Diatoms	Bacillariophyceae	*Rhizosolenia fallax*			X		
Diatoms	Bacillariophyceae	*Rhizosolenia imbricata*	X		X		
Diatoms	Bacillariophyceae	*Skeletonema dohrnii*		X		X	
Diatoms	Bacillariophyceae	*Skeletonema marinoi*	X				X
Diatoms	Bacillariophyceae	*Sundstroemia setigera*			X		
Diatoms	Bacillariophyceae	*Thalassionema frauenfeldii*			X		
Diatoms	Bacillariophyceae	*Thalassionema nitzschioides*					X
Diatoms	Bacillariophyceae	*Thalassiosira profunda*	X		X		
Diatoms	Bacillariophyceae	*Thalassiosira rotula*				X	X
Dinoflagellates	Dinophyceae	*Akashiwo sanguinea*	X		X		X
Dinoflagellates	Dinophyceae	*Alexandrium andersonii*		X		X	
Dinoflagellates	Dinophyceae	*Alexandrium catenella*				X	
Dinoflagellates	Dinophyceae	*Alexandrium hiranoi*		X	X	X	
Dinoflagellates	Dinophyceae	*Alexandrium insuetum*				X	
Dinoflagellates	Dinophyceae	*Alexandrium margalefii*	X	X			
Dinoflagellates	Dinophyceae	*Alexandrium minutum*	X				
Dinoflagellates	Dinophyceae	*Ansanella granifera*		X			
Dinoflagellates	Dinophyceae	*Archaeperidinium saanichi*			X	X	
Dinoflagellates	Dinophyceae	*Biecheleria brevisulcata*		X			
Dinoflagellates	Dinophyceae	*Biecheleria cincta*	X				
Dinoflagellates	Dinophyceae	*Biecheleriopsis adriatica*	X	X			
Dinoflagellates	Dinophyceae	*Blastodinium spinulosum*	X				
Dinoflagellates	Dinophyceae	*Blixaea quinquecornis*	X		X		
Dinoflagellates	Dinophyceae	*Dinophysis sacculus*					X

Dinoflagellates	Dinophyceae	*Fragilidium duplocampaniforme*	X	X			
Dinoflagellates	Dinophyceae	*Fragilidium mexicanum*	X	X			
Dinoflagellates	Dinophyceae	*Gonyaulax bohaiensis*	X				
Dinoflagellates	Dinophyceae	*Gonyaulax digitale*	X	X			
Dinoflagellates	Dinophyceae	*Gonyaulax fragilis*	X		X		
Dinoflagellates	Dinophyceae	*Gonyaulax polygramma*	X	X			
Dinoflagellates	Dinophyceae	*Gonyaulax spinifera*		X		X	X
Dinoflagellates	Dinophyceae	*Gonyaulax whaseongensis*	X	X			
Dinoflagellates	Dinophyceae	*Grammatodinium tongyeonginum*	X		X		
Dinoflagellates	Dinophyceae	*Gymnodinium catenatum*	X			X	
Dinoflagellates	Dinophyceae	*Gymnodinium dorsalisulcum*	X				
Dinoflagellates	Dinophyceae	*Gymnodinium impudicum*	X	X	X	X	
Dinoflagellates	Dinophyceae	*Gymnodinium smaydae*		X		X	
Dinoflagellates	Dinophyceae	*Gyrodiniellum shiwhaense*		X			
Dinoflagellates	Dinophyceae	*Gyrodinium dominans*	X				
Dinoflagellates	Dinophyceae	*Gyrodinium fusiforme*	X				
Dinoflagellates	Dinophyceae	*Gyrodinium jinhaense*	X				
Dinoflagellates	Dinophyceae	*Islandinium tricingulatum*	X				
Dinoflagellates	Dinophyceae	*Karenia mikimotoi*				X	
Dinoflagellates	Dinophyceae	*Karenia papilionacea*					X
Dinoflagellates	Dinophyceae	*Kofoidinium pavillardii*	X				
Dinoflagellates	Dinophyceae	*Kofoidinium velleloides*					X
Dinoflagellates	Dinophyceae	*Lebouridinium glaucum*	X				
Dinoflagellates	Dinophyceae	*Leiocephalium pseudosanguineum*				X	
Dinoflagellates	Dinophyceae	*Lepidodinium viride*				X	
Dinoflagellates	Dinophyceae	*Lessardia elongata*	X		X		
Dinoflagellates	Dinophyceae	*Levanderina fissa*	X	X	X	X	
Dinoflagellates	Dinophyceae	*Lingulodinium polyedra*				X	X
Dinoflagellates	Dinophyceae	*Margalefidinium fulvescens*	X		X		
Dinoflagellates	Dinophyceae	*Noctiluca scintillans*	X	X	X	X	X
Dinoflagellates	Dinophyceae	*Oxyphysis oxytoxoides*				X	X
Dinoflagellates	Dinophyceae	*Oxytoxum caudatum*					X
Dinoflagellates	Dinophyceae	*Oxytoxum elongatum*					X
Dinoflagellates	Dinophyceae	*Oxytoxum laticeps*					X
Dinoflagellates	Dinophyceae	*Oxytoxum scolopax*					X
Dinoflagellates	Dinophyceae	*Paragymnodinium shiwhaense*		X	X	X	
Dinoflagellates	Dinophyceae	*Pelagodinium bei*	X				
Dinoflagellates	Dinophyceae	*Phalacroma rotundatum*					X
Dinoflagellates	Dinophyceae	*Polykrikos geminatus*	X	X		X	
Dinoflagellates	Dinophyceae	*Polykrikos hartmannii*		X		X	
Dinoflagellates	Dinophyceae	*Polykrikos kofoidii*	X		X		
Dinoflagellates	Dinophyceae	*Polykrikos schwartzii*	X		X		
Dinoflagellates	Dinophyceae	*Prorocentrum compressum*					X
Dinoflagellates	Dinophyceae	*Prorocentrum cordatum*					X
Dinoflagellates	Dinophyceae	*Prorocentrum dentatum*				X	
Dinoflagellates	Dinophyceae	*Prorocentrum micans*					X

Dinoflagellates	Dinophyceae	*Prorocentrum triestinum*					X
Dinoflagellates	Dinophyceae	*Protoceratium reticulatum*	X	X	X	X	X
Dinoflagellates	Dinophyceae	*Protodinium simplex*	X			X	
Dinoflagellates	Dinophyceae	*Protoperidinium bipes*	X		X		X
Dinoflagellates	Dinophyceae	*Protoperidinium claudicans*					X
Dinoflagellates	Dinophyceae	*Protoperidinium diabolus*					X
Dinoflagellates	Dinophyceae	*Protoperidinium divergens*					X
Dinoflagellates	Dinophyceae	*Protoperidinium ovum*					X
Dinoflagellates	Dinophyceae	*Protoperidinium steinii*					X
Dinoflagellates	Dinophyceae	*Pseliodinium fusus*	X				
Dinoflagellates	Dinophyceae	*Pyrophacus steinii*	X		X		
Dinoflagellates	Dinophyceae	*Scrippsiella acuminata*	X				
Dinoflagellates	Dinophyceae	*Scrippsiella trochoidea*				X	
Dinoflagellates	Dinophyceae	*Spatulodinium pseudonoctiluca*			X		
Dinoflagellates	Dinophyceae	*Syltodinium listii*	X				
Dinoflagellates	Dinophyceae	*Triadinium polyedricum*		X		X	
Dinoflagellates	Dinophyceae	*Tripos extensus*					X
Dinoflagellates	Dinophyceae	*Tripos furca*	X				X
Dinoflagellates	Dinophyceae	*Tripos fusus*	X	X			X
Dinoflagellates	Dinophyceae	*Tripos tenuis*		X		X	
Dinoflagellates	Dinophyceae	*Wangodinium sinense*	X				
Dinoflagellates	Dinophyceae	*Yihiella yeosuensis*	X	X			
Phytoflagellates	Bicosoecophyceae	*Bicosoeca petiolata*		X			
Phytoflagellates	Bicosoecophyceae	*Bicosoeca vacillans*		X			
Phytoflagellates	Bolidophyceae	*Triparma laevis*	X				
Phytoflagellates	Bolidophyceae	*Triparma mediterranea*	X	X			
Phytoflagellates	Bolidophyceae	*Triparma pacifica*	X	X	X	X	
Phytoflagellates	Bolidophyceae	*Triparma strigata*			X		
Phytoflagellates	Chlorarachniophyceae	*Bigelowiella natans*				X	
Phytoflagellates	Chlorarachniophyceae	*Chlorarachnion reptans*	X	X		X	
Phytoflagellates	Chlorarachniophyceae	*Lotharella oceanica*				X	
Phytoflagellates	Chlorarachniophyceae	*Minorisa minuta*	X	X			
Phytoflagellates	Chlorarachniophyceae	*Partenskyella glossopodia*		X			
Phytoflagellates	Chlorodendrophyceae	*Tetraselmis convolutae*	X				
Phytoflagellates	Chlorodendrophyceae	*Tetraselmis marina*		X	X		
Phytoflagellates	Chlorophyceae	*Chlamydomonas kuwadae*	X				
Phytoflagellates	Chlorophyceae	*Chlorochytrium lemnae*				X	
Phytoflagellates	Chlorophyceae	*Desmodesmus abundans*		X		X	
Phytoflagellates	Choanoflagellatea	*Helgoeca nana*		X			
Phytoflagellates	Chrysophyceae	*Chrysolepidomonas dendrolepidota*	X		X		
Phytoflagellates	Chrysophyceae	*Dinobryon faculiferum*					X
Phytoflagellates	Chrysophyceae	*Dinobryon porrectum*					X
Phytoflagellates	Chrysophyceae	*Ochromonas distigma*			X		
Phytoflagellates	Chrysophyceae	*Paraphysomonas foraminifera*		X			
Phytoflagellates	Chrysophyceae	*Paraphysomonas imperforata*			X	X	
Phytoflagellates	Chrysophyceae	*Poterioochromonas malhamensis*	X				

Phytoflagellates	Coccolithophyceae	*Chrysochromulina acantha*		X			
Phytoflagellates	Coccolithophyceae	*Chrysochromulina campanulifera*		X			
Phytoflagellates	Coccolithophyceae	*Chrysochromulina cymbium*		X			
Phytoflagellates	Coccolithophyceae	*Chrysochromulina leadbeateri*	X	X	X		
Phytoflagellates	Coccolithophyceae	*Chrysochromulina rotalis*	X	X			
Phytoflagellates	Coccolithophyceae	*Chrysochromulina scutellum*		X			
Phytoflagellates	Coccolithophyceae	*Chrysochromulina simplex*		X			
Phytoflagellates	Coccolithophyceae	*Chrysochromulina spinifera*	X	X	X		
Phytoflagellates	Coccolithophyceae	*Chrysochromulina tobinii*		X			
Phytoflagellates	Coccolithophyceae	*Haptolina herdlensis*		X			
Phytoflagellates	Coccolithophyceae	*Phaeocystis cordata*	X	X			
Phytoflagellates	Cryptophyceae	*Geminigera cryophila*				X	
Phytoflagellates	Cryptophyceae	*Hemiselmis aquamarina*	X				
Phytoflagellates	Cryptophyceae	*Hemiselmis cryptochromatica*			X		
Phytoflagellates	Cryptophyceae	*Hemiselmis rufescens*			X	X	
Phytoflagellates	Cryptophyceae	*Rhinomonas nottbecki*			X		
Phytoflagellates	Cryptophyceae	*Teleaulax acuta*	X	X			
Phytoflagellates	Cryptophyceae	*Teleaulax amphioxeia*	X	X	X		
Phytoflagellates	Dictyochophyceae	*Dictyocha fibula*					X
Phytoflagellates	Dictyochophyceae	*Dictyocha octonaria*			X		
Phytoflagellates	Dictyochophyceae	*Dictyocha speculum*	X	X		X	
Phytoflagellates	Dictyochophyceae	*Pedinella elastica*	X	X			
Phytoflagellates	Dictyochophyceae	*Pseudochattonella farcimen*		X			
Phytoflagellates	Dictyochophyceae	*Pseudochattonella verruculosa*			X	X	
Phytoflagellates	Dictyochophyceae	*Rhizochromulina marina*		X			
Phytoflagellates	Dictyochophyceae	*Vicicitus globosus*	X				
Phytoflagellates	Eustigmatophyceae	*Microchloropsis gaditana*			X		
Phytoflagellates	Eustigmatophyceae	*Microchloropsis salina*				X	
Phytoflagellates	Goniomonadophyceae	*Goniomonas amphinema*	X				
Phytoflagellates	Imbricatea	*Paulinella chromatophora*		X			
Phytoflagellates	Mamiellophyceae	*Bathycoccus prasinos*	X				
Phytoflagellates	Mamiellophyceae	*Dolichomastix tenuilepis*	X	X	X	X	
Phytoflagellates	Mamiellophyceae	*Mamiella gilva*	X	X	X	X	
Phytoflagellates	Mamiellophyceae	*Mantoniella squamata*		X			
Phytoflagellates	Mamiellophyceae	*Micromonas bravo*	X	X			
Phytoflagellates	Mamiellophyceae	*Micromonas commoda*	X	X			
Phytoflagellates	Mamiellophyceae	*Micromonas pusilla*			X		
Phytoflagellates	Mamiellophyceae	*Microrhizoidea pickettheapsiorum*		X			
Phytoflagellates	Mamiellophyceae	*Ostreococcus mediterraneus*	X	X			
Phytoflagellates	Mamiellophyceae	*Ostreococcus tauri*	X				
Phytoflagellates	Pelagophyceae	*Aureococcus anophagefferens*	X				
Phytoflagellates	Pelagophyceae	*Pelagomonas calceolata*	X				
Phytoflagellates	Pinguiophyceae	*Phaeomonas parva*	X	X	X	X	
Phytoflagellates	Pyramimonadophyceae	*Cymbomonas tetramitiformis*	X	X		X	
Phytoflagellates	Pyramimonadophyceae	*Pterosperma cristatum*	X		X		
Phytoflagellates	Pyramimonadophyceae	*Pycnococcus provasolii*	X	X	X	X	

Phytoflagellates	Pyramimonadophyceae	*Pyramimonas aurea*		X			
Phytoflagellates	Pyramimonadophyceae	*Pyramimonas australis*			X		
Phytoflagellates	Pyramimonadophyceae	*Pyramimonas disomata*		X			
Phytoflagellates	Pyramimonadophyceae	*Pyramimonas olivacea*		X		X	
Phytoflagellates	Pyramimonadophyceae	*Pyramimonas parkeae*		X		X	
Phytoflagellates	Pyramimonadophyceae	*Pyramimonas tetrarhynchus*		X		X	
Phytoflagellates	Raphidophyceae	*Chattonella subsalsa*	X		X	X	
Phytoflagellates	Raphidophyceae	*Fibrocapsa japonica*	X	X	X	X	
Phytoflagellates	Telonemea	*Telonema antarcticum*	X	X		X	
Phytoflagellates	Telonemea	*Telonema subtile*	X				
Phytoflagellates	Trebouxiophyceae	*Chloroidium saccharophilum*				X	
Phytoflagellates	Trebouxiophyceae	*Picochlorum maculatum*		X	X	X	
Phytoflagellates	Trebouxiophyceae	*Prototheca wickerhamii*		X			
Phytoflagellates	Trebouxiophyceae	*Trebouxia crenulata*		X			

DISCUSSION

In this study, microscopy analysis is coupled with metabarcoding using two markers of the 18S rRNA gene (V4 and V9) and two databases (PR2 and SILVA). Compared to the known phytoplankton community of the study area identified so far, comprising 479 species (Neri et al., 2023; Totti et al., 2019, 2005, 2002), this study, using metabarcoding too, identified additional 99 genera (8 diatoms, 32 dinoflagellates, 1 coccolithophore, and 58 phytoflagellates) and 151 species (23 diatoms, 50 dinoflagellates, 1 coccolithophore, and 77 phytoflagellates).

The phytoplankton community strongly change when microscopy and metabarcoding approaches were used. High percentages values of dinoflagellate and diatom genera were identified through microscopy, while high percentages of phytoflagellates were found by metabarcoding, followed by dinoflagellates and diatoms.

On the other hand, for diatoms, dinoflagellates and phytoflagellates, metabarcoding reported taxa never observed so far. The reasons why metabarcoding can identify different genera and species have been reported in previous studies (Bilbao et al., 2023; Mordret et al., 2018; Pierce et al., 2023; Piredda et al., 2017; Santi et al., 2021). Firstly, microscopy analysis of phytoplankton involves observation of fixed samples under a light microscope. This technique allows for the identification of phytoplankton cells based on their morphology, but it has some intrinsic limitations. Formalin, commonly used to preserve samples, can damage or destroy organisms without cell walls (e.g., naked dinoflagellates or raphidophytes), leading to an underestimation of their presence. Small organisms, such as phytoflagellates, require the use of scanning electron microscopy (SEM) or transmission electron microscopy (TEM) for proper identification, as the ultrastructural details necessary for their determination are not visible under a light microscope. Furthermore, the volume of seawater used for microscopic analysis (from 0.05 to 2 ml) is significantly smaller than that processed by metabarcoding analysis (2 L in this study) (Bilbao et al., 2023; Gran-Stadniczeñko et al., 2019; Piredda et al., 2017). This reduces the likelihood of detecting rare taxa, parasites, and symbionts (e.g., *Pelagodinium bei*,

Blastodinium spp., *Zooxanthella* spp.) through microscopy. Finally, the accuracy of identification strongly depends on the skill and experience of the operator, introducing a factor of subjectivity that can lead to errors.

However, it is important to consider that, despite its advantages, metabarcoding also has some sources of error. Indeed, the higher number of genera and/or species detected might not be entirely accurate or real, leading to an overestimation caused by various technical errors occurring during different stages of the process, including polymerase chain reaction (which can introduce errors affecting the accuracy of species identification), the sequencing process, and data analysis through bioinformatics pipelines (Behnke et al., 2011; Ershova et al., 2023; Marinchel et al., 2023; Preston et al., 2022; Santoferrara, 2019). PCR can amplify some taxa more efficiently than others due to differences in target sequences and DNA conformation, leading to a distortion in abundance estimates (Behnke et al., 2011; Ershova et al., 2023; Marinchel et al., 2023; Preston et al., 2022; Santoferrara, 2019) compared to microscopic analysis, which is not influenced by such amplification effects.

It is also important to consider that the ability to detect and correctly identify all taxa present in a sample through metabarcoding can be limited by factors such as the specificity of universal primers (Kelly et al., 2019; Pawlowski et al., 2011; Stoeck et al., 2010) and the similarity of marker sequences among different taxa (Gran-Stadniczeñko et al., 2019; Mordret et al., 2018; Piganeau et al., 2011). In the first case, although primers are called "universal," they do not always amplify all sequences with the same efficiency. Some taxa may have variants in the regions where primers should bind, resulting in lower amplification efficiency for these taxa. In the second case, if marker sequences are too similar, the taxonomic assignment process may fail to correctly separate the taxa, leading to identification errors or the failure to detect some taxa (Mordret et al., 2018). For these reasons, the primers used in this study were verified to have no mismatches, confirming effective binding to the target sequences. Therefore, the non-detectability of certain taxa cannot be attributed to issues with the primers themselves (Stadhouders et al., 2010).

Although the use of different genetic markers (V4 and V9 regions) and databases (PR2 and SILVA) has allowed for the detection of greater diversity than that detected by microscopy, the latter identified some organisms that metabarcoding did not detect, both among genera (3 diatoms, 3 dinoflagellates, 4 coccolithophores, and 4 phytoflagellates), species (17 diatoms, 18 dinoflagellates, 6 coccolithophores, and 3 phytoflagellates). The absence of these sequences highlights the urgent need to update databases (Mordret et al., 2023; Piredda et al., 2017; Santi et al., 2021; Turk Dermastia et al., 2023) to better represent diversity, allowing for more comprehensive and representative studies of communities. One of the most surprising results of this study was the almost total absence of coccolithophore genera and species revealed by metabarcoding analysis. This is primarily because only a number of coccolithophore taxa are lacking from the reference databases, probably due to the difficulty of cultivation already reported for this group (Probert & Houdan, 2004). Indeed, there are few studies that combine morphological and molecular analyses (Gran-Stadniczeñko et al., 2017), preventing the updating of databases.

The typical annual trend of the phytoplankton community of the northwestern Adriatic Sea has been highlighted in previous studies: diatom blooms occur in winter, spring, and autumn, and between these blooms, phytoplankton communities are dominated by heterogeneous communities of small phytoflagellates, while dinoflagellates increase only in spring and summer. Coccolithophorids are a minor but persistent component of winter communities, with a peak in spring (Bernardi Aubry et al., 2004; Totti et al., 2019, 2005). The assessment of the abundance trend is possible only using microscopy, as metabarcoding approaches do not allow the estimation of abundances.

Considering the community structure (in terms of percent groups), it significantly changes when microscopy and metabarcoding approaches are used. While microscopy showed that all months were dominated by phytoflagellates, metabarcoding indicated that dinoflagellates were the most prevalent group in every month except February and September, where diatoms were more abundant. This could be explained considering that some species have a significantly different number of gene copies

compared to others, affecting the number of sequences obtained through metabarcoding (Mäki et al., 2017; Not et al., 2009). For this reason, to better compare the relative abundances obtained from microscopic analysis and the GCN derived from metabarcoding, the conversion factors to convert GCN to abundance and biomass described in the study by Lapeyra Martin et al. (2022) was applied. After conversion, a greater similarity between the relative abundances obtained by both microscopy and metabarcoding is easily noticeable, leading to an increase of phytoflagellates, and a decrease of dinoflagellates. This can be explained by the hypothesis that cells with large DNA content, such as those of dinoflagellates (Hackett et al., 2004; LaJeunesse et al., 2005; Wang et al., 2024), provide many reads during sequencing. In contrast, the conversion factor seems to underestimate the relative abundances of diatoms. This could be explained by the fact that diatoms have highly variable biovolumes, ranging from 10^1 a 10^9 μm^3 (Vasselon et al., 2018), and as the biovolume increases, so do the copies of the 18S rRNA gene (Godhe et al., 2008). This large natural variability in biovolume and GCN makes it challenging to develop and apply a CF that is accurate for all diatoms; it might be useful to use CFs at the genus level (Vasselon et al., 2018). Regarding coccolithophores, the use of CF improves the match between microscopy and metabarcoding. Therefore, the use of CFs for relative abundances made the values of metabarcoding more similar to those of microscopy, compared to the data without the use of CFs, as also highlighted by the NMDS analysis.

Regarding the biomasses, considering that metabarcoding measures the quantity of sequences present in a sample, larger cells (e.g., dinoflagellates) with more copies of the 18S rRNA gene (Godhe et al., 2008; Prokopowich et al., 2003; Zhu et al., 2005) are likely overestimated. Therefore, using biomass in microscopy analyses makes the results more comparable with metabarcoding data because both methods give greater weight to larger organisms, as highlighted by this study. However, only in a few months (May, June, and October) the different approaches reported a similar community composition, with a marked dominance of dinoflagellates. In the other months, in terms of biomass, MB suggested that diatoms prevailed in the community, whereas, following microscopy, dinoflagellates were the

dominating group. This is in contrast with previous studies (Piredda et al., 2017; Santi et al., 2021), where dinoflagellates consistently appeared with higher percentages in metabarcoding. Phytoflagellates appear throughout the study period with higher percentages in metabarcoding compared to microscopy. This is explained because they are difficult to recognize due to their small size (Caron et al., 2012; Cavalier-Smith & Chao, 2006).

In an attempt to standardize the results obtained from microscopy and metabarcoding, we decided to apply the second conversion factor proposed by Lapeyra Martin et al. (2022) to our results, but in this case, the conversion factor did not smooth the differences between the two approaches. Using the CF in the metabarcoding value, the percentage of phytoflagellates increased in each month, compared to the lower biomass obtained by microscopy, as did diatoms (except in June and July). Dinoflagellates showed lower biomass values with metabarcoding than with microscopy, in contrast to the study by Lapeyra Martin et al. (2022). The large genome of dinoflagellates generates more reads, and by applying the conversion factor, the expected result was a percentage closer to microscopy.

Biomass estimates in phytoplankton samples can be affected by several sources of error, including errors in cell size measurements, morphological variations due to the use of fixatives (Godhe et al., 2008), and variations in carbon density between cells of the same group and species but with different sizes (e.g., diatoms at different life cycle stages) (Harrison et al., 2015). These biases limit the effectiveness of conversion factors in improving the accuracy of biomass estimates obtained by metabarcoding. Furthermore, as highlighted by Lapeyra Martin et al. (2022), CF values were obtained from organisms in their reference database, and this could affect replicability, making it difficult to apply the same approach in other areas or with other phytoplankton communities.

Therefore, the use of CFs for biomass emphasized the differences between metabarcoding and microscopy, compared to the data without the use of CFs, as also highlighted by the NMDS analysis.

CONCLUSIONS

In conclusion, this study highlighted the importance of combining microscopy and metabarcoding for a more comprehensive understanding of phytoplankton communities. Indeed, metabarcoding represents a valid approach to implement the evaluation of phytoplankton diversity leading to recognise cryptic/pseudocryptic species and/or low abundant (often potentially toxic) species. However, different regions and databases should still be combined with microscopy to have a more detailed information on the community and to counteract the drawbacks of metabarcoding. Undoubtedly, the resolution provided by metabarcoding is constantly increasing thanks to the rapidity with which the molecular approach is improving. However, the metabarcoding approach still exhibits significant gaps in the reference databases, especially for underrepresented groups such as coccolithophores. These gaps could be addressed by incorporating sequences of missing species, ideally obtained from the same type locality as in the original species description.

Finally, this study has demonstrated the effectiveness of the combined approach of microscopy analysis and metabarcoding for the abundances and biomasses of the phytoplankton community. Microscopy analysis tends to overestimate or underestimate the abundances or the biomass of some groups. The use of conversion factors to compare relative abundances and biomasses between microscope and metabarcoding gave interesting but not always concordant results, indicating that further improvements are needed. In particular, the difficulty of applying accurate conversion factors for groups with large variations in biovolume, such as diatoms, highlights the complexity of standardizing estimates between different methods. In this study, the conversion factors allow for smoothing differences between microscope and metabarcoding only regarding abundances, but not biomasses. In this latter case, it is still not clear which biomass values (those estimated from metabarcoding and those from microscopy) were more accurate.

REFERENCES

Amaral-Zettler, L.A., McCliment, E.A., Ducklow, H.W., Huse, S.M., 2009. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of Small-Subunit Ribosomal RNA Genes. PLoS ONE 4, e6372. https://doi.org/10.1371/journal.pone.0006372

Amato, A., Montresor, M., 2008. Morphology, phylogeny, and sexual cycle of *Pseudo-nitzschia mannii* sp. nov. (Bacillariophyceae): a pseudo-cryptic species within the *P. pseudodelicatissima* complex. Phycologia 47, 487–497. https://doi.org/10.2216/07-92.1

Amato, A., Kooistra, W., Levialdighiron, J., Mann, D., Proschold, T., Montresor, M., 2007. Reproductive isolation among sympatric cryptic species in marine diatoms. Protist 158, 193–207. https://doi.org/10.1016/j.protis.2006.10.001

Andersson, A., Jurgensone, I., Rowe, O.F., Simonelli, P., Bignert, A., Lundberg, E., Karlsson, J., 2013. Can humic water discharge counteract eutrophication in coastal waters? PLoS ONE 8, e61293. https://doi.org/10.1371/journal.pone.0061293

Andrews, S., 2010. FastQC: a quality control tool for high throughput sequence data.

Artegiani, A., Paschini, E., Russo, A., Bregant, D., Raicich, F., Pinardi, N., 1997a. The Adriatic Sea General Circulation. Part I: Air–Sea Interactions and Water Mass Structure. J. Phys. Oceanogr. 27, 1492–1514. https://doi.org/10.1175/1520-0485(1997)027<1492:TASGCP>2.0.CO;2

Artegiani, A, Paschini, E., Russo, A., Bregant, D., Raicich, F., Pinardi, N., 1997b. The Adriatic Sea general circulation. Part II: baroclinic circulation structure. J. Phys. Oceanogr. 27, 1515–1532. https://doi.org/10.1175/1520-0485(1997)027<1515:TASGCP>2.0.CO;2

Barnes, M.A., Turner, C.R., 2016. The ecology of environmental DNA and implications for conservation genetics. Conserv. Genet. 17, 1–17. https://doi.org/10.1007/s10592-015-0775-4

Beardall, J., Stojkovic, S., Larsen, S., 2009. Living in a high CO_2 world: impacts of global climate change on marine phytoplankton. Plant Ecol. Divers. 2, 191–205. https://doi.org/10.1080/17550870903271363

Behnke, A., Engel, M., Christen, R., Nebel, M., Klein, R.R., Stoeck, T., 2011. Depicting more accurate pictures of protistan community complexity using pyrosequencing of hypervariable SSU rRNA gene regions. Environ. Microbiol. 13, 340–349. https://doi.org/10.1111/j.1462-2920.2010.02332.x

Bensi, M., Cardin, V., Rubino, A., Notarstefano, G., Poulain, P.M., 2013. Effects of winter convection on the deep layer of the Southern Adriatic Sea in 2012. J. Geophys. Res. Oceans 118, 6064–6075. https://doi.org/10.1002/2013JC009432

Berdalet, E, Fleming, Gowen, R, Davidson, K. Hess, P., Backer, L.C., Moore, S.K., Hoagland, P., Enevoldsen, H., 2016. Marine harmful algal blooms, human health and wellbeing: challenges and opportunities in the 21st century. J. Mar. Biol. Assoc. U. K. 96(1), 61–91.

Bernardi Aubry, F.B., Berton, A., Bastianini, M., Socal, G., Acri, F., 2004. Phytoplankton succession in a coastal area of the NW Adriatic, over a 10-year sampling period (1990–1999). Cont. Shelf Res. 24, 97–115. https://doi.org/10.1016/j.csr.2003.09.007

Bernardi Aubry, F., Pugnetti, A., Roselli, L., Stanca, E., Acri, F., Finotto, S., Basset, A., 2017. Phytoplankton morphological traits in a nutrient-enriched, turbulent Mediterranean microtidal lagoon. J. Plankton Res. 39, 564–576. https://doi.org/10.1093/plankt/fbx008

Bernardi Aubry, F., Acri, F., Finotto, S., Pugnetti, A., 2021. Phytoplankton Dynamics and Water Quality in the Venice Lagoon. Water 13, 2780. https://doi.org/10.3390/w13192780

Bilbao, J., Pavloudi, C., Blanco-Rayón, E., Franco, J., Madariaga, I., Seoane, S., 2023. Phytoplankton community composition in relation to environmental variability in the Urdaibai estuary (SE Bay of Biscay): Microscopy and eDNA metabarcoding. Mar. Environ. Res. 191, 106175. https://doi.org/10.1016/j.marenvres.2023.106175

Boldrin, A., Langone, L., Miserocchi, S., Turchetto, M., Acri, F., 2005. Po River plume on the Adriatic continental shelf: Dispersion and sedimentation of dissolved and suspended matter during different river discharge rates. Mar. Geol. 222–223, 135–158. https://doi.org/10.1016/j.margeo.2005.06.010

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., Van Der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., Von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9

Boni, L., Pompei, M., Reti, M., 1983. The occurrence of *Gonyaulax tamarensis* Lebour bloom in the Adriatic Sea along the coast of Emilia-Romagna. G. Bot. Ital. 117, 115–120. https://doi.org/10.1080/11263508309427964

Bukin, Y.S., Mikhailov, I.S., Petrova, D.P., Galachyants, Y.P., Zakharova, Y.R., Likhoshway, Y.V., 2023. The effect of metabarcoding 18S rRNA region choice on diversity of microeukaryotes including phytoplankton. World J. Microbiol. Biotechnol. 39, 229. https://doi.org/10.1007/s11274-023-03678-1

Buljan, M., Zore-Armanda, M., 1976. Oceanographical properties of the Adriatic Sea. Ocean. Mar. Biol. Ann. Rev., 14, 11–98.

Cabrini, M., Fornasaro, D., Cossarini, G., Lipizer, M., Virgilio, D., 2012. Phytoplankton temporal changes in a coastal northern Adriatic site during the last 25 years. Estuar. Coast. Shelf Sci. 115, 113–124. https://doi.org/10.1016/j.ecss.2012.07.007

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869

Campanelli, A., Grilli, F., Paschini, E., Marini, M., 2011. The influence of an exceptional Po River flood on the physical and chemical oceanographic properties of the Adriatic Sea. Dyn. Atmospheres Oceans 52, 284–297. https://doi.org/10.1016/j.dynatmoce.2011.05.004

Campbell, L., Thompson, P.A., 2023. Trends in phytoplankton over space and time. J. Plankton Res. 45, 144–145. https://doi.org/10.1093/plankt/fbac065

Caporaso, J.G., Paszkiewicz, K., Field, D., Knight, R., Gilbert, J.A., 2012. The Western English Channel contains a persistent microbial seed bank. ISME J. 6, 1089–1093. https://doi.org/10.1038/ismej.2011.162

Caron, D.A., Countway, P.D., Jones, A.C., Kim, D.Y., Schnetzer, A., 2012. Marine protistan diversity. Annu. Rev. Mar. Sci. 4, 467–493. https://doi.org/10.1146/annurev-marine-120709-142802

Carugati, L., Corinaldesi, C., Dell'Anno, A., Danovaro, R., 2015. Metagenetic tools for the census of marine meiofaunal biodiversity: An overview. Mar. Genomics 24, 11–20. https://doi.org/10.1016/j.margen.2015.04.010

Castellani, C., Edwards, M., 2017. Marine plankton: a practical guide to ecology, methodology, and taxonomy. Oxford University Press.

Cavalier-Smith, T., Chao, E.E.-Y., 2006. Phylogeny and megasystematics of phagotrophic heterokonts (Kingdom Chromista). J. Mol. Evol. 62, 388–420. https://doi.org/10.1007/s00239-004-0353-8

Cerino, F., Fornasaro, D., Kralj, M., Giani, M., Cabrini, M., 2019. Phytoplankton temporal dynamics in the coastal waters of the north-eastern Adriatic Sea (Mediterranean Sea) from 2010 to 2017. Nat. Conserv. 34, 343–372. https://doi.org/10.3897/natureconservation.34.30720

Chepurnov, V.A., Mann, D.G., Sabbe, K., Vyverman, W., 2004. Experimental studies on sexual reproduction in diatoms. Int. Rev. Cytol., 237, 91–154. https://doi.org/10.1016/S0074-7696(04)37003-8

Cloern, J.E., Jassby, A.D., 2010. Patterns and scales of phytoplankton variability in estuarine–coastal ecosystems. Estuaries Coasts 33, 230–241. https://doi.org/10.1007/s12237-009-9195-3

Cozzi, S., Lipizer, M., Cantoni, C., Catalano, G., 2002. Nutrient balance in the ecosystem of the Northwestern Adriatic Sea. Chem. Ecol. 18, 1–12. https://doi.org/10.1080/02757540212685

Cozzi, S., Cabrini, M., Kralj, M., De Vittor, C., Celio, M., Giani, M., 2020. Climatic and anthropogenic impacts on environmental conditions and phytoplankton community in the Gulf of Trieste (Northern Adriatic Sea). Water 12, 2652. https://doi.org/10.3390/w12092652

Cucchiari, E., Pistocchi, R., Pezzolesi, L., Penna, A., Battocchi, C., Cerino, F., Totti, C., 2010. Resting cysts of *Fibrocapsa japonica* (Raphidophyceae) from coastal sediments of the northern Adriatic Sea (Mediterranean Sea). Harmful Algae 10, 81–87. https://doi.org/10.1016/j.hal.2010.07.003

Cushman-Roisin, B., Gacic, M., Poulain, P.M., Artegiani, A., 2013. Physical oceanography of the Adriatic Sea: past, present and future. Springer Sci. Bus. Media.

Dagenais-Bellefeuille, S., Morse, D., 2013. Putting the N in dinoflagellates. Front. Microbiol. 4. https://doi.org/10.3389/fmicb.2013.00369

De Clerck, O., Bogaert, K.A., Leliaert, F., 2012. Diversity and evolution of algae. Adv. Bot. Res. 64, 55–86. https://doi.org/10.1016/B978-0-12-391499-6.00002-5

de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., Lara, E., Berney, C., Le Bescot, N., Probert, I., Carmichael, M., Poulain, J., Romac, S., Colin, S., Aury, J.-M., Bittner, L., Chaffron, S., Dunthorn, M., Engelen, S., Flegontova, O., Guidi, L., Horák, A., Jaillon, O., Lima-Mendez, G., Lukeš, J., Malviya, S., Morard, R., Mulot, M., Scalco, E., Siano, R., Vincent, F., Zingone, A., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis, S., Acinas, S.G., Bork, P., Bowler, C., Gorsky, G., Grimsley, N., Hingamp, P., Iudicone, D., Not, F., Ogata, H., Pesant, S., Raes, J., Sieracki, M.E., Speich, S., Stemmann, L., Sunagawa, S., Weissenbach, J., Wincker, P., Karsenti, E., Boss, E., Follows, M., Karp-Boss, L., Krzic, U., Reynaud, E.G., Sardet, C., Sullivan, M.B.,

Velayoudon, D., 2015. Eukaryotic plankton diversity in the sunlit ocean. Science 348, 6237. https://doi.org/10.1126/science.1261605

Debroas, D., Domaizon, I., Humbert, J.-F., Jardillier, L., Lepère, C., Oudart, A., Taïb, N., 2017. Overview of freshwater microbial eukaryotes diversity: a first analysis of publicly available metabarcoding data. FEMS Microbiol. Ecol. 93, fix023. https://doi.org/10.1093/femsec/fix023

Degobbis, D., Malej, A., Fonda Umani, S., 1999. The mucilage phenomenon in the northern Adriatic Sea. A critical review of the present scientific hypotheses. Ann Ist Super Sanità 35, 81–373.

Degobbis, D., Precali, R., Ivancic, I., Smodlaka, N., Fuks, D., Kveder, S., 2000. Long-term changes in the northern Adriatic ecosystem related to anthropogenic eutrophication. Int. J. Environ. Pollut. 13, 495–533. https://doi.org/10.1504/IJEP.2000.002332

Deiner, K., Walser, J.-C., Mächler, E., Altermatt, F., 2015. Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. Biol. Conserv. 183, 53–63. https://doi.org/10.1016/j.biocon.2014.11.018

Deiner, K., Yamanaka, H., Bernatchez, L., 2021. The future of biodiversity monitoring and conservation utilizing environmental DNA. Environ. DNA 3, 3–7. https://doi.org/10.1002/edn3.178

Desrosiers, C., Leflaive, J., Eulin, A., Ten-Hage, L., 2013. Bioindicators in marine waters: Benthic diatoms as a tool to assess water quality from eutrophic to oligotrophic coastal ecosystems. Ecol. Indic. 32, 25–34. https://doi.org/10.1016/j.ecolind.2013.02.021

Didaskalou, E.A., Trimbos, K.B., Stewart, K.A., 2022. Environmental DNA. Curr. Biol. 32, R1250–R1252. https://doi.org/10.1016/j.cub.2022.09.052

Djakovac, T., Degobbis, D., Supić, N., Precali, R., 2012. Marked reduction of eutrophication pressure in the northeastern Adriatic in the period 2000–2009. Estuar. Coast. Shelf Sci. 115, 25–32. https://doi.org/10.1016/j.ecss.2012.03.029

Edgar, R.C., 2016. SINTAX: a simple non-Bayesian taxonomy classifier for 16S and ITS sequences. bioRxiv. https://doi.org/10.1101/074161

Edler, L., Elbrachter, M., 2010. The Utermöhl method for quantitative phytoplankton analysis. In: Karlson, B., Cusack, C., Bresnan, E. (Eds.), Microscopic and Molecular Methods for Quantitative Phytoplankton Analysis, vol. 55. IOC UNESCO, Manuals and Guides, pp. 13–20.

Eikrem, W., Medlin, L.K., Henderiks, J., Rokitta, S., Rost, B., Probert, I., Throndsen, J., Edvardsen, B., 2016. Haptophyta. Handbook of the Protists. Springer International Publishing, Cham, pp. 1–61. https://doi.org/10.1007/978-3-319-32669-6_38-1

Ershova, E.A., Wangensteen, O.S., Falkenhaug, T., 2023. Mock samples resolve biases in diversity estimates and quantitative interpretation of zooplankton metabarcoding data. Mar. Biodivers. 53, 66. https://doi.org/10.1007/s12526-023-01372-x

European Parliament, 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive)

Ficetola, G.F., Miaud, C., Pompanon, F., Taberlet, P., 2008. Species detection using environmental DNA from water samples. Biol. Lett. 4, 423–425. https://doi.org/10.1098/rsbl.2008.0118

Field, C.B., Behrenfeld, M.J., Randerson, J.T., Falkowski, P., 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281, 237–240. https://doi.org/10.1126/science.281.5374.237

Fischer, A., Moberg, E., Alexander, H., Brownlee, E., Hunter-Cevera, K., Pitz, K., Rosengard, S., Sosik, H., 2014. Sixty years of Sverdrup: a retrospective of progress in the study of phytoplankton blooms. Oceanography 27, 222–235. https://doi.org/10.5670/oceanog.2014.26

Fonseca, V.G., Kirse, A., Giebner, H., Vause, B.J., Drago, T., Power, D.M., Peck, L.S., Clark, M.S., 2022. Metabarcoding the Antarctic Peninsula biodiversity using a multi-gene approach. ISME Commun. 2. https://doi.org/10.1038/s43705-022-00118-3

Fuhrman, J.A., McCallum, K., Davis, A.A., 1993. Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific Oceans. Appl. Environ. Microbiol. 59, 1294–1302. https://doi.org/10.1128/aem.59.5.1294-1302.1993

Gačić, M., Kovačević, V., Cosoli, S., Mazzoldi, A., Paduan, J.D., Mancero-Mosquera, I., Yari, S., 2009. Surface current patterns in front of the Venice Lagoon. Estuar. Coast. Shelf Sci. 82, 485–494. https://doi.org/10.1016/j.ecss.2009.02.012

Gai, F.F., Hedemand, C.K., Louw, D.C., Grobler, K., Krock, B., Moestrup, Ø., Lundholm, N., 2018. Morphological, molecular and toxigenic characteristics of Namibian *Pseudo-nitzschia* species – including *Pseudo-nitzschia bucculenta* sp. nov. Harmful Algae 76, 80–95. https://doi.org/10.1016/j.hal.2018.05.003

Giani, M., Rinaldi, A., Degobbis, D., 2005. Mucilages in the Adriatic and Tyrrhenian Sea: An introduction. Sci. Total Environ. 353, 3–9. https://doi.org/10.1016/j.scitotenv.2005.09.006

Giani, M., Djakovac, T., Degobbis, D., Cozzi, S., Solidoro, C., Umani, S.F., 2012. Recent changes in the marine ecosystems of the northern Adriatic Sea. Estuar. Coast. Shelf Sci. 115, 1–13. https://doi.org/10.1016/j.ecss.2012.08.023

Giering, S.L.C., Sanders, R., Lampitt, R.S., Anderson, T.R., Tamburini, C., Boutrif, M., Zubkov, M.V., Marsay, C.M., Henson, S.A., Saw, K., Cook, K., Mayor, D.J., 2014. Reconciliation of the carbon budget in the ocean's twilight zone. Nature 507, 480–483. https://doi.org/10.1038/nature13123

Giovannoni, S.J., Britschgi, T.B., Moyer, C.L., Field, K.G., 1990. Genetic diversity in Sargasso Sea bacterioplankton. Nature 345, 60–63. https://doi.org/10.1038/345060a0

Glibert, P.M., Berdalet, E., Burford, M.A., Pitcher, G.C., Zhou, M. (2018). Harmful Algal Blooms and the Importance of Understanding Their Ecology and Oceanography. In: Glibert, P., Berdalet, E., Burford, M., Pitcher, G., Zhou, M. (eds) Global Ecology and Oceanography of Harmful Algal Blooms. Ecological Studies, vol 232. Springer, Cham. https://doi.org/10.1007/978-3-319-70069-4_2

Godhe, A., Asplund, M.E., Härnström, K., Saravanan, V., Tyagi, A., Karunasagar, I., 2008. Quantification of diatom and dinoflagellate biomasses in coastal marine seawater samples by Real-Time PCR. Appl. Environ. Microbiol. 74, 7174–7182. https://doi.org/10.1128/AEM.01298-08

Gonzalez, J.M., Portillo, M.C., Belda-Ferre, P., Mira, A., 2012. Amplification by PCR artificially reduces the proportion of the rare biosphere in microbial communities. PLoS ONE 7, e29973. https://doi.org/10.1371/journal.pone.0029973

Gopalakrishnakone, P., Haddad, V., Tubaro, A., Kim, E., Kem, W.R. (Eds.), 2016. Marine and freshwater toxins. Springer Netherlands, Dordrecht. https://doi.org/10.1007/978-94-007-6419-4

Graham, L.E., Kaneko, Y., Renzaglia, K., 1991. Subcellular structures of relevance to the origin of land plants (embryophytes) from green algae. Crit. Rev. Plant Sci. 10, 323–342. https://doi.org/10.1080/07352689109382315

Graham L.E., Graham J.M, Wilcox L.W., Cook M.E. 2016. Algae 3rd edition. LJLM Press.

Gran-Stadniczeñko, S., Egge, E., Hostyeva, V., Logares, R., Eikrem, W., Edvardsen, B., 2019. Protist diversity and seasonal dynamics in Skagerrak plankton communities as revealed by metabarcoding and microscopy. J. Eukaryot. Microbiol. 66, 494–513. https://doi.org/10.1111/jeu.12700

Gran-Stadniczeñko, S., Šupraha, L., Egge, E.D., Edvardsen, B., 2017. Haptophyte diversity and vertical distribution explored by 18S and 28S ribosomal RNA gene metabarcoding and scanning electron microscopy. J. Eukaryot. Microbiol. 64, 514–532. https://doi.org/10.1111/jeu.12388

Grbec, B., Dulcic, J., Morovic, M., 2002. Long-term changes in landings of small pelagic fish in the eastern Adriatic-possible influence of climate oscillations over the Northern Hemisphere. Clim. Res. 20, 241–252. https://doi.org/10.3354/cr020241

Grilli, F., Paschini, E., Precali, R., Russo, A., Supić, N., 2005. Circulation and horizontal fluxes in the northern Adriatic Sea in the period June 1999–July 2002. Part I: Geostrophic circulation and current measurement. Sci. Total Environ. 353, 57–67. https://doi.org/10.1016/j.scitotenv.2005.09.008

Grilli, F., Accoroni, S., Acri, F., Bernardi Aubry, F., Bergami, C., Cabrini, M., Campanelli, A., Giani, M., Guicciardi, S., Marini, M., Neri, F., Penna, A., Penna, P., Pugnetti, A., Ravaioli, M., Riminucci, F., Ricci, F., Totti, C., Viaroli, P., Cozzi, S., 2020. Seasonal and interannual trends of oceanographic parameters over 40 years in the Northern Adriatic Sea in relation to nutrient loadings using the EMODnet Chemistry Data Portal. Water 12, 2280. https://doi.org/10.3390/w12082280

Grisogono, B., Belušić, D., 2009. A review of recent advances in understanding the meso- and microscale properties of the severe Bora wind. Tellus A 61, 1–16. https://doi.org/10.1111/j.1600-0870.2008.00369.x

Guillou, L., Eikrem, W., Chrétiennot-Dinet, M.-J., Le Gall, F., Massana, R., Romari, K., Pedrós-Alió, C., Vaulot, D., 2004. Diversity of picoplanktonic prasinophytes assessed by direct nuclear SSU rDNA sequencing of environmental samples and novel isolates retrieved from oceanic and coastal marine ecosystems. Protist 155, 193–214. https://doi.org/10.1078/143446104774199592

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., del Campo, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Holzmann, M., Kooistra, W.H.C.F., Lara, E., Le Bescot, N., Logares, R., Mahé, F., Massana, R., Montresor, M., Morard, R., Not, F., Pawlowski, J., Probert, I., Sauvadet, A.-L., Siano, R., Stoeck, T., Vaulot, D., Zimmermann, P., Christen, R., 2013. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. Nucleic Acids Res. 41, D597–D604. https://doi.org/10.1093/nar/gks1160

Guiry, M.D., Guiry, G.M., 2024. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Visited 12/07/2024.

Hackett, J.D., Anderson, D.M., Erdner, D.L., Bhattacharya, D., 2004. Dinoflagellates: a remarkable evolutionary experiment. Am. J. Bot. 91, 1523–1534. https://doi.org/10.3732/ajb.91.10.1523

Hadziavdic, K., Lekang, K., Lanzen, A., Jonassen, I., Thompson, E.M., Troedsson, C., 2014. Characterization of the 18S rRNA gene for designing universal eukaryote specific primers. PLoS ONE 9, e87624. https://doi.org/10.1371/journal.pone.0087624

Hallegraeff, G.M., Anderson, D.M., Cembella, A.D., Enevoldsen, H.O., 2004. Manual on harmful marine microalgae in monographs on oceanographic methodology, 11 (2nd ed.), in: UNESCO. pp. 25–50.

Hasle, G.R., Syvertsen, E.E., 1996. Marine Diatoms. In: C.R Tomas. (ed.), Identifying Marine Diatoms and Dinoflagellates, Academic Press: 5-385.Elsevier, pp. 5–385. https://doi.org/10.1016/B978-012693015-3/50005-X

Hansen, A.N., Visser, A.W., 2019. The seasonal succession of optimal diatom traits. Limnol. Oceanogr. 64, 1442–1457. https://doi.org/10.1002/lno.11126

Harrison, P.J., Zingone, A., Mickelson, M.J., Lehtinen, S., Ramaiah, N., Kraberg, A.C., Sun, J., McQuatters-Gollop, A., Jakobsen, H.H., 2015. Cell volumes of marine phytoplankton from globally distributed coastal data sets. Estuar. Coast. Shelf Sci. 162, 130–142. https://doi.org/10.1016/j.ecss.2015.05.026

Heimdal, B.R. 1997. Modern Coccolithophorids. In: Tomas C.R. (ed.), Identifying Marine Phytoplankton. Academic Press, San Diego, pp. 731-833.

Hibberd, D.J., Greenwood, A.D., Griffiths, H.B., 1971. Observations on the ultrastructure of the flagella and periplast in the Cryptophyceae. Br. Phycol. J. 6, 61–72. https://doi.org/10.1080/00071617100650071

Honsell, G., Boni, L., Cabrini, M., Pompei, M., 1992. Toxic or potentially toxic dinoflagellates from the Northern Adriatic Sea, in: Marine Coastal Eutrophication. Elsevier, pp. 107–114. https://doi.org/10.1016/B978-0-444-89990-3.50015-8

Hopkins, T.S., Artegiani, A., Kinder, C.A., Pariante, R.1999. A discussion of the northern Adriatic circulation and flushing as determinated by the ELNA hydrography. Ecosyst. Res. Rep. Adriat. Ses 87–106.

Horiguchi, T., 2016. Raphidophyceae (Raphidophyta). In: Archibald, J.M., Simpson, A.G.B., Slamovits, C.H., Margulis, L., Melkonian, M., Chapman, D.J., Corliss, J.O. (Eds.), Handbook of the Protists. Springer International Publishing, Cham, pp. 1–26. https://doi.org/10.1007/978-3-319-32669-6_37-1

Kelly, R.P., Shelton, A.O., Gallego, R., 2019. Understanding PCR processes to draw meaningful conclusions from environmental DNA studies. Sci. Rep. 9, 12133. https://doi.org/10.1038/s41598-019-48546-x

Kembel, S.W., Wu, M., Eisen, J.A., Green, J.L., 2012. Incorporating 16S gene copy number information improves estimates of microbial diversity and abundance. PLoS Comput. Biol. 8, e1002743. https://doi.org/10.1371/journal.pcbi.1002743

Kooistra, W.H.C.F., Gersonde, R., Medlin, L.K., Mann, D.G., 2007. the origin and evolution of the diatoms: their adaptation to a planktonic existence, in evolution of primary producers in the sea. Elsevier, pp. 207–249. https://doi.org/10.1016/B978-012370518-1/50012-6

La Jeunesse, T.C., Lambert, G., Andersen, R.A., Coffroth, M.A., Galbraith, D.W., 2005. *Symbiodinium* (Pyrrhophyta) genome sizes (DNA content) are smallest among dinoflagellates. J. Phycol. 41, 880–886. https://doi.org/10.1111/j.0022-3646.2005.04231.x

Lapeyra Martin, J., Santi, I., Pitta, P., John, U., Gypens, N., 2022. Towards quantitative metabarcoding of eukaryotic plankton: an approach to improve 18S rRNA gene copy number bias. Metabarcoding Metagenomics 6, e85794. https://doi.org/10.3897/mbmg.6.85794

Le Bescot, N., Mahé, F., Audic, S., Dimier, C., Garet, M., Poulain, J., Wincker, P., de Vargas, C., Siano, R., 2016. Global patterns of pelagic dinoflagellate diversity across protist size classes unveiled by metabarcoding. Environ. Microbiol. 18, 609–626. https://doi.org/10.1111/1462-2920.13039

Longhurst, A.R., Glen Harrison, W., 1989. The biological pump: Profiles of plankton production and consumption in the upper ocean. Prog. Oceanogr. 22, 47–123. https://doi.org/10.1016/0079-6611(89)90010-4

López-García, P., Rodríguez-Valera, F., Pedrós-Alió, C., Moreira, D., 2001. Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature 409, 603–607. https://doi.org/10.1038/35054537

Lundholm, N., Bates, S.S., Baugh, K.A., Bill, B.D., Connell, L.B., Léger, C., Trainer, V.L., 2012. Cryptic and pseudo-cryptic diversity in diatoms - with descriptions of *Pseudo-nitzschia hasleana* sp. nov. and *P. fryxelliana* sp. nov. J. Phycol. 48, 436–454. https://doi.org/10.1111/j.1529-8817.2012.01132.x

Lundholm, N., Moestrup, Ø., Hasle, G.R., Hoef-Emden, K., 2003. A study of the *Pseudo-nitzschia Pseudodelicatissima/cuspidata complex* (bacillariophyceae): what is *P. pseudodelicatissima* ? J. Phycol. 39, 797–813. https://doi.org/10.1046/j.1529-8817.2003.02031.x

Lundholm, N., Moestrup, Ø., Kotaki, Y., Hoef-Emden, K., Scholin, C., Miller, P., 2006. Inter- and intraspecific variation of the *Pseudo-nitzschia delicatissima* complex (bacillariophyceae) illustrated by rRNA probes, morphological data and phylogenetic analyses. J. Phycol. 42, 464–481. https://doi.org/10.1111/j.1529-8817.2006.00211.x

Machado, K.B., Andrade, A.T. de, Almeida, M.F. de, Nabout, J.C., 2023. Systematic mapping of phytoplankton literature about global climate change: revealing temporal trends in research. Hydrobiologia 850, 167–182. https://doi.org/10.1007/s10750-022-05052-y

Mäki, A., Salmi, P., Mikkonen, A., Kremp, A., Tiirola, M., 2017. Sample preservation, DNA or RNA extraction and data analysis for high-throughput phytoplankton community sequencing. Front. Microbiol. 8, 1848. https://doi.org/10.3389/fmicb.2017.01848

Manca, B.B., Budillon, G., Scarazzato, P., Ursella, L., 2003. Evolution of dynamics in the eastern Mediterranean affecting water mass structures and properties in the Ionian and Adriatic Seas. J. Geophys. Res. Oceans 108. https://doi.org/10.1029/2002JC001664

Mantziafou, A., Lascaratos, A., 2008. Deep-water formation in the Adriatic Sea: Interannual simulations for the years 1979–1999. Deep Sea Res. Part I: Oceanogr. Res. Pap. 55, 1403–1427. https://doi.org/10.1016/j.dsr.2008.06.005

Marcinko, C.L.J., Painter, S.C., Martin, A.P., Allen, J.T., 2013. A review of the measurement and modelling of dinoflagellate bioluminescence. Prog. Oceanogr. 109, 117–129. https://doi.org/10.1016/j.pocean.2012.10.008

Marić, D., Kraus, R., Godrijan, J., Supić, N., Djakovac, T., Precali, R., 2012. Phytoplankton response to climatic and anthropogenic influences in the north-eastern Adriatic during the last four decades. Estuar. Coast. Shelf Sci. 115, 98–112. https://doi.org/10.1016/j.ecss.2012.02.003

Marinchel, N., Marchesini, A., Nardi, D., Girardi, M., Casabianca, S., Vernesi, C., Penna, A., 2023. Mock community experiments can inform on the reliability of eDNA metabarcoding data: a case study on marine phytoplankton. Sci. Rep. 13, 20164. https://doi.org/10.1038/s41598-023-47462-5

Marini, M., Jones, B.H., Campanelli, A., Grilli, F., Lee, C.M., 2008. Seasonal variability and Po River plume influence on biochemical properties along western Adriatic coast. J. Geophys. Res. Oceans 113, C05S90. https://doi.org/10.1029/2007JC004370

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10. https://doi.org/10.14806/ej.17.1.200

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8, e61217. https://doi.org/10.1371/journal.pone.0061217

Moon-van der Staay, S.Y., De Wachter, R., Vaulot, D., 2001. Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. Nature 409, 607–610. https://doi.org/10.1038/35054541

Mordret, S., Piredda, R., Vaulot, D., Montresor, M., Kooistra, W.H.C.F., Sarno, D., 2018. A curated dinoflagellate (Dinophyceae) reference database for the 18S rRNA gene. Mol. Ecol. Resour. 18, 974–987. https://doi.org/10.1111/1755-0998.12781

Mordret, S., Piredda, R., Zampicinini, G., Kooistra, W.H.C.F., Zingone, A., Montresor, M., Sarno, D., 2023. Metabarcoding reveals marked seasonality and a distinctive winter assemblage of dinoflagellates at a coastal LTER site in the Gulf of Naples. Mar. Ecol. 44, e12758. https://doi.org/10.1111/maec.12758

Mozetič, P., Solidoro, C., Cossarini, G., Socal, G., Precali, R., Francé, J., Bianchi, F., De Vittor, C., Smodlaka, N., Fonda Umani, S., 2010. Recent trends towards oligotrophication of the northern adriatic: evidence from chlorophyll a time series. Estuaries Coasts 33, 362–375. https://doi.org/10.1007/s12237-009-9191-7

Needham, D.M., Fuhrman, J.A., Cram, J.A., Fuhrman, J.A., Sun, F., 2016. Pronounced daily succession of phytoplankton, archaea and bacteria following a spring bloom. Nat. Microbiol. 1 16005.

Neri, F., Romagnoli, T., Accoroni, S., Campanelli, A., Marini, M., Grilli, F., Totti, C., 2022. Phytoplankton and environmental drivers at a long-term offshore station in the northern Adriatic Sea (1988–2018). Cont. Shelf Res. 242, 104746. https://doi.org/10.1016/j.csr.2022.104746

Neri, F., Romagnoli, T., Accoroni, S., Ubaldi, M., Garzia, A., Pizzuti, A., Campanelli, A., Grilli, F., Marini, M., Totti, C., 2023. Phytoplankton communities in a coastal and offshore stations of the northern Adriatic Sea approached by network analysis and different statistical descriptors. Estuar. Coast. Shelf Sci. 282, 108224. https://doi.org/10.1016/j.ecss.2023.108224

Not, F., Del Campo, J., Balagué, V., De Vargas, C., Massana, R., 2009. New insights into the diversity of marine picoeukaryotes. PLoS ONE 4, e7143. https://doi.org/10.1371/journal.pone.0007143

Oddo, P., Pinardi, N., Zavatarelli, M., 2005. A numerical study of the interannual variability of the Adriatic Sea (2000–2002). Sci. Tot. Environ. 353, 39–56. https://doi.org/10.1016/j.scitotenv.2005.09.061

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.-H., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C.J.F., Weedon, J., 2022. vegan: Community Ecology Package. https://doi.org/10.32614/CRAN.package.vegan

Orlic M AND Gacic, M.A.N.D.L.P., 1992. The currents and circulation of the Adriatic Sea. Oceanol. Acta 15, 109–124.

Ovchinnikov IM, Z.V., Krivosheya VG, Udodov AI, 1985. A forming of deep eastern Mediterranean water in the Adriatic Sea. Okeanol. 25 911–917.

Pasarić, Z., Belušić, D., Klaić, Z.B., 2007. Orographic influences on the Adriatic sirocco wind. Ann. Geophys. 25, 1263–1267. https://doi.org/10.5194/angeo-25-1263-2007

Pawlowski, J., Christen, R., Lecroq, B., Bachar, D., Shahbazkia, H.R., Amaral-Zettler, L., Guillou, L., 2011. Eukaryotic richness in the abyss: insights from pyrotag sequencing. PLoS ONE 6, e18169. https://doi.org/10.1371/journal.pone.0018169

Pearman, J.K., Biessy, L., Thomson-Laing, G., Waters, S., Vandergoes, M.J., Howarth, J.D., Rees, A., Moy, C., Pochon, X., Wood, S.A., 2020. Local factors drive bacterial and microeukaryotic community composition in lake surface sediment collected across an altitudinal gradient. FEMS Microbiol. Ecol. 96, fiaa070. https://doi.org/10.1093/femsec/fiaa070

Pereira, L., Neto, J.M. (Eds.), 2014. Marine algae, biodiversity, taxonomy, environmental assessment, and biotechnology ed. CRC Press. https://doi.org/10.1201/b17540

Pierce, E., Torano, O., Lin, Y., Schnetzer, A., Marchetti, A., 2023. Comparison of advanced methodologies for diatom identification within dynamic coastal communities. Limnol. Oceanogr. Methods 21, 687–702. https://doi.org/10.1002/lom3.10575

Piganeau, G., Eyre-Walker, A., Grimsley, N., Moreau, H., 2011. How and why DNA barcodes underestimate the diversity of microbial eukaryotes. PLoS ONE 6, e16342. https://doi.org/10.1371/journal.pone.0016342

Piredda, R., Claverie, J.-M., Decelle, J., de Vargas, C., Dunthorn, M., Edvardsen, B., Eikrem, W., Forster, D., Kooistra, W.H.C.F., Logares, R., Massana, R., Montresor, M., Not, F., Ogata, H., Pawlowski, J., Romac, S., Sarno, D., Stoeck, T., Zingone, A., 2018. Diatom diversity through HTS-metabarcoding in coastal European seas. Sci. Rep. 8, 18059. https://doi.org/10.1038/s41598-018-36345-9

Piredda, R., Tomasino, M.P., D'Erchia, A.M., Manzari, C., Pesole, G., Montresor, M., Kooistra, W.H.C.F., Sarno, D., Zingone, A., 2017. Diversity and temporal patterns of planktonic protist assemblages at a Mediterranean Long Term Ecological Research site. FEMS Microbiol. Ecol. 93, fiw200. https://doi.org/10.1093/femsec/fiw200

Poulain P.-M., Cushman-Roisin, B, 2001. Circulation. In: Cushman-Roisin, B., Gačić,M Pierre-Marie Poulain, Antonio Artegiani (eds) Physical Oceanography of the Adriatic Sea. Springer Netherlands, Dordrecht. pp. 67-109. https://doi.org/10.1007/978-94-015-9819-4

Poulain, P.-M., Raicich, F., 2001. Forcings. In: Cushman-Roisin, B., Gačić,M Pierre-Marie Poulain, Antonio Artegiani (eds) Physical Oceanography of the Adriatic Sea. Springer Netherlands, Dordrecht, pp. 45–65. https://doi.org/10.1007/978-94-015-9819-4_2

Preston, M., Fritzsche, M., Woodcock, P., 2022. Report understanding and mitigating errors and biases in metabarcoding: an introduction for non-specialists. JNCC Report 699.

Probert, I., Houdan, A., 2004. The laboratory culture of coccolithophores, in: Thierstein, H.R., Young, J.R. (Eds.), Coccolithophores. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 217–249. https://doi.org/10.1007/978-3-662-06278-4_9

Prokopowich, C.D., Gregory, T.R., Crease, T.J., 2003. The correlation between rDNA copy number and genome size in eukaryotes. Genome 46, 48–50. https://doi.org/10.1139/g02-103

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219

Quijano-Scheggia, S.I., Garcés, E., Lundholm, N., Moestrup, Ø., Andree, K., Camp, J., 2009. Morphology, physiology, molecular phylogeny and sexual compatibility of the cryptic *Pseudo-nitzschia delicatissima complex* (Bacillariophyta), including the description of *P. arenysensis* sp. nov. Phycologia 48, 492–509. https://doi.org/10.2216/08-21.1

Raven, J.A., 1994. Carbon fixation and carbon availability in marine phytoplankton. Photosynth. Res. 39, 259–273. https://doi.org/10.1007/BF00014587

Rizzoli, P.M., Bergamasco, A., 1983. The dynamics of the coastal region of the Northern Adriatic Sea. J. Phys. Oceanogr. 13, 1105–1130. https://doi.org/10.1175/1520-0485(1983)013<1105:TDOTCR>2.0.CO;2

Robinson, A.R., Leslie, W.G., Theocharis, A., Lascaratos, A., 2001. Mediterranean Sea circulation. Encyclopedia of Ocean Sciences. 1689–1705. https://doi.org/10.1006/rwos.2001.0376

Roh, C., Villatte, F., Kim, B.-G., Schmid, R.D., 2006. Comparative study of methods for extraction and purification of environmental DNA from soil and sludge samples. Appl. Biochem. Biotechnol. 134, 97–112. https://doi.org/10.1385/ABAB:134:2:97

Roselli, L., Basset, A., 2015. Decoding size distribution patterns in marine and transitional water phytoplankton: from community to species level. PLOS ONE 10, e0127193. https://doi.org/10.1371/journal.pone.0127193

Round, F.E., Crawford, R.M., Mann, D.G., 1990. Diatoms: biology and morphology of the genera. Camb. Univ. Press.

Rubino, A., Hainbucher, D., 2007. A large abrupt change in the abyssal water masses of the eastern Mediterranean. Geophys. Res. Lett. 34. https://doi.org/10.1029/2007GL031737

Ruiz-González, C., Logares, R., Sebastián, M., Mestre, M., Rodríguez-Martínez, R., Galí, M., Sala, M.M., Acinas, S.G., Duarte, C.M., Gasol, J.M., 2019. Higher contribution of globally rare bacterial taxa reflects environmental transitions across the surface ocean. Mol. Ecol. 28, 1930–1945. https://doi.org/10.1111/mec.15026

Ruppert, K.M., Kline, R.J., Rahman, M.S., 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Glob. Ecol. Conserv. 17, e00547. https://doi.org/10.1016/j.gecco.2019.e00547

Russo, A., Maccaferri, S., Djakovac, T., Precali, R., Degobbis, D., Deserti, M., Paschini, E., Lyons, D.M., 2005. Meteorological and oceanographic conditions in the northern Adriatic Sea during the period June 1999–July 2002: Influence on the mucilage phenomenon. Sci. Total Environ. 353, 24–38. https://doi.org/10.1016/j.scitotenv.2005.09.058

Sáez, A.G., Probert, I., Young, J.R., Edvardsen, B., Eikrem, W., Medlin, L.K. (2004). A review of the phylogeny of the Haptophyta. In: Thierstein, H.R., Young, J.R. (eds) Coccolithophores. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-06278-4_10.

Santi, I., Kasapidis, P., Karakassis, I., Pitta, P., 2021. A comparison of DNA metabarcoding and microscopy methodologies for the study of aquatic microbial eukaryotes. Diversity 13, 180. https://doi.org/10.3390/d13050180

Santoferrara, L.F., 2019. Current practice in plankton metabarcoding: Optimization and error management. J. Plankton Res. 41, 571–582. https://doi.org/10.1093/plankt/fbz041

Sarno, D., Zingone, A., Montresor, M., 2010. A massive and simultaneous sex event of two *Pseudo-nitzschia* species. Deep Sea Res. Part II Top. Stud. Oceanogr. 57, 248–255. https://doi.org/10.1016/j.dsr2.2009.09.012

Segura, A.M., Kruk, C., Calliari, D., García-Rodriguez, F., Conde, D., Widdicombe, C.E., Fort, H., 2013. Competition drives clumpy species coexistence in estuarine phytoplankton. Sci. Rep. 3, 1037. https://doi.org/10.1038/srep01037

Simon, N., Cras, A.L., Foulon, E., Lemée, R., 2009. Diversity and evolution of marine phytoplankton. Comptes Rendus - Biol. 332, 159–170. https://doi.org/10.1016/j.crvi.2008.09.009

Smetacek, V., Cloern, J.E., 2008. On Phytoplankton Trends. Science 319, 1346–1348. https://doi.org/10.1126/science.1151330

Smith, K.F., Kohli, G.S., Murray, S.A., Rhodes, L.L., 2017. Assessment of the metabarcoding approach for community analysis of benthic-epiphytic dinoflagellates using mock communities. N. Z. J. Mar. Freshw. Res. 51, 555–576. https://doi.org/10.1080/00288330.2017.1298632

Sogin, M.L., Morrison, H.G., Huber, J.A., Welch, D.M., Huse, S.M., Neal, P.R., Arrieta, J.M., Herndl, G.J., 2006. Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc. Natl. Acad. Sci. 103, 12115–12120. https://doi.org/10.1073/pnas.0605127103

Stadhouders, R., Pas, S.D., Anber, J., Voermans, J., Mes, T.H.M., Schutten, M., 2010. The effect of primer-template mismatches on the detection and quantification of nucleic acids using the 5′ nuclease assay. J. Mol. Diagn. 12, 109–117. https://doi.org/10.2353/jmoldx.2010.090035

Stockner, J.G., Antia, N.J., 1986. Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. Can. J. Fish. Aquat. Sci. 43, 2472–2503. https://doi.org/10.1139/f86-307

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D.M., Breiner, H., Richards, T.A., 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol. Ecol. 19, 21–31. https://doi.org/10.1111/j.1365-294X.2009.04480.x

Stoeck, T., Breiner, H., Filker, S., Ostermaier, V., Kammerlander, B., Sonntag, B., 2014. A morphogenetic survey on ciliate plankton from a mountain lake pinpoints the necessity of lineage-specific barcode markers in microbial ecology. Environ. Microbiol. 16, 430–444. https://doi.org/10.1111/1462-2920.12194

Strassert, J.F.H., Jamy, M., Mylnikov, A.P., Tikhonenkov, D.V., Burki, F., 2019. New phylogenomic analysis of the enigmatic phylum Telonemia further resolves the eukaryote tree of life. Mol. Biol. Evol. 36, 757–765. https://doi.org/10.1093/molbev/msz012

Stuart, J., Ryan, K.G., Pearman, J.K., Thomson-Laing, J., Hampton, H.G., Smith, K.F., 2024. A comparison of two gene regions for assessing community composition of eukaryotic marine microalgae from coastal ecosystems. Sci. Rep. 14, 6442. https://doi.org/10.1038/s41598-024-56993-4

Supić, N., Orlić, M., Degobbis, D., 2000. Istrian coastal countercurrent and its year-to-year variability. Estuar. Coast. Shelf Sci. 51, 385–397. https://doi.org/10.1006/ecss.2000.0681

Taberlet, P., Bonin, A., Zinger, L., Coissac, E., 2018. Environmental DNA. Oxford University PressOxford. https://doi.org/10.1093/oso/9780198767220.001.0001

Theriot, E.C., 2012. Diatoms, in: Encyclopedia of Life Sciences. Wiley. https://doi.org/10.1002/9780470015902.a0000330.pub2

Throndsen, J., 1978. Phytoplankton manual. Monographs on oceanographic methodology.

Throndsen, J. 1997. The planktonic marine flagellates. In: Tomas C.R. (ed.), Identifying Marine Phytoplankton. Academic Press, San Diego, pp. 591-729.

Totti, C., Cangini, M., Ferrari, C., Kraus, R., Pompei, M., Pugnetti, A., Romagnoli, T., Vanucci, S., Socal, G., 2005. Phytoplankton size-distribution and community structure in relation to mucilage occurrence in the northern Adriatic Sea. Sci. Total Environ. 353, 204–217. https://doi.org/10.1016/j.scitotenv.2005.09.028

Totti, C., Cucchiari, E., Romagnoli, T., 2002. Intra and interannual variability of phytoplankton in coastal area of Senigallia (northern Adriatic Sea) from 1988 to 2000. Biol. Mar. Mediterr. 9, 391–399.

Totti, C., Romagnoli, T., Accoroni, S., Coluccelli, A., Pellegrini, M., Campanelli, A., Grilli, F., Marini, M., 2019. Phytoplankton communities in the northwestern Adriatic Sea: Interdecadal variability over a 30-years period (1988–2016) and relationships with meteoclimatic drivers. J. Mar. Syst. 193, 137–153. https://doi.org/10.1016/j.jmarsys.2019.01.007

Townsend, D.W., Cammen, L.M., Holligan, P.M., Campbell, D.E., Pettigrew, N.R., 1994. Causes and consequences of variability in the timing of spring phytoplankton blooms. Deep Sea Res. Part Oceanogr. Res. Pap. 41, 747–765. https://doi.org/10.1016/0967-0637(94)90075-2

Turk Dermastia, T., Vascotto, I., Francé, J., Stanković, D., Mozetič, P., 2023. Evaluation of the rbcL marker for metabarcoding of marine diatoms and inference of population structure of selected genera. Front. Microbiol. 14, 1071379. https://doi.org/10.3389/fmicb.2023.1071379

Turk, V., Hagström, Å., Kovač, N., Faganeli, J., 2010. Composition and function of mucilage macroaggregates in the northern Adriatic. Aquat. Microb. Ecol. 61, 279–289. https://doi.org/10.3354/ame01447

Ursella, L., Kovačević, V., Gačić, M., 2011. Footprints of mesoscale eddy passages in the Strait of Otranto (Adriatic Sea). J. Geophys. Res. 116, C04005. https://doi.org/10.1029/2010JC006633

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G.H., Geniez, P., Pont, D., Argillier, C., Baudoin, J., Peroux, T., Crivelli, A.J., Olivier, A., Acqueberge, M., Le Brun, M., Møller, P.R., Willerslev, E., Dejean, T., 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Mol. Ecol. 25, 929–942. https://doi.org/10.1111/mec.13428

van der Loos, L.M., Nijland, R., 2021. Biases in bulk: DNA metabarcoding of marine communities and the methodology involved. Mol. Ecol. 30, 3270–3288. https://doi.org/10.1111/mec.15592

Vasselon, V., Bouchez, A., Rimet, F., Jacquet, S., Trobajo, R., Corniquel, M., Tapolczai, K., Domaizon, I., 2018. Avoiding quantification bias in metabarcoding: Application of a cell biovolume correction factor in diatom molecular biomonitoring. Methods Ecol. Evol. 9, 1060–1069. https://doi.org/10.1111/2041-210X.12960

Verri, G., Furnari, L., Gunduz, M., Senatore, A., Santos da Costa, V., De Lorenzis, A., Fedele, G., Manco, I., Mentaschi, L., Clementi, E., Coppini, G., Mercogliano, P., Mendicino, G., Pinardi, N., 2024. Climate projections of the Adriatic Sea: role of river release. Front. Clim. 6, 1368413. https://doi.org/10.3389/fclim.2024.1368413

Vilibić, I., Orlić, M., 2002. Adriatic water masses, their rates of formation and transport through the Otranto Strait. Deep Sea Res. Part I: Oceanogr. Res. 49, 1321–1340. https://doi.org/10.1016/S0967-0637(02)00028-6

Vilibić, I., Mihanović, H., Janeković, I., Šepić, J., 2016. Modelling the formation of dense water in the northern Adriatic: Sensitivity studies. Ocean Model. 101, 17–29. https://doi.org/10.1016/j.ocemod.2016.03.001

Viprey, M., Guillou, L., Ferréol, M., Vaulot, D., 2008. Wide genetic diversity of picoplanktonic green algae (Chloroplastida) in the Mediterranean Sea uncovered by a phylum-biased PCR approach. Environ. Microbiol. 10, 1804–1822. https://doi.org/10.1111/j.1462-2920.2008.01602.x

Wang, C., Lan, C.Q., 2018. Effects of shear stress on microalgae – A review. Biotechnol. Adv. 36, 986–1002. https://doi.org/10.1016/j.biotechadv.2018.03.001

Wang, H., Wu, P., Xiong, L., Kim, H.-S., Kim, J.H., Ki, J.-S., 2024. Nuclear genome of dinoflagellates: Size variation and insights into evolutionary mechanisms. Eur. J. Protistol. 93, 126061. https://doi.org/10.1016/j.ejop.2024.126061

Wang, Z., Liu, L., Tang, Y., Li, A., Liu, C., Xie, C., Xiao, L., Lu, S., 2022. Phytoplankton community and HAB species in the South China Sea detected by morphological and metabarcoding approaches. Harmful Algae 118, 102297. https://doi.org/10.1016/j.hal.2022.102297

Wickham, H., 2016. Getting Started with ggplot2, in: Ggplot2, Use R! Springer International Publishing, Cham, pp. 11–31. https://doi.org/10.1007/978-3-319-24277-4_2

Widdicombe, C.E., Eloire, D., Harbour, D., Harris, R.P., Somerfield, P.J., 2010. Long-term phytoplankton community dynamics in the Western English Channel. J. Plankton Res. 32, 643–655. https://doi.org/10.1093/plankt/fbp127

Wurzbacher, C., Attermeyer, K., Kettner, M.T., Flintrop, C., Warthmann, N., Hilt, S., Grossart, H., Monaghan, M.T., 2017. DNA metabarcoding of unfractionated water samples relates phyto-, zoo- and bacterioplankton dynamics and reveals a single-taxon bacterial bloom. Environ. Microbiol. Rep. 9, 383–388. https://doi.org/10.1111/1758-2229.12540

Xiao, X., Sogge, H., Lagesen, K., Tooming-Klunderud, A., Jakobsen, K.S., Rohrlack, T., 2014. Use of high throughput sequencing and light microscopy show contrasting results in a study of phytoplankton occurrence in a freshwater environment. PLoS ONE 9, e106510. https://doi.org/10.1371/journal.pone.0106510

Zhu, F., Massana, R., Not, F., Marie, D., Vaulot, D., 2005. Mapping of picoeucaryotes in marine ecosystems with quantitative PCR of the 18S rRNA gene. FEMS Microbiol. Ecol. 52, 79–92. https://doi.org/10.1016/j.femsec.2004.10.006