Functional Diversity of Marine Protists in Coastal Ecosystems

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Diversité Fonctionnelle des Protistes Marins dans l'Ecosystème Côtier

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"imagining for a moment that we understand

the many complex contingencies on which

the existence of each species depends."

Charles Darwin

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INTRODUCTION

1) Preamble: Ecology

Ecology, as every science, comes from the intrinsic curiosity of mankind. It is our ability to recognize patterns that makes us question "why" and "how" does the things that surrounds us exist and do what they do. Ecology really is this particular question applied to life on earth. If the term coined by Ernst Haeckel (1834-1919) defined the science as the study ('logos') of life's habitat ('oikos'), it is now firmly believed that life, as we observe it in a given space and time, is the result of past and ongoing co-evolution of the living and its environment.

To define the "living" is not an easy task. A recent study evidenced the earliest traces of life on earth among sedimentary rocks that are in between 3 770 and 4 280 million years old (Dodd *et al.*, 2017). The "tracks" consisted in the observations of produce from an allegedly biogenic reaction that reduced ferric iron in order to carry out the catabolism (i.e. the breaking down) of carbonaceous material. According to these proofs, life can be defined by its metabolic activity, i.e. entities that displace and transform chemicals and uses the energy from these reactions to "live". This capability represents one of the "seven pillars" that defines life according to a review by Koshland Jr. (2002). Other pillars include: 1/ a "Program" that codes for the activities of an organism. The program is coded with successive nucleotides that forms a macromolecule of Deoxyribonucleic Acid (DNA) constituting the genome, portions of DNA are transformed into strands of Ribonucleic acid (RNA) which are then translated into proteins that are the seat of chemical reactions; 2/ "Compartmentalization" resulting in a finite volume in which a) the program and metabolic activity are kept from deleterious chemicals and b) that serves as membrane favoring the kinetic of metabolic activity (e.g. the cell, organs or organelles); 3/ the capability to "Improvise" i.e. to change the "program" to possibly achieve better efficiency in survival and energy acquisition (e.g. DNA mutations and selections); 4/ "Regeneration" to compensate the decline of metabolic activity due to the organism progressive oxidation, i.e. whether the living organism is repaired or a new one is created by reproduction; 5/ "Adaptability" i.e. mechanisms that allow the survival of the organism when the metabolic activity is sub-optimal; and 6/ the

"Seclusion" of an organism's activities to prevent short-circuits in the metabolism (e.g. to create proteins specialized in few reactions, to create further compartmentalization like organs or organelles, or to code for an operating system that arranges each metabolic pathway). Throughout these principles, the retro-active processes linking the organisms and their environment are clearly highlighted. The metabolic exchanges of chemicals regulate both the survival of an organism and, in a larger timescale, the chemical content of its environment. This joint evolution has driven the creation of organisms adapted to the various conditions observed on earth in the last billion years, some of which still dominate the modern earth (Falkowski *et al.*, 1998, 2004; Litchman *et al.*, 2007). Nowadays the role of interactions among organisms in shaping the evolution of the living is also increasingly brought forward. These interactions have various effect on the organisms (positive, negative or neutral; Faust and Raes, 2012) and take place at different scales, a) whether molecular, with horizontal (Keeling and Palmer, 2008; Husnik and McCutcheon, 2017) or viral (Villareal, 2004; Koonin and Wolf, 2012) exchanges of DNA, or b) at the level of the organism, with predation or parasitism (Dodson and Brooks, 1965; Lafferty and Kuris, 2002; Dobson *et al.*, 2008), past and ongoing symbioses (Falkowski *et al.*, 2004; Selosse *et al.*, 2016), and competition (Hardin, 1960; Chesson, 2000). Organisms thus undergoes further specialization driven by interactions with each other.

The contemporaneous area where both environment-organism and organism-organism interactions occur is called an "ecosystem". To compare life across these ecosystems the ecologist needs a systematic classification. Early classification of the living originates in the works of the ancient Greek philosopher Aristotles (384–322 BC) and of the Swedish naturalist Carl von Linné (1707-1778) that sorted organisms into groups according to their shape (i.e. morphology) or their mechanical functioning (i.e. physiology). Later with Charles Darwin's (1809-1882) idea that organisms were the result of evolution process and natural selection, taxonomy has been rooted into a theorized Universally Common Ancestor (UCA) (Theobald, 2010). In modern taxonomy, organisms are spread hierarchically into 'clades' according to their 'relatedness' to the UCA and other organisms (Ruggiero *et al.*, 2015). We now measure the 'relatedness' between organisms with morphological, genetic or ecological synapomorphy, but also on the evolutionary relationships of organisms with each other (i.e. phylogeny). The name of the "clades" are inherited

from the Linnaean taxonomy (Domain, Kingdom, Phylum, Class, Order, Genus and Species), they represent increasingly smaller groups of organisms clustered together on the basis of their relatedness, with the species as the smallest unit of taxonomy. Over the last century, the species concept has been extensively debated (de Queiroz, 2005). A first established proposition arose from Ernst Mayr (1904-2005) that defined the "biological species concept" as "groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups". However, this definition could not satisfy microbiologists (Cohan, 2002), that challenged the reproductive isolation by observing exchanges of characters, recombination processes and gene transfer across distinct species (Koonin *et al*., 2001; Keeling and Palmer, 2008; Koonin and Wolf, 2012). The modern consensus of the species concept is still inspired by Ernst Mayr and integrates more of the evolutionary relationships between organisms. Species are now defined as *metapopulation lineage*, in which *lineage* refers to a series of ancestor and descendant while *metapopulation* refers to an inclusive population made up of connected subpopulations possessing the same gene pool (de Queiroz, 2005; De Queiroz, 2007). Speciation is every evolutionary process (i.e. mutation, natural selection, migration or genetic drift) that mark a distinction in the genome, the morphological characters or the ecological preferences of two organisms (De Queiroz, 2007; Fišer *et al*., 2018). Accordingly, the influence of speciation can be delayed; whether influencing first the genome, morphological characters and/or the ecological preferences of two organisms; and two species under ongoing speciation constitutes cryptic species (Fišer *et al*., 2018).

In measuring and comparing the living in an ecosystem, ecology uses the species concept, taxonomy and the evolutionary history of species to understand how the living is structured by its environment and retro-actively how the environment is structured by the living.

2) Marine Protistan Diversity

Marine protists are relevant both for the science of life evolution and for contemporaneous ecology. Protists are members of Eukaryota, one of the two 'domains' that are recognized nowadays, i.e. Eukaryota and Prokaryota (Ruggiero *et al*., 2015). Investigations of the most ancient eukaryotic fossils estimate the apparition of protists to date back to ca 2 billion years ago or beyond (Caron *et al*., 2012 and references therein). The peculiarity of protists is that they are capable of existence as single cells and are perceptible mostly at the microscopic scale (one to thousand microns) (Adl *et al*., 2012; Caron *et al*., 2012), which place them among the microbial world. Earliest descriptions of protists probably date back to the 17th century with the birth of the first microscopes and microbiology [alternatively, by Antonie van Leeuwenhoek (Netherlands, 1632-1723) or Athanasius Kircher (Germany, 1602-1690) (Wainwright, 2003)]. Starting from this point, microorganisms were increasingly described and classified with morphological criteria. During the 20th century, with the successive advent of electron microscopy (1930s) and genomics (1980s) researchers further classified microorganisms (Cavalier-Smith, 1993; Adl *et al*., 2005) and, by comparing their genomes, started to reconstruct the phylogenic history of eukaryotic life (Woese and Fox, 1977; Cavalier-Smith, 1982; Baldauf, 2003).

Protists are still extensively studied nowadays because they bear the stigma of eukaryotic evolution. Comparative genomics based on protists indicate that the last eukaryotic common ancestor probably originates from the fusion between now extinct ancestors of Archea and Bacteria (both higher clades of prokaryotes) (Katz, 2012). As every eukaryote, and contrary to prokaryotes, protists have a nucleus in which DNA is stored and a complex cell architecture supported by a cytoskeleton. The emergence of these features is yet unknown, currently hypotheses for the origin of the nucleus focuses mostly on an autogenous creation, while it has been proposed that the cytoskeleton could be issued from an endosymbiosis with an ancestral spirochete (a bacterium with a filamentous shape) (Katz, 2012). The advantages of these two features during eukaryotic evolution are however better understood. The

nucleus protects DNA from other deleterious materials, separates DNA transcription and translation (process by which genes are converted into ribonucleic acid RNA and proteins) and allows the 'compartmentalization' and 'seclusion' of the cell for other functions to occur; in return the cytoskeleton allows diversification of the cell structure, motility, as well as phagocytosis (Katz, 2012). Phagocytosis, i.e. engulfment of particles within the cell, have later helped eukaryotes to acquire the mitochondria and photosynthetic plastids by favoring endosymbiosis (Figure 1) (Cavalier-Smith, 1982; Katz, 2012; McFadden, 2014), although note that it is still under debate whether the endosymbioses arrived before or after the existence of eukaryotic specific features (Archibald, 2015). These two organelles are the place of chemical reactions from which most eukaryotes gain energy. According to genetic and morphologic criteria, the mitochondria is probably issued from a single endosymbiosis with an Alphaproteobacteria (Katz, 2012). This endosymbiosis seems to have appeared before the diversification of eukaryotes since all clades of protists contains species with mitochondria. Reversely, the first plastids among eukaryotes were issued from a primary endosymbiosis with a Cyanobacteria, but eukaryotes diversified several times following successive plastid losses, secondary and even tertiary endosymbiosis (with small eukaryotes) that occurred varyingly across eukaryotic evolution (Falkowski *et al*., 2004; Katz, 2012) (Figure 1). In consequence, contemporaneous protistan diversity is composed of both plastid bearing species, with a wide array of plastid types, and non-plastidic species. There are also non-plastid bearing protists with genetic traces of plastids within their genomes or protists with non-functional plastids (Adl *et al*., 2012), this observation has led to postulate the existence of secondary loss of plastids among numerous protists (Katz, 2012). To create new organic matter (i.e. anabolism), protists thus rely upon a) the mitochondria, which uses the catabolism of organic matter as a reactive power (i.e. 'respiration' with oxidation of dioxygen), and b) the plastids, which converts light into a reactive power (i.e. photosynthesis with assimilation of carbon dioxide). Accordingly, non-plastid bearing protists rely mostly on catabolism and have thus adapted various strategies in order to engulf preys or organic matter (i.e. heterotrophs), while plastid-bearing protists (i.e. phototrophs or "phytoplankton" in marine ecosystems) have optimized strategies for light and nutrient acquisition. Contrarily to prokaryotes that have multiplied all sorts of metabolism to produce energy (Falkowski *et al*., 2008; Massana and Logares, 2013), the strength of

eukaryotes lies on a great diversity of cell structures and trophic processes that have increased the efficiency of respiration and photosynthesis (Massana and Logares, 2013; Keeling and del Campo, 2017). A great evidence of the adaptability at the cellular level of marine protist is found in mixotrophic processes, where protists appears to be flexible in their heterotrophy and phototrophy (Stoecker *et al*., 2017; Mitra, 2018). This strategy is indeed increasingly recognized and supposed to be widespread within eukaryotes (Selosse *et al*., 2016).

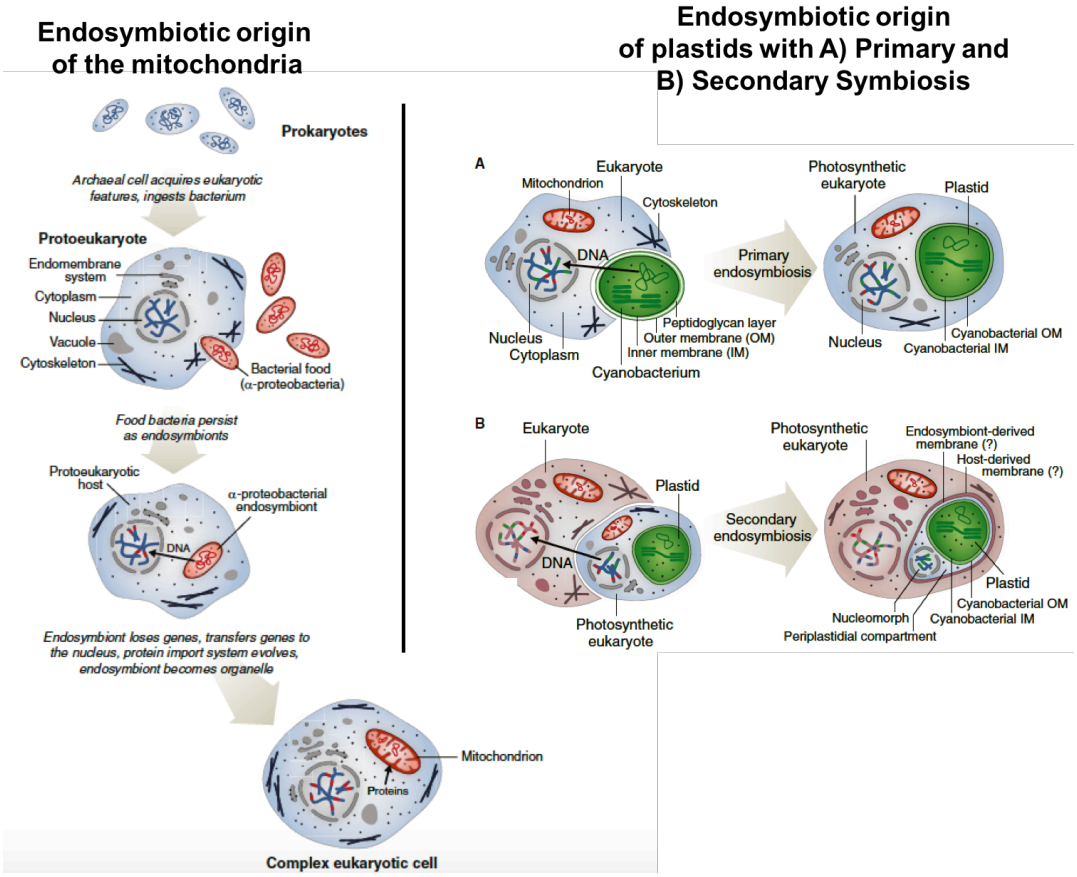

Figure 1: Schematic interpretation of the endosymbiosis origin of the eukaryotic mitochondria (left) and plastids (right), according to Archibald (2015).

Contemporaneous eukaryotic diversity is dominated by at least 8 super-groups, all contain protists from which the diversity and few marine, both pelagic and benthic, representative will be detailed here (more details can be found in Falkowski *et al*., 2004; Caron *et al*., 2012; Katz, 2012; also note that a phylogenetic tree with 7 super-groups is presented in Figure 2 and inspired by Worden *et al*., 2015, distinctions are commented in the text). 'Opisthokonts' regroups the multicellular animals (Metazoan), Fungi, but also the protistan and marine Choanoflagellates as well as parasitic Mesomycetozoan. Most are heterotrophic and

their name reflects the posterior position of their flagella, a cellular structure involved in motility. 'Amoebozoa' contains the protistan lobose and testate amoeba, they are amorphous heterotrophs that feeds with pseudopodia (cellular projections used for phagocytosis and gliding locomotion), 'Amoebozoa' also contains the multicellular slime-molds. Some Amoebozoan can be found in marine benthic sediments. 'Archaeplastida' unites eukaryotes that have retained green (e.g. Plants, Ulvophytes or protistan Chlorophytes and Prasinophytes) or red pigments (i.e. Rhodophytes) from the primary endosymbiosis with a cyanobacteria (Falkowski *et al*., 2004). Chlorophytes are notable features of the small eukaryotic phytoplankton in the ocean, their distribution and diversity have been only recently highlighted by genetic surveys during the last 10 years (Not *et al*., 2005; Vaulot *et al*., 2008; Massana, 2011). The 'Chromalveolata' group is strongly debated but is supposed to cluster together the Alveolates (with alveolar sacs within the cell membrane) and the Stramenopiles (with two structurally distinct flagella during at least a part of the life cycle). These two clades include numerous taxa present in marine ecosystems. Within the 'Stramenopiles', marine Bacillariophyta, or diatoms, are a great share of marine phytoplankton. Most of diatoms have lost their flagella and contains red plastids derived from a secondary endosymbiosis with a red algae (Falkowski *et al*., 2004), another notable feature of most diatoms is their structurally complex (and elegant) silicate cover (Hallegraeff, 1986). Stramenopiles includes other taxa from the MAarine STtramenopiles group (MAST), small bacterivorous protists which have been discovered and described only recently (Massana *et al*., 2006; Massana, 2011). Within the Alveolates, marine Ciliophora, or ciliates, are heterotrophs covered with cilia involved in their locomotion, many are found to retain plastids from their prey (i.e. kleptoplastidy) which makes them potential mixotrophs (Sanders, 1991; Stoecker *et al*., 2017). Dinophyta, or dinoflagellates, also Alveolates, are flagellates with sometimes complex coverings, they have retained plastids from a secondary endosymbiosis with alternatively a green or a red algae but also with Coccolitophorids (here tertiary endosymbiosis) (Falkowski *et al*., 2004). Dinoflagellates also have a wide range of trophic strategies, comprising all sorts of mixotrophy and complex organelles involved in predation (Jeong *et al*., 2010). Members of the Marine ALVeolate groups (MALV) are also a widespread member of marine ecosystems, most seem to be parasites (Guillou *et al*., 2008; Siano *et al*., 2011; Strassert *et al*., 2018). 'Rhizaria' contains pseudopodia-forming members of

Radiolaria, Foraminifera, and Acantharia, that respectively produce skeletal structures of silica, calcium-carbonate, and strontium-sulfate. Their ecology is poorly known but the group contains both heterotrophs and non-constitutive symbiotic phototrophs (Decelle *et al*., 2012). 'Excavates' and 'Discicristates' have been merged together in Figure 2, these supergroups contain numerous small bacterivorous taxa usually not abundant in the sea. However, 'Discicristates' also contains Euglenozoan, alternatively heterotrophic, phototrophic or mixotrophic organisms found in coastal ecosystems. Clades that are at the time unresolved by modern phylogeny, called 'Oprhans' due to their lack of known ancestors, includes 'Haptophytes' that comprises the mixotrophic and calcitic Coccolitophorids (Young *et al*., 2005; Unrein *et al*., 2014), as well as the phototrophic and foam forming *Phaeocystis*. Finally, the position of Cryptophytes is even less resolved, but this clade contains phototrophs with very variable pigmentation and found abundant in numerous marine surveys (Massana *et al*., 2004; Massana, 2011). Mixotrophy by Cryptophytes has been reported in freshwater (Grujcic *et al*., 2018), but this aspect of their biology as not been investigated in marine systems.

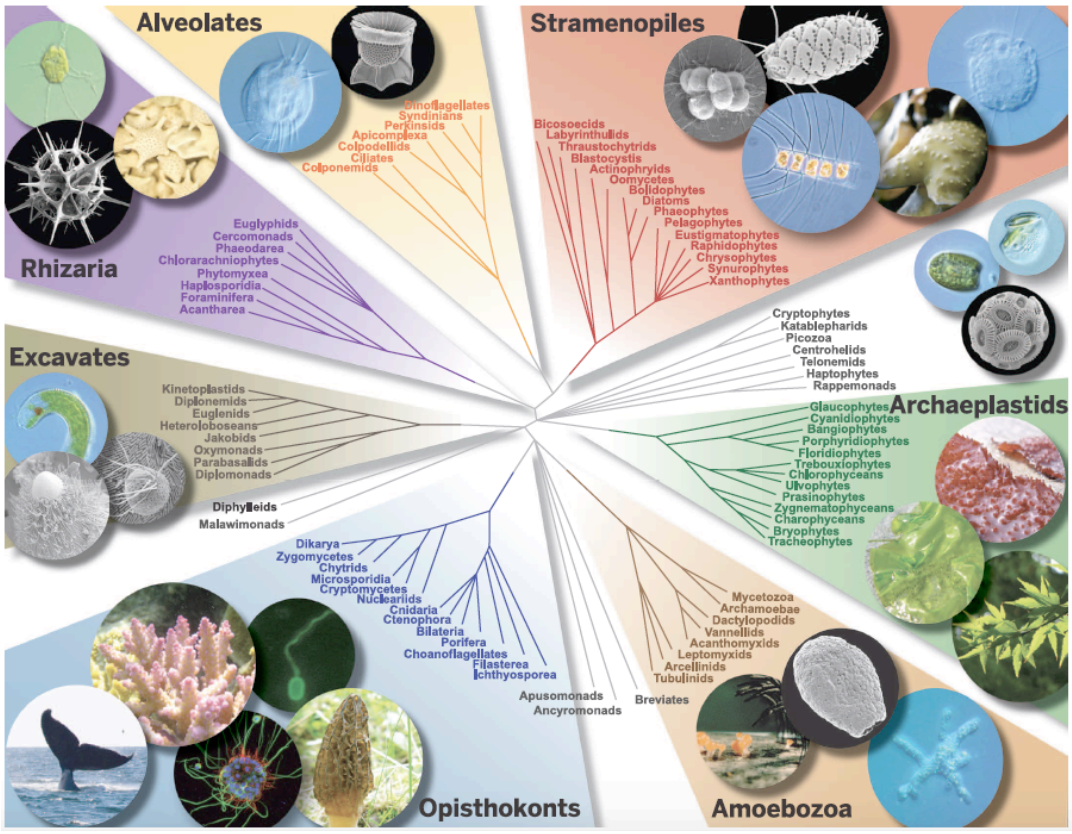

Figure 2: The eukaryotic tree of life with 7 supergroups all containing marine protists, pictures highlights eukaryotic diversity, as depicted in Worden et al., (2015).

Although many aspects of the history of eukaryotes remain uncertain (e.g. origin of the nucleus, unknown taxa, unresolved phylogeny), protists are found everywhere on earth. Notably within the ocean, where they constitute most of eukaryotic diversity (de Vargas *et al*., 2015). Still, protistan taxa usually do not occur at the same time, intricate changes in the diversity of this bulk of organisms strongly shape ecosystems and researchers have long tried to explain them.

3) Marine Protistan Ecology: State of the Art

In accordance with their incapability to move against currents in aquatic systems, marine microbial organisms are referred to as "plankton" from the Greek "planktos", meaning drifter. The terms phytoplankton and zooplankton are further used for describing phototrophic and heterotrophic plankton, these names are derived from the Greek "phyton", for plant, and "zoon", for animal. Marine microbial diversity is composed of multiple protists and prokaryotes, while zooplankton contains also metazoans, the term plankton thus regroups all these organisms, unicellular and pluricellulars. In this section, a parallel history of ecological paradigms and their application to marine protists will be introduced. It is non-exhaustive and sometimes focuses more on "plankton", and often on phytoplankton ecology rather than generally on protists. The following sections deal with theories and paradigms that have been proposed in plankton ecology, that are partially applied and discussed to the data and results generated during the PhD work.

a) Everything is everywhere but the environment selects

The debate of "Everything is everywhere but the environment selects", is perhaps the first meeting point between microbiology and ecology. Nowadays, the theory is attributed to the microbiologist Lourens G.M. Baas-Becking (1895-1963), but premises of the question are found in Carl von Linné's work on biogeography, the science of species spatial distribution, and the several debates that it generated. This debate has indeed spread across several historic figures going from Linnaeus himself, to his principal detractor Comte de Buffon (1707-1788), botanist Augustin de Candolle (1778-1841), Charles Lyell (1797-1841), botanist Joseph Hooker (1817-1911), Charles Darwin (1809-1882) along with Alfred Wallace (1823-1913) and finally microbiologist Martimus Willem Beijerinck (1851-1931), whose work probably inspired the most Baas-Becking's famous formulation (O'Malley, 2007). The general concept supposes that species are ubiquitous but the environment, in a given space and time, "chooses" the dominating species by presenting specific

conditions that favors the most competitive species. The major counter-argument before microbiology arrived, was that the greater part of known species was, at-least regionally, endemic and consequently "not everywhere". What botanist and microbiologist later realized was that the theory was possible only when comprising the parameter of dispersion (as a deterministic or stochastic process), in fact a species can be everywhere only if it is able to go everywhere. With time the theory was proven truer for the biogeography of micro-organism than for macro-organism. During the voyage of the Beagle, Charles Darwin sampled marine protists worldwide and promoted the veracity of the theory for the microbial world (O'Malley, 2007). Later Baas Becking did more justice to the theory, saying that: "in a given environmental setting most of the microbial species are only latently present. Hence, on a small scale, most microbial biodiversity is hidden from our observation, because most species will occur at densities below our limit of detection" (De Wit and Bouvier, 2006). Nowadays, searchers recognize that protists seem indeed to occupy larger geographic ranges than multicellular organisms, but 1/ a great share of their diversity is still undescribed, 2/ species that do not produce resting-stages tend to have weaker dispersal, although this statement is increasingly questioned by the wide dispersal ability of small protists (Šlapeta *et al*., 2006; Worden *et al*., 2009; Read *et al*., 2013), and 3/ protists are evolutionary older that multicellular organisms and thus have had more historical chances to colonize new environments (Foissner, 2006). In addition, the existence of strong barriers for the dispersal of marine protists is increasingly recognized, notably the saline gradient (Foissner, 2006; Logares *et al*., 2009). The paradigm is still under debate and as notably been further improved with the advent of genetic-based taxonomy. If morphology, stressed the occurrence of numerous and common shapes across various ecosystems, genetic a) determined that these morphological organisms sometimes constituted distinct species and b) proved that protistan communities (abundant and rare) were structured distinctly across various environments (Caron, 2009; Logares *et al*., 2014). Researchers further recognize that answers to this paradigm depends mostly on the scales of time and space studied (Dolan, 2005). Indeed, it still is not possible to prove the complete absence of an organism at a given place (Fuhrman, 2009), and perhaps it will never be.

b) Redfield Ratio

The work of Alfred C. Redfield (1890-1983) provides a second theoretical framework still in use nowadays. Briefly, his results demonstrated that the average plankton (comprising protists) and the global ocean had similar atomic ratios of Carbon, Nitrogen and Phosphorus (C:N:P = 106:16:1) (Redfield, 1934). According to Redfield, along time, the plankton forced the stoichiometry of the ocean to meet its requirements through the fixation of C, N and P (Redfield, 1958; Klausmeier *et al*., 2004). This led him to hypothesize that the plankton was a "biological control of chemical factors in the environment" and that the nitrate present in seawater as well as the oxygen of the atmosphere are regulated by organic activity (Redfield, 1958). Apart from being one of the major study to highlight the existence of retroactive chemical processes between plankton and the abiotic ocean, the work of Redfield has been integrated in many tools used in today's ecology. Although corrected by in-situ observations, this ratio is still the basis of mathematical stoichiometry used in plankton modelling, notably with the use of "Cell-Quota" equations (Droop, 1968; Sommer, 1991). The ratio in N:P also distinguishes distinct strategies within the phytoplankton (Klausmeier *et al*., 2004), species that invests on a rapid population growth rate develop in nutrient-rich environments and have lower N:P, while species with slow growth rates invests on better adaptability to environments that are N-depleted and have higher N:P (Figure 3). The N:P ratio across species seem indeed to originate in a molecular-involved homeostasis between investments in uptake-proteins richer in N and ribosomes richer in P (Loladze and Elser, 2011). This ratio thus represents a 'biological trait' that distinguishes species on the basis of their physiology and can be used to predict an organism's success in a given condition.

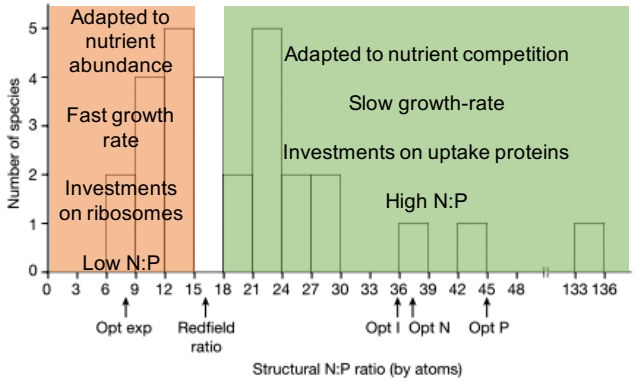

Figure 3: Phytoplankton strategies to distinct environmental conditions and their effect on the N:P of the species, adapted from Klausmeier et al., (2004).

c) Trophic Ecology

One of the next historical junctions between protists and theoretical ecology come from the work of Raymond Lindeman (1915-1942). His last publication "The trophic-dynamic aspect of ecology" probably launched trophic ecology (Lindeman, 1942). In this paper, Lindeman resumed his observations of community successions with lakes. He sorted organisms into trophic levels (or guilds, see the Δ in Figure 4) representing: 1/ Producers, corresponding to photosynthetic organisms able to produce organic matter out of nutrients and light, here comprising phytoplankton with phototrophic prokaryotes and protists (level 1; Figure 4); 2/ Consumers, differentiated in primary, secondary or tertiary consumers, according to whether they feed on primary producers or higher predators, here heterotrophic protists of increasing size, metazoan heterotrophs (or zooplankton) and higher predators (levels 2, 3, 4; Figure 4); and finally 3/ Decomposers, that feeds on fecal or dead matter from the two previous levels and remineralize organic matter. In the paper of Lindeman, bacteria played the role of decomposers, however in another section we will develop the role of heterotrophic protists in remineralization. In a similar fashion to the earlier Lotka-Volterra model (Volterra, 1926), Lindeman observed that there existed a delay in the growth of each trophic level in comparison to their lower trophic level (or main resource), this delay explained patterns of succession of both the pelagic and the benthic communities within lake ecosystems. Finally, Lindeman noticed that the higher the species trophic level was, the higher the organic matter assimilated was allocated to maintain the metabolism rather than to biomass production. To compensate for this phenomenon, Lindeman postulated that predators had developed complex strategies to be more efficient in their predation to still maintain biomass production. Overall the work of Lindeman has been criticized for being too reductive, however it is within Lindeman's framework that researchers have later added the effect of the microbial loop (Pomeroy, 1974), parasites (Dobson *et al*., 2008) or intra-guild interactions (Polis and Holt, 1992).

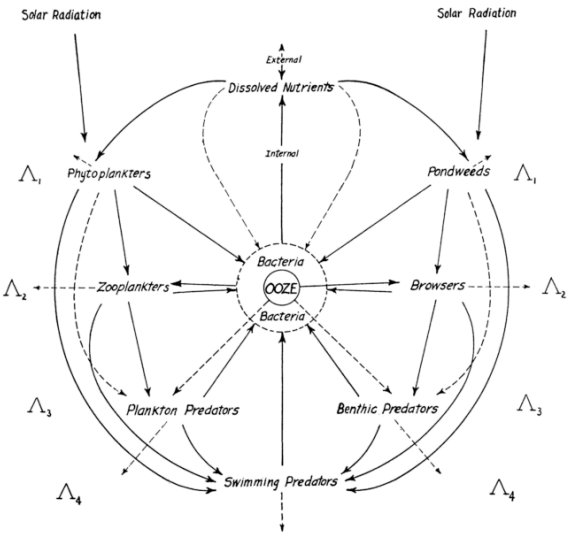

Figure 4: Trophic levels and interactions among the pelagic community (left) and the benthic community (right) of lake ecosystems, as depicted in Lindeman (1942).

d) Competitive Exclusion and the Paradox of the Plankton

From 1940 to 1960, ecologists discussed what Garrett Hardin (1915-2003) later called "the Competitive Exclusion Principle" (Hardin, 1960). The theory was initiated by the work of Georgii-Frantsevich Gause (1910-1986) on the study of the coexistence of two *Paramecium* species in culture (Gause, 1932). Roughly the theory explains that two species A and B, a) with the same ecological niche (or equivalent resource requirements), b) within the same geographical location, c) with one population (A) growing faster than the other (B), should come to the conclusion that A dominates the environment and B becomes extinct. This issue triggered lots of reflection among contemporaries which resulted notably in one of the seminal work of George Evelyn Hutchinson (1903-1991). Hutchinson applied the Competition Exclusion principles to the marine plankton in an article named "The paradox of the plankton" (Hutchinson, 1961). The case of plankton, even if Hutchinson discussed particularly of phytoplankton, is the exact antithesis of what the principle approximates. Namely, plankton exhibits a wide diversity of species all competing on the same resources. Even worse, those resources are scarcely present in the environment which should accentuate species competition. Hutchinson considered the experimental design of Gause's experiment, and questioned what the differences with the natural habitat of plankton were. He highlighted the influence of both the environmental and species interactions influences. Hutchinson, underlined that the

marine environment was complex and nearly never in an equilibrium that could theoretically lead to competitive exclusion. The aquatic environments, especially coastal systems, are indeed among the most changing environments on earth, one can think for example of tides and/or light availability varying in time and space. Furthermore, species interactions like symbiosis or commensalism could help the less efficient species while predation could limit the growth of the supposedly dominant species, leading to the survival of various competitors. The theory later found resonance in the "Intermediate Disturbance Hypothesis" (Padisak, 1993; Reynolds *et al*., 1993). Briefly, in accordance with Hutchinson's work, this theory stated that strong constant abiotic disturbances selected few resistant organisms while weak constant disturbances allowed competitive exclusion to occur and to decrease species richness. Therefore, at intermediate levels of disturbances, a higher diversity of organisms could be found because competition and stress were reduced (Li, 2002). In addition, due to cycles of variable conditions no species dominates long enough to exclude the others (Huisman, 2010). In this context of abiotic fluctuations, species-species interactions, comprising non-exclusive competition, predation or symbiosis, are increasingly recognized to add even more variability, this causes an even higher unpredictability in the composition of the planktonic community that has been called "chaos" (Huisman and Welssing, 1999; Benincá *et al*., 2008). Nowadays, the hypotheses of "the Paradox of the plankton" and of the "intermediate disturbances" have been strongly criticized because they do not prevent competitive exclusion to happen at the timescale of evolution (Fox, 2013). Notwithstanding, at shorter timescales and in marine surveys these concepts are still consistently observed (Li, 2002).

e) Plankton species successions and ecosystem maturity

During the sixties, Ramon Margalef (1919-2004) synthesized the hypotheses dominating the contemporaneous aquatic research in a principle he called the "maturity of an ecosystem" (Margalef, 1963). According to Margalef, the structure of community "becomes more complex, more-rich, as time passes", he proposed the term maturity as a metric of this increase in complexity in any undisturbed ecosystem. He defined that the maturity of a system held a great relation to the availability of resources for primary production. Indeed, he observed that in a

"simple system", replete in nutrients and where light is available, the community was dominated by few fast-growing and competitive photosynthetic species (e.g. during a bloom). Conversely, "complex systems" were dominated by slow-growing species, more efficient under lower resources availability, and these systems left more space for the apparition of more heterotrophs. Accordingly, species richness was also higher in complex ecosystems. Margalef applied these hypotheses to the spatial and temporal variations of marine ecosystems, and focused on the vertical gradients of the water column and the typical phytoplankton succession. He noticed that diatom species where occurring when turbulent mixing brought nutrient to the surface, and supposed that diatoms were adapted to nutrient competition and unable to maintain on surface without hydrodynamic (because un-motile). He opposed them to the flagellates (motile), whom survived on lower nutrients and lower hydrodynamic and that co-occurred with other trophic levels. Based on these observations, Margalef supposed that among pelagic ecosystems there existed a progression towards a more mature ecosystem with the coupled decrease in hydro-dynamism and nutrient inputs. The concepts developed by Margalef for the plankton bears similitudes with other works found in benthic (Pearson and Rosenberg, 1978) or in plant communities (Connell and Slatyer, 1977). For these communities, successions tend generally to an ecological climax, or a peak in maturity. However, in the marine plankton, Margalef noted that such peaks could never be found. Indeed, Margalef hypothesized that the marine environment presented favorable and harsh conditions for marine plankton within short timescales, this process enclosed plankton communities into cycles of maturation without ever being maintained at a climax.

f) The microbial loop

During the rest of the sixties, the consecutive developments of community-metabolism measurements (e.g. respirometer) (Pomeroy and Johannes, 1966), water pumps, and fine-mesh water filters (Beers *et al.*, 1967), allowed to target more easily the smallest size-classes of plankton. These methods conducted to decisive observations (Beers and Stewart, 1969; Malone, 1971) that resulted in a change of paradigm for the plankton trophic organization (Pomeroy, 1974). Until then, the pelagic community was still thought in trophic levels, with as described by Pomeroy (1974): 1) diatoms (phytoplankton); 2) copepods (metazoan zooplankton); 3) Krill;

4) Fishes; 5) Fish Predators. But the new approaches highlighted the influence of the understudied, then called, "nanno-plankton" (< 60 μm), dominated by small protists and prokaryotes. Starting with phototrophic organisms, Pomeroy, based on a bibliographical study, showed that previously ignored organisms (< 60μm) represented between 40 to 99% (90% in average) of the global ocean photosynthesis. For example, in the Sargasso Sea, *Coccolithus huxleyi* (later named *Emiliana huxleyi*), a "nanno-plankter", was responsible for most of annual photosynthesis activity. This made Pomeroy propose that the nanno-plankton was a constant photosynthetic "background" able to grow on low nutrient conditions, while dinoflagellates and diatoms were the more visible organisms growing only during seasonal pulses of nutrients. For heterotrophic organisms, Beers & Stewart (1969) distinguished zooplankton (> 200 μm) from micro-zooplankton (< 200 μm) on the basis of filtration methods. They noticed that both in terms of abundance and total bio-volume, micro-zooplankton could represent up to 90% of total zooplankton and was usually dominated by Ciliates. Based on evidence from both phyto- and zooplankton, Pomeroy (1974) integrated new compartments to the pelagic trophic web. Phytoplankton was divided into "net-phytoplankton" (> 60μm) and "nanno-plankton" (< 60μm), while heterotrophic protists were integrated as a complex, that contained dissolved, particulate organic matter (DOM and POM) and Bacteria, that interacted with the rest of the plankton community (Figure 5).

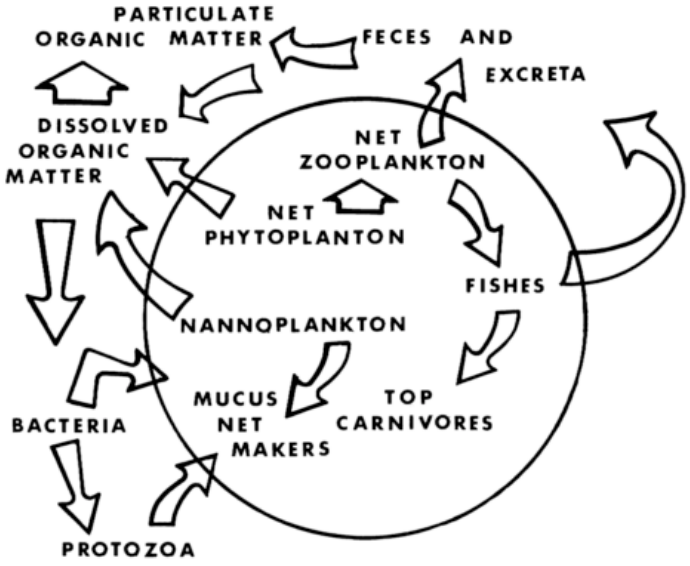

Figure 5: The 'classical' schematic interpretation of the pelagic foodweb (inside the circle) and the new pathways considered by the microbial loop (outside), as proposed by Pomeroy (1974).

At the time, Pomeroy's assumptions were rejected by the scientific community (Kirchman, 2008). However later on, with the birth of many new techniques (epi-fluorescence microscopy, chemical methods, radioactivity, microcosm), the scientific community came to take a second look at Pomeroy's work. On the basis of more robust observations, Azam *et al*. (1983) reformulated Pomeroy's theory into "The Microbial Loop". Azam's review stressed the inherent competition for nutrients in between phytoplankton and bacteria. The authors went on to state that bacteria were better competitors for nutrients than phytoplankton because of better kinetics and greater abundances. They proposed that heterotrophic protists represented a substantial grazing impact on naturally abundant bacteria, which left enough space for the occurrence of larger phytoplankton. Ducklow (1983), also noticed that bacteria alone could not explain the remineralization process evidenced in the sea. Ducklow stressed the role of the catabolism from heterotrophic protists in the remineralization, cementing the concept of the microbial-loop as the cumulated activity of bacteria and micro-zooplankton. At this point, the general scheme affirmed that 1) the DOM synthesized by the growth and decay of phytoplankton, 2) was first processed by Bacteria, themselves eaten by 3) small flagellates further predated by 4) Ciliates that allowed the recycling of dead organic matter. The fate of this secondary production was later highlighted by Sherr & Sherr (1988), whom showed that the metazoan zooplankton could fed on the heterotrophic protists of the microbial loop. Those last authors also supposed that in oligotrophic conditions with a domination of smaller phytoplankton, metazoan could thrive by feeding on larger heterotrophic protists. Compared with the linear food-chain of Raymond Lindeman, the current trophic scheme of pelagic plankton thus represented more of a food-web, with numerous intra- and extra-guild interactions.

By the dawn of the 90's, few advents complicated even more the marine microbial scheme (Figure 6). Some studies indeed highlighted the existence of mixotrophy and thus the impact of supposed phototrophs on grazing and reversely the impact of heterotrophic protists on plankton photosynthesis (see Sanders (1991) for a contemporaneous review). From then, Bratbak *et al*. (1994) also integrated viruses to the microbial loop. These authors first estimated that viral lysis had a major effect in reducing bacterial abundance in the ocean. Considering this unfinished predation, Bratback *et al*. estimated that the lysed cells could release

nutrients and DOM that fueled the microbial loop and the recycling of organic matter. In addition, they supposed that viruses could only infect dense populations of bacteria, according to Bratbak *et al.*, this phenomenon helped to maintain equivalent diversity across the community of organism that the viruses infected. This last supposition was later formalized by the formula "killing the winner" by Thingstad & Lignell (1997), that offered yet another explanation to Hutchinson's paradox. Finally, the last organisms that integrated the microbial loop were the protistan parasites. Although many species were described throughout the 20th century; see e.g. the works of Edouard Chatton (1883-1947), of Jean (1922-1989) and Monique Cachon (1928-2011) (Coats, 1999); their ecology within pelagic ecosystems is still poorly understood. Early studies focused on the parasitism of Dinoflagellates (Coats *et al*., 1996), after infecting a host these parasites were evidenced to release numerous zoospores as well as to destroy their host's cell (Erard-Le Denn *et al*., 2000). These processes turned abundant hosts into more available DOM and POM for the microbial loop, as it was shown that ciliates could feed on the zoospores released (Johansson and Coats, 2002). As there also seem to exist a prey density regulation of parasitism (Holt *et al*., 2003), the effects of parasites on protistan diversity were hypothesized be the same as the one of viruses on their bacterial preys. Parasitism was more recently highlighted as particularly useful in order to make available some otherwise inedible preys, notably diatoms (Scholz *et al*., 2016). Furthermore, genetic-based sampling stressed the high abundance of protistan parasites within the ocean (López-García *et al*., 2001; Lefèvre *et al*., 2008; de Vargas *et al*., 2015). This diversity of parasites was hypothesized to support many species interactions within the plankton (Lima-mendez *et al*., 2015), the effect of an increase in species connectance is not fully understood but has been postulated to increase ecosystem stability (Lafferty *et al*., 2008).

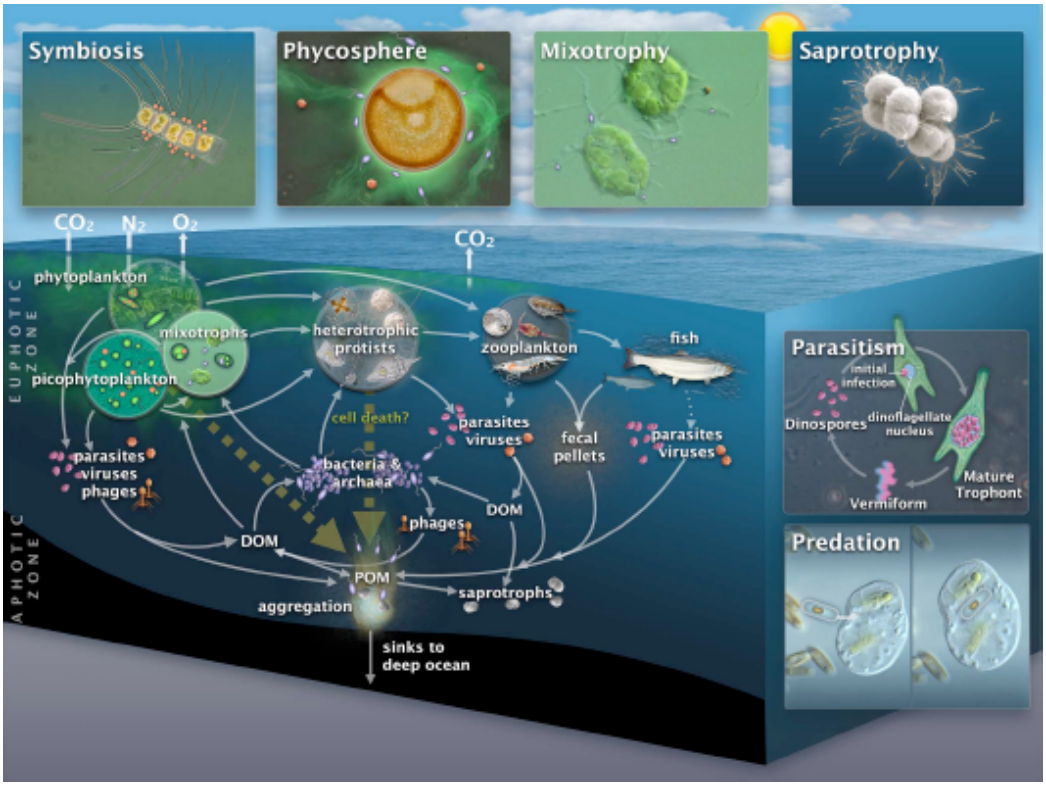

Figure 6: The pelagic foodweb integrating all major advances in protistan ecology, as described by Worden et al., (2015).

g) Transitions in pelagic ecosystems

By combining the amount of information about the microbial loop and marine production, Legendre & Rassoulzadegan's (1995) synthesized a typology of marine pelagic ecosystem that is highly influential on nowadays understanding of plankton ecology. The authors reviewed contemporaneous scientific knowledge and stressed the existence of two different pathways for plankton production: the herbivorous and the microbial food webs (Figure 7). The two pathways answered to opposite biotic conditions, the herbivorous chain took place within conditions replete in nutrients, while the microbial food web appeared in waters depleted in nutrients. The two processes also had a strong effect on the production of marine pelagic ecosystem, indeed the herbivorous chain was created by new inputs of nutrients fueling large phytoplankton (e.g. diatoms) and a food-web composed of larger organisms, this consisted in "new production". Conversely, the microbial food web thrived on fewer nutrients concentrations regenerated by the microbial food-web that alimented only small food-webs and small phytoplankton organisms, consisting in a "regenerated production". The authors stressed that pelagic ecosystems were more a continuum in between these two states. They divided this continuum within four major trophic states: 1) "the herbivorous web" where the system shows high nutrients and domination by large phyto- and zooplankton species; 2) "the microbial loop" where the system is nearly dominated by bacteria competing with phytoplankton on low nutrients and a sparse microbial loop; and two transient states: 3) "the multivorous web" similar to the "herbivorous web" but combined with a small microbial loop; 4) "the microbial web" similar to the "microbial loop" but with an important share of protozoa that regulates production of bacteria and that can support the development of phytoplankton and higher zooplankton. The decisive factor in the fluctuation between these states was nutrient availability and especially the competition in between small plankton, good competitor under depleted environments, and large phytoplankton better competitor under replete conditions. Legendre and Rassoulzadegan only found one exception to this rule: High Nutrients Low Chlorophyll zones (HNLC). Indeed, in those zones the high nutrient content paradoxically did not lead to an herbivorous or multivorous web. Based on a review of other studies they highlighted that the lack of iron was a supplementary limiting factor for primary production in these zones. Indeed, iron is especially required to

create the enzymes involved in the uptake of larger phytoplankton species, hence in its absence those systems were kept in a microbial-loop state. This conception of the pelagic system will later be fully integrated and be verified by multiple and various articles and is still highly influential nowadays (Duarte *et al*., 2000; Vidussi *et al*., 2001; Sherr and Sherr, 2002; Harmelin-Vivien *et al*., 2008; Tortajada *et al*., 2012).

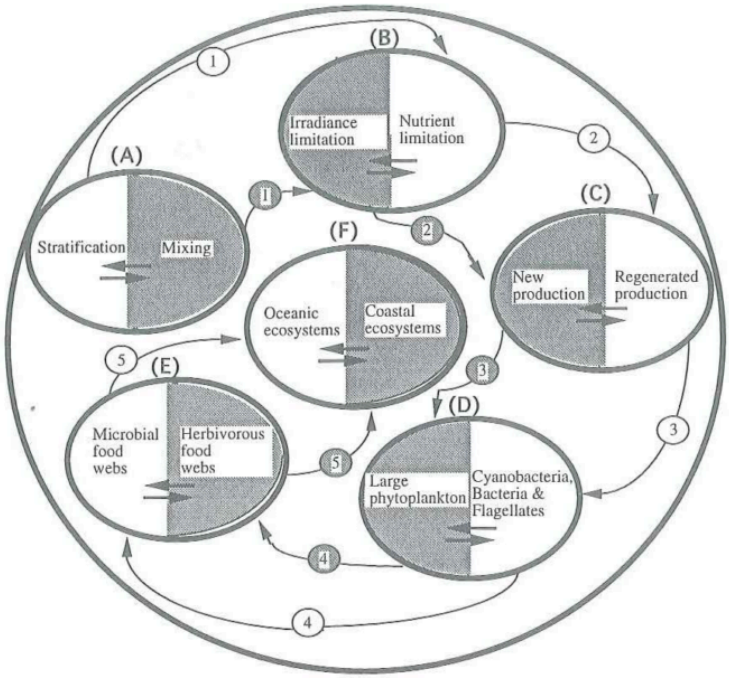

Figure 7: Alternation between herbivorous food-web (gray) and the microbial foodweb (white) and the 6 factors that favors the development in between food webs; A. Hydrodynamic, B. The origin of nutrients, C. The type of primary production, D. The community developed, E. The resulting food-web and F. The type of ecosystem in which they are favored. Legendre et Rassoulzadegan (1995).

h) Neutral theory and dispersal

The last theory that shook the community of marine planktonologists, is the neutral theory of biodiversity postulated by Stephen P. Hubbell (2001). In every science, neutral theories suppose that "nothing happens", these theories are indeed not made to explain a phenomenon but simply to bring attention to the processes in a phenomenon that are not deterministic. Hubbel proposed that for biodiversity the neutral theory would imply that all the hypothesis leading to competitive exclusion and other interaction processes would be absent, species among a same trophic level would be identical in their probabilities of giving birth, death, migration, and speciation. As a consequence, species distribution would be determined only by demographic stochasticity, random dispersal, and random speciation. Thereafter, in

marine plankton ecology the stochasticity of species distribution was evidenced by species counting under the microscope, for both heterotrophic protists (Dolan *et al*., 2007) and phytoplankton (Chust *et al*., 2013), but also by phytoplankton phenotype modelling investigations (Barton *et al*., 2010; Clayton *et al*., 2013) and more recently by a genetic-based taxonomy approach of all marine protists (Ser-Giacomi *et al*., 2018). All authors stressed the vast influence of marine currents and oceanic transport on species distribution. However, the process of dispersal only co-influenced species distribution, in addition with resource availability. Barton *et al*., (2010) notably formalized that ultimately phytoplankton local diversity was explained by a balance in between the timescale of competitive exclusion and dispersal. Indeed, in environments at equilibrium (constant high or low nutrient concentrations) species would usually be subjected to competitive exclusion for resources and the less competitive species would tend to a local extinction. However, in this same environment, if dispersal dilutes the abundance of the most competitive species or simply displaces the two species before competitive exclusion ends, then competitive exclusion does not happen. Barton *et al*., (2010) also supposed that oceanic phytoplankton diversity hotspots were probably most influenced by dispersal at the interface between distinct oceanic basins, where dispersal would influence the formation of an ecotone increasing local diversity.

i) Conclusion

Much of the knowledge that exists about marine protistan ecology arose with studies on phytoplankton, and thus focused mainly on phototrophic protists. However, as described here other protists interacts with phytoplankton. In 2002, Sherr & Sherr have reviewed current knowledge of heterotrophic protists. In pelagic systems, protozoans were represented by small flagellates, larger ciliates and dinoflagellates, with punctual appearance of amoeba-like organisms. At the light of those same studies, Sherr and Sherr supposed that these organisms played major roles among plankton food-webs e.g. predation on bacteria, phytoplankton, other heterotrophic species, consumption by meta-zooplankton species, increase of remineralization rates of the microbial loop and even primary production with some mixotrophic species. More recently Caron *et al*. (2012) added to the protozoan community an underestimated number of very small heterotrophic organisms feeding on bacteria

(e.g. in Massana *et al*., 2006), some protistan parasites (e.g. in Guillou *et al*., 2008) and other symbionts (e.g. in Gómez *et al*., 2011). The diversity of these organisms has been unveiled by sequencing methods and only few descriptions of these organisms and their ecology are available, However, now that ecologists have easier access to the protistan diversity of the ocean, and in regard of their influence on the pelagic system, it is time to study the community of marine protists in its entirety.

4) Methodological developments in the sampling of marine protists

As evidenced in the previous section, there is several ways to sample marine protists and all advents in sampling technology helped to the comprehension of the planktonic community. The first method probably arose with optical microscopy. Through the lens of light microscopy scientists first described marine protists, structured the modern protistan taxonomy and studied species distribution among marine samples (Wainwright, 2003). Later, electron microscopy helped to describe species with further precision (Adl *et al.,* 2005), while nets with various mesh-sizes allowed to target distinct size-classes of the plankton (Sieburth *et al.,* 1978). Culturing methods were also inherent to microscopic investigation of newly found taxa, cultures indeed allowed to study the growth, life-cycles, ways of feeding of these organisms and to better understand their ecology. For studying cells natural abundance, flow-cytometry now study cells one by one, which allows to give information about cells abundance but also their fluorescence (related to plastid composition) or size; the method notably underlined the high abundances of pico-phytoplankton in the marine ecosystem (Li, 1994). The natural concentration of pigments specific to certain taxa can also be used to get a proxy of the taxonomic composition of phytoplankton (Vidussi *et al.,* 2001; Ansotegui *et al.,* 2003). However, all methods are limited, e.g. microscopy in the identification of small species inscrutable under microscope, pigment and flow-cytometry methods on the taxonomy of the organisms they investigate, in addition not all species can be cultured or sampled enough times to allow a good description.

DNA-based taxonomy made a major breakthrough in marine microbial diversity at the dawn of the 21st century. The basis of this method consists in 1/ extracting DNA from a cell, 2/ amplifying one or multiple genes, i.e. genetic barcodes, with PCR, 3/ tagging the DNA sequences with the organism known species name or, conversely, 4/ looking for sequence homology in a larger DNA database with a curated taxonomy of the gene(s) studied (Tautz *et al.,* 2003). This method notably allowed the discovery of numerous uncultured organisms within the

environment and also gave a taxonomy to some under-considered taxa, e.g. heterotrophic protists, parasites or small organisms (López-García *et al.,* 2001; Massana *et al.,* 2004). These methods are now adapted to study the composition of full communities of organisms, an approach that have been called metabarcoding. Some uncertainties in this method remains important, however for marine protists, most of these biases have been overcome. a) To compare the diversity within a community, a single marker needs to be chosen. This marker needs to be conservative across the community and enough variable to distinguish existent taxa. For eukaryotic diversity, the identified markers are components of the DNA that codes to the small subunit of ribosomes (SSU rDNA), they are called V4 and the V9 (Stoeck *et al.,* 2010). In addition, unsupervised bioinformatics methods allow to detect and cluster together markers highly similar (Mahé *et al.,* 2015), this constitutes the species level of DNA based-taxonomy called an Operational Taxonomic Unit (OTU). b) A database comprising the sequences of all the organisms within the community needs to be created. A database of the V4 and V9 regions of eukaryotic SSU rDNA has been constructed on the basis of all known eukaryotes (Guillou *et al.,* 2013), and further developments helps to find the closest taxonomic relatives of unknown taxa. c) PCR amplifies all sequences exponentially but at distinct speed according to sequence length, this bias force the expression of abundance as relative abundance. An increasing number of methods in ecology have been adapted to study species ecology and interactions based on computational datasets (Langfelder and Horvath, 2008; Legendre and Legendre, 2012; Kurtz *et al.,* 2015; Quinn *et al.,* 2017). d) Relative abundance can be calculated only on the assumption that organisms have the same number of sequence copies by cell. For marine plankton, a strong assumption that is made is that organisms within the same size range can have the same number of copy by cell (de Vargas *et al.,* 2015), however this might not be the case for all organisms (Decelle *et al.,* 2014). Nevertheless, genetic-based taxonomy is increasingly used in marine surveys to describe microbial communities. However, the method has been criticized for decoupling the taxonomy and the functional diversity of the organisms investigated (Stec *et al.,* 2017). In fact, many protists are discovered with this approach, their phylogeny is traced back and we have a good representation of their natural abundance. Still the role these organisms in their environment as well as how the environment influences their distribution remains poorly understood.

5) A perspective for marine protists: functional ecology

Functional ecology attempts to describe the various strategies that species have adapted to survive in their environment and that ultimately influence their role within an ecosystem. If the functional roles of marine protists are now quite known (e.g. primary producers, predators, parasites, decomposers) the strategy that they have adapted are still poorly understood. As a first step, few theories about marine protists strategies will be presented. Then, other specialized frameworks will be introduced and we argue for the development of a trait approach to the functional diversity of marine protists.

a) Strategies of marine protists in aquatic ecosystems

General ecology has strongly shaped the modern approach of functional ecology. The origin of species "strategies" can perhaps be traced back to the work of MacArthur and Wilson (1967) that defined the concept of *r* and *K* strategies. The theory is based on the observation that species invests distinctly in population growth rate and lifetime duration. The *r* strategy represents species with a fast growth-rate but a short lifetime, that typically invades environments full of dedicated resources; while the *K* strategy has a slower growth-rate, a longer lifetime, but is more efficient in its resource use and can thrive on lower resource availability (Pianka, 1970).

This framework was later refined with phototrophic organisms in mind during the work of Grime (1974) on larger terrestrial plants. Grime distinguished three strategies of plants adapted to 1/ Competition, when resources were abundant (equivalent to *r* strategies), 2/ Stress, when resources were scarce (equivalent to *K* strategies) and 3/ Disturbances, when resources were present but a process of any type limited its use (a new strategy). Grime indeed noted that disturbances limiting the growth of competitors where often present in agriculture, either by the means of predation, pathogens or human-induced withdrawal, and that stressed environment were stillinhabited. In a similar fashion to *r* and *K* strategies, each of the three strategies was traceable by distinct morphology, growth rate or longevity, and the relative abundance of these strategies on the environment was determined by the balance of resource and disturbance observed in the field studied. Among

phytoplankton, Margalef (1978) and Reynolds (1980) were quick to recognize similar patterns (Figure 8). Both authors highlighted the influence of two major parameters on the growth of phytoplankton. Nutrients were the limiting resource, while disturbances were implemented by any parameter that limited light availability. Interestingly there existed strategies adapted to the predominance of each hydrologic condition. Noting the interplay between water turbulences and nutrient inputs in marine ecosystems, the authors evidenced the domination of Diatoms in well mixed and nutrient-rich waters. Dinoflagellates were found at the other side of the environmental gradient, when water turbulence was low, stratification occurred, and when nutrient were generally depleted. Only Reynolds noted that environments with high nutrients but low light availability existed, e.g. in winter, in deeper and highly turbulent water columns or in turbid waters, and these conditions showed a domination by small nano-pico-phytoplankton (Reynolds C.S., 2003). Reynolds later defined these strategies following a terminology defined by Grime (Grime, 1974; Reynolds, 2006):

- *Competitors* (C), good competitors at high nutrient concentrations, e.g. Diatoms (previous strategy *r*).

- *Ruderals* (R), with a fast growth rate but a low longevity, able to thrive under disturbed conditions with low light. e.g. small green algae, Chlorophyta or Cyanobacteria (new strategy).

- *Stress tolerant* (S), with a slow growth rate but high longevity, present at low concentrations of nutrients. e.g. Dinophyta (previous strategy *K*).

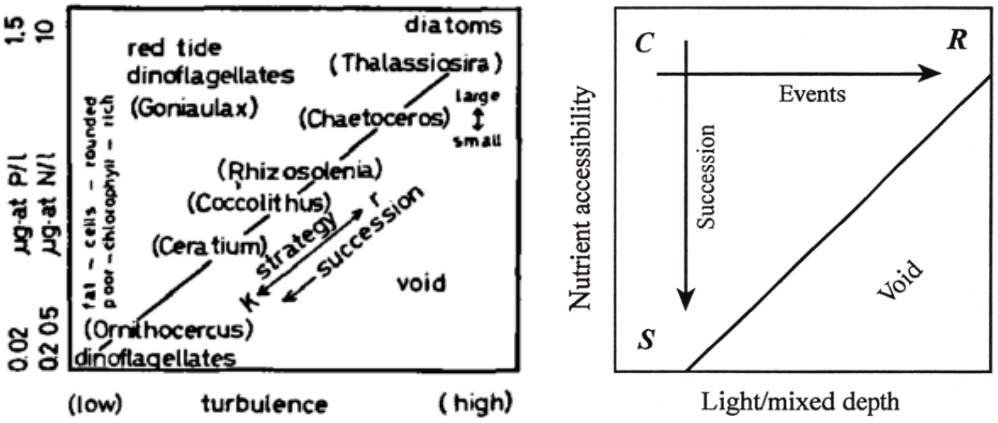

Figure 8: Margalef's (left) and Reynolds (right) schematic interpretation of phytoplnakton strategies within aquatic ecosystems, note that Margalef (1978) only recognized a nutrient gradient coincident with turbulence and distinguishing only diatoms and dinoflagellates, while Reynolds (2003) added light and mixed depth (light is decreasing with mixed depth from left to right) as a constraint favoring the ruderals.

b) Lifeforms and successions

Both Margalef (1978) and later Reynolds (1984) recognized that each strategy had distinct "life-forms" adapted to the conditions coincident with their environmental preferences. For example, large un-motile diatoms generally dominated turbulent environments, while motile dinoflagellates thrived in stratified waters. Later it was highlighted that cell-size reduction was also an advantage of small organisms to better grow under light limitation (Raven, 1998; Marañón, 2015). If motility maintained the organisms on the surface against sedimentation, Margalef (1978) also postulated that it was an investment against oncoming predators, as well as swimming could favor water renewal around the cells (and by thus nutrient diffusion). He also emphasized on the importance of cell morphology (size, length or bulk), presence of spines, mucilaginous covers, colonies, rigid or toxic membranes against predation. By the end of his paper Margalef synthesized a differential model where the dynamic of phytoplankton was governed by: growth rate of various populations, predation, sedimentation and dispersal. Margalef noted that it was the diverse investment of phytoplankton species in relation to these parameters that created a diversity of species able to run successions parallel to nutrients and turbulence mutual decrease.

c) Patterns of succession and functional groups

Focusing more on patterns of phytoplankton successions within lakes, Reynolds (1980) regrouped organisms in functional groups representative of lifeforms, that, he noticed, had also similar ecologies and physiologies (Figure 9). He hypothesized that these groups distinguished with further precision the various strategies adapted to gradients in turbulence and nutrients. The 14 functional groups contained: (1, 2) Diatoms dominating the spring bloom (1 appeared in poor lakes, 2 in richer lakes) and (7, 8) other Diatoms occurring at the end of summer (7 appeared in poor lakes and 8 in richer lakes). When the waters started to stratify after the spring bloom of diatoms, the lakes were often dominated by green algae (3, 4, 5) with presence at the end of cyanobacteria (6). During harsh stratification, swimming dinoflagellates (10: *Ceratium*) or other cyanobacteria dominated (9: *Microcystis*). Other groups were observed frequently but never dominated, notably the (11) small colonial

Chlorophytes (*Pediastrum*) and the filamentous cyanobacteria *Oscillatoria* (12) that dominated during lower productivity periods, while other poorly identified nano-algae (X) and *Cryptomonads* (Y) were present every time but at low abundances. As Margalef, Reynolds recognized that there were succession phases more repeatable than others and attributed these perturbations either to the allogenic changes in the physical environment or to predation. Later on, Reynolds used performance traits (i.e. measure of the success of a species in certain conditions) rather than functional trait (i.e. a selective advantage that impacts a species success) (Violle *et al*., 2007) and complicated even further his scheme, with the recognition of more than 31 functional groups of freshwater phytoplankton (Reynolds *et al*., 2002).

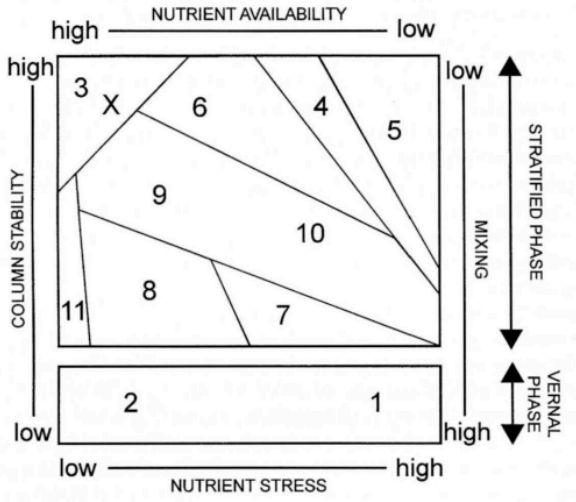

Figure 9: Reynolds' (1980) work on functional groups of phytoplankton and their environmental preferences in lake ecosystems, numbers and letters represent the functional groups detailed in the text.

d) Beyond phytoplankton: heterotrophic protists

In parallel to these works on phytoplankton Fenchel (1980a, 1980b, 1982a, 1982b) helped to define the functional diversity of heterotrophic protists. In a first series of paper Fenchel focused on Ciliates (Fenchel, 1980a, 1980b). He noted that Ciliates had developed complex ways of feeding involving cilia that concentrated the suspended food close their mouth (i.e. cytosome). The efficiency of this mechanisms was involved in the clearance rate (food items ingested per predator per unit of time) of Ciliates. The optimal size of food items for ciliates was a function of the clearance rate, the size of the mouth of the ciliate species and food concentration. The success of heterotrophic protists in conditions of various prey abundance could thus be

estimated by measurable morphological characters (Fenchel, 1980b). With help from the study of their functional response to small food items, Fenchel estimated that Ciliates could not be efficient removers of bacteria as proposed in the microbial loop concept (Pomeroy, 1974; Fenchel, 1980a). Instead, Fenchel studied the functional response of smaller heterotrophic flagellates and proposed them as regulators of bacteria in marine environments (Fenchel, 1982a). Within the small heterotrophs that he investigated, most fed with a flagellar apparatus that brought food particles towards their cytosome or to their pseudopodia. He noted that the size of their preferential food was determined by their clearance rates, distinct motile or attached ways of living, as well as the abundance, size and motility of their prey (Fenchel, 1982a). The functional response of small heterotrophic protists to small particle size indicated that they could feed on the natural bacterial abundances of the marine environment (Fenchel, 1982b). These results were later integrated to the microbial loop as depicted by Azam *et al*. (1983), where bacteria were eaten by small heterotrophic flagellates and ciliates were more efficient in the size range of preys such as small heterotrophic flagellates.

It is thus by using functional traits that researcher better understood protistan ecological strategies. The study of these strategies than improved the knowledge of the succession patterns, the composition and the functional roles of plankton communities.

e) Contemporaneous Functional Ecology

The functional approach gained wide interest among ecologists when Tilman *et al*. (1997) showed that functional diversity influenced more ecosystem functioning than species diversity. Gathering different plant traits, these authors regrouped species among functional groups and studied the effects of community structure on productivity. The results showed that species productivity was influenced more by the number of functional groups than by species diversity, however species diversity within functional groups, or functional redundancy, still increased productivity. By cumulating distinct strategies there was thus a better utilization of resources within the ecosystem which allowed to increase plants productivity. Since the work of Tilman and colleagues, the functional approach enriched by harvesting traits among various communities and this lead to recent publications about, among others:

benthic systems (Rigolet *et al*., 2014), zooplankton community (Barnett *et al*., 2007; Kiørboe, 2011; Litchman *et al*., 2013; Benedetti *et al*., 2015), fishes (Mouillot *et al*., 2013, 2014; Villéger *et al*., 2013), microbial litter (Allison, 2012) or even amphibians (Tsianou and Kallimanis, 2015).

For marine plankton, there has been major reviews of relevant phytoplankton traits by Litchman & Klausmeier (2008), but also zooplankton traits (Litchman *et al*., 2013) and microbial traits (Litchman *et al*., 2015). The reviewing work of Litchman and colleagues gave much importance into sorting traits according to 1/ their typology (i.e. whether involving morphology, physiology, behavior or life-history) and 2/ their effect on ecological functions (i.e. reproduction, resource acquisition and avoidance of predation) (Figure 10). It is necessary to note the distinction between ecological function and functional role, for phytoplankton the functional role depending on the question can be e.g. primary production, while the ecological functions are proxies estimating the chance of a phytoplankton species to thrive, and to carry out primary production, under certain conditions (i.e. fitness, Violle *et al*., 2007). Another interesting focus of Litchman and colleagues was the recognition of trade-offs as an interrelation of traits, as they noted, the interdependence in these traits defined distinct ecological strategies based on investments into the distinct ecological functions (i.e. reproduction, resource acquisition and avoidance of predation). For example, a *K* strategy species have a long lifetime but this is only possible at the cost of a slower development.

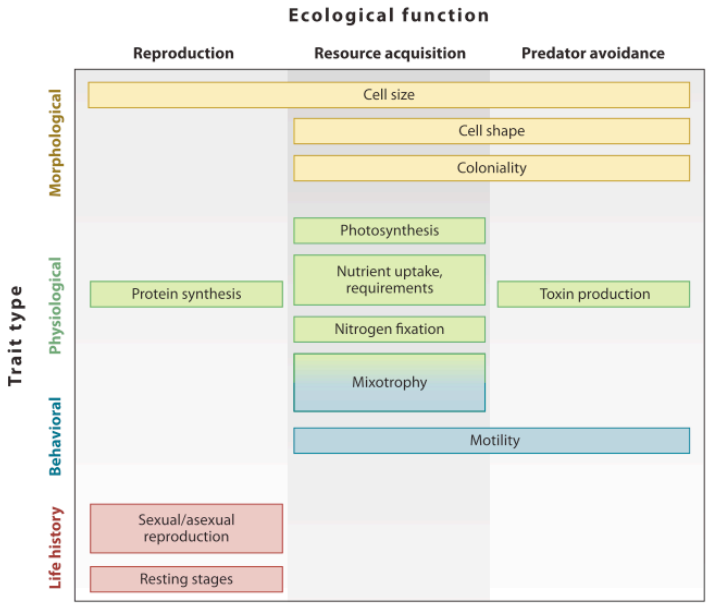

Figure 10: The theoretical trait framework of Litchman and Klausmeier (2008) for phytoplankton.

These reviews have fueled further works on nutrient utilization traits (Edwards *et al*., 2012, 2013a, 2013b) and morphological groups (Kruk *et al*., 2010, 2011) which helped to better understand the dynamics of phytoplankton community structure within aquatic ecosystems. The functional diversity of heterotrophic has been less treated by researchers. Recent works focused on refining the functional response of model species (Weisse *et al*., 2016) or on building functional groups that distinguished protistan parasites and heterotrophs according to their size (Genitsaris *et al*., 2015, 2016). Perhaps the gathering of relevant functional traits for heterotrophic protists have been overshadowed by the increasing recognition of mixotrophy within marine protists (Caron, 2016; Stoecker *et al*., 2017; Mitra, 2018). This trophic strategy complicates our understanding of functional traits, e.g. in mixotrophs nutrient acquisition and trade-offs are still poorly understood (Våge *et al*., 2013). The existence of mixotrophs also justifies to study the protist community as a whole and not as a strictly dichotomous assemblage (Flynn *et al*., 2013). However, the reunion of both phototrophic and heterotrophic protists calls for a common set of traits that is necessarily restrictive at the species level in order to be the most inclusive at the community level, indeed trophic-specific traits cannot resume the diversity of all protists. If traits of resource acquisition or growth rates cannot be measured for all protists yet, some simpler parameters can be gathered from the specialized literature. Many protists are starting to be described and sometimes simple morphological groups can better describe the community than other classifications (Kruk *et al*., 2011).

6) Marine Coastal Ecosystems

In the ocean, the production of marine plankton is triggered by the combination of few environmental processes. Briefly, during winter and periods of high hydrodynamic, the deep waters enriched in nutrients (due to past remineralization) are mixed with the surface depleted waters. When sunlight becomes more available in early spring, phytoplankton organisms, comprising an important share of protists, uses nutrients present at surface to grow exponentially and this triggers the spring-bloom (Sverdrup, 1953). Later on, a summer stratification settles in due to heat exchange with the atmosphere and the warming of the surface, this stratification prevents the input of new nutrients at surface which becomes rapidly depleted and dominated by organisms from the microbial-loop (Legendre and Rassoulzadegan, 1995). Coastal ecosystems typically follow a similar functioning, however there is multi-various source of nutrients, including river inputs, benthic remineralization, variability in hydrodynamic, and higher water turbulence with associated upwelling (Cloern, 1996; Capone and Hutchins, 2013; Maguer *et al.,* 2015), that also influences protistan production. In addition, coastal ecosystems are submitted to strong inputs of terrestrial organic matter (Liénart *et al.,* 2018), that can decrease the light availability for primary production (Cloern, 1987), but also fuel the remineralization processes of the microbial-loop (Hedges *et al.,* 1997). In addition, the salinity gradient represented by estuaries is supposed to carry species with distinct adaptations (Logares *et al.,* 2009; Telesh *et al.,* 2013), and sampling these ecosystems might help studying a large diversity of organisms in few iterations. The accumulation of all these phenomena usually explains the low predictability of microbial population among marine coastal ecosystems (Cloern and Jassby, 2008; Martin-Platero *et al.,* 2018), but also the wide diversity of protists found in these environments (Massana *et al.,* 2015; Hu *et al.,* 2016), that supposedly also corresponds to a high functional diversity (McGill *et al.,* 2006).

In addition, coastal ecosystems support anthropogenic activities and pressures since at least 20 000 years (Rick and Erlandson, 2009). Nowadays, 40 to 60% of the human population is concentrated within the first 100km between terrestrial

environments and the shoreline (Vitousek *et al.,* 1997; Martìnez *et al.,* 2007). The ecosystem services, i.e. the economic benefits that humans derive from earth natural habitats and ecosystem processes, provided by coastal zones to human population are such that they could represent one third of earth's economic value (Costanza *et al.,* 1997). Furthermore, the presence of human populations causes anthropogenic pressure that affects protistan communities and their functional role. These effects range from the global eutrophication of coasts and its influence on the growth of toxic protists (Heisler *et al.,* 2008), the introduction of invasive species (Hallegraeff and Bolch, 1992), the removal of top-predators or habitat degradations (Borja *et al.,* 2010), the increasing of anoxia events (do Rosario Gomes *et al.,* 2014; Levin and Breitburg, 2015), to the global effect of climate change (Harley *et al.,* 2006; Hutchins and Fu, 2017).

In this sense, prior to scientific curiosity and challenges in microbiology, better understanding the role of protistan communities and their dynamic within coastal ecosystems represents also a major issue for human activities and ecosystem management.

OVERVIEW AND OBJECTIVES

The main purpose of this PhD was to help the understanding of marine protists ecology within coastal ecosystems by the use of a functional approach. To do so, I have combined a DNA-based taxonomy approach to marine protistan diversity and a trait approach to study the functional diversity of protists. A functional classification combining both autotrophic and heterotrophic protists is proposed, the distribution of species and their traits in the environment was studied, the patterns of marine protist's functional diversity were analyzed, the way the environment can influence this functional diversity and how protists functional groups can influence community assembling are discussed.

Waters from various coastal ecosystems from the French coast were sampled from 2009 to 2015. In addition, a campaign was carried out in 2015 across the coasts of Senegal within waters influenced by the Senegalese upwelling. During the three years of my PhD, I had the possibility to be formed to the whole process of protist genetic biodiversity study. I had the possibility to participate to a sampling cruise, to learn the difficulties of sampling protists in the sea (results from these campaigns are not presented in this manuscript). I have notably taken part in the laboratory work to understand the bias and limitations of the genetic analyses at the basis of the metabarcoding approach (DNA extraction, PCR amplification of genetic markers, DNA purification and library construction). I have processed the samples from the campaigns of Daoulex 2015, Senegal 2015 and M2BIPAT (September 2014, March, July and September 2015), corresponding to 610 distinct samples (out of a total of 1145). All samples were then sequenced and processed with bioinformatics, that were carried out respectively by the Genotoul sequencing platform (https://get.genotoul.fr/) and Stéphane Audic at the Station Biologique de Roscoff.

Based on functional traits highlighted by various reviewing works, I have then carried out a literature survey, comprising 717 distinct sources, to propose available traits relevant to annotate protistan taxa. Using the taxonomic information of the molecular Operational Taxonomic Units (OTUs) found in the vast dataset assembled, I have annotated the OTUs with functional traits (details about this work can be found in supplementary material of **Chapter I**). On the basis of a community (samples x OTUs) and a trait table (OTUs x traits), I was then able to study the functional diversity and ecology of marine protists within the coastal ecosystems sampled. This work was focused on few targeted objectives and problematics. These different approaches are separated in the three main chapters of this manuscript.

In **Chapter I**, have tried to introduce marine protists to the debate about the functional redundancy of microbial communities. For this study, I have analyzed all our samples and OTUs and formulated the following questions: Can marine protists be united under relevant and coherent functional groups in the coastal ecosystem? Are these groups containing taxa from various genetic clades of protists? What is the dynamic of these groups across size-fractions and their environment? Most importantly, I have tried to understand if the marine protistan taxonomic and functional diversity were co-variating in their environment or if the changes in taxonomic composition resulted in invariable patterns of protists' functional groups, supposing that a change in species diversity would not alter the biological functions occurring in the ecosystem. For this analysis, I have developed unsupervised statistics inspired from functional analyses of other biological compartments (e.g. benthic macro-fauna, fishes). Finally, I have used multivariate statistics to test the proposed hypotheses. This chapter have been submitted to 'Environmental Microbiology'.

In **Chapter II**, I have tried to understand if and how the physics of marine ecosystems influence the functional diversity of protists. I have focused on the dynamic of marine protists across a tidal front, a submescoscale physical phenomenon that appears in coastal ecosystems. The following problematics were postulated: Does environmental fluctuations at the submesoscale structure the functional and taxonomic diversity of marine protists? And how? I have used our functional approach to sort organisms according to their trophic strategy (phototrophs vs. heterotrophs) and studied the effects of a tidal front on the OTUs richness of these two groups. Then, I have used ecological concepts to explain the distribution of OTUs and their traits across the tidal front. The first section of this chapter will soon be submitted to Frontiers in Microbiology.

In **Chapter III**, I have focused on a single functional group, the parasites, and investigated how the taxa playing the functional role of parasitism influenced the phenology of a single dinoflagellate species (*Alexandrium minutum*). Three blooms of the dinoflagellate were sampled in 2013, 2014 and 2015 in the bay of Brest (Brittany, France). I have analyzed if a host dinoflagellate population could be associable to a recurrent parasite community across distinct blooms. I have used our newly developed functional approach and a DNA sequence homology strategy to select the parasite OTUs of our dataset and study their co-occurrence within the

blooms of *Alexandrium minutum*. I have then used a statistical approach to infer the parasitic interactions with the dinoflagellate. The stability and repeatability of the co-occurrence along the blooms was further studied to contribute to the study of the function of parasitism in marine protistan communities. This chapter is still in preparation.

Each chapter is enlarged by some analysis and figures, included as supplementary material. The general results will be further discussed at the end of the manuscript, in a general **Conclusion**. Finally, I will discuss the potential research perspectives in the field of functional ecology of marine protists.

CHAPTER I: COUPLING BETWEEN TAXONOMIC AND FUNCTIONAL DIVERSITY IN PROTISTAN COASTAL

Résumé (en français)

La génétique permet désormais d'étudier les espèces présentes dans l'environnement à une profondeur d'échantillonnage jusqu'ici inégalée. Cette méthode contribue à la découverte de nombreux nouveaux protistes (i.e. organismes eucaryotes unicellulaires) dans l'océan, mais le rôle de ces organismes dans l'écosystème marin reste encore méconnu. En nous inspirant d'approches développées pour les plantes terrestres, les poissons ou de la macrofaune benthique, nous avons sélectionné des traits (e.g. mesure morphologique, physiologique, comportementale ou du cycle de vie) permettant de décrire le rôle fonctionnel des protistes marins ainsi que les différentes stratégies adoptées par les protistes pour survivre dans l'environnement marin. En regroupant de nombreux échantillons de l'écosystème côtier, nous avons identifié des protistes marins par une approche génétique, puis par un travail bibliographique nous avons essayé de décrire les traits de ces protistes. En comparant la diversité taxonomique (issue de la génétique) et fonctionnelle (issue de notre approche de trait) des protistes marins nous avons pu mettre en évidence le fort lien entre la composition des communautés de protistes et le rôle qu'elles jouent dans les milieux marins. Ces résultats contrastent avec de récentes études sur les procaryotes démontrant peu de causalité entre fonction et composition de communauté, nous discutons donc des différences fondamentales entre les eucaryotes et les procaryotes. Finalement, nous observons que les protistes des plus petites fractions de taille démontrent une plus grande richesse taxonomique et fonctionnelle, nous supposons que ce phénomène est lié au maintien d'une communauté moins fluctuante car moins limitée en ressources.

Context

By coupling metabarcoding and a trait-based approach, patterns of protistan taxonomic and functional diversity were investigated across three size-fractions of marine plankton. In contrast with the theories advancing a decoupling between the functions and the taxonomy of marine prokaryotes, we showed that changes in the taxonomic composition of micro-eukaryotic communities corresponded to variations of both ecological strategies and functional roles. The coupling between functional and taxonomic diversity was conservative across different protist size-classes. However, differences emerged between larger and smaller plankton communities. Functional groups relative contribution and taxonomic diversity were significantly more equitable and less variable in pico-nano-plankton than in micro-plankton communities. This suggests the existence of a larger taxonomy and functional diversity of the smallest plankton communities, and corroborates the idea that nano and pico-plankton are part of an ocean's veil on which larger protists and metazoans might develop.

Author contributions

The samples used in this chapter have been retrieved by distinct surveys or sampling campaigns that were carried out prior to the beginning of the PhD. I took part in the genetic procedures but other samples were processed by Sophie Schmitt and Lauriane Madec (Ifremer de Brest), as well as Sarah Romac and Fabienne Rigaut-Jalabert (Station Biologique de Roscoff, SBR). Environmental variables have been measured by the SOMLIT monitoring network, members of the Ifremer and LEMAR staff. All samples were sequenced by the Genotoul platform and bioinformatics were carried out by Stéphane Audic (SBR). The theoretical trait framework was established during discussions with the presence of Cédric Berney, Nicolas Henry and all the members of the PhD (This work can be found in the Supplementary material 1 of this chapter). I have carried out the trait annotation and all the analyses presented here. I have written this manuscript under the supervision of Raffaele Siano and Marc Sourisseau, this manuscript has been submitted to Environmental Microbiology.

Coupling between taxonomic and functional diversity in protistan coastal communities

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Abstract

The study of protistan functional diversity is crucial to understand the dynamic of oceanic ecological processes. We combined metabarcoding data of various coastal ecosystems and a newly developed trait-based approach to study the link between the taxonomic and functional diversity across marine protistan communities of different size classes. Environmental DNA has been extracted, and the V4-18s-rDNA genomic region was amplified and sequenced. In parallel, we developed a new theoretical framework of 30 biological traits that covers the diversity and the variety of marine protistan ecological strategies. Operational Taxonomic Units (OTUs) from our metabarcoding dataset were associated to 13 biological traits, using published and accessible information on protists. Trade-offs between traits were depicted and functional groups, describing ecological strategies and functional roles of marine protists, were identified by means of unsupervised statistical methods. We demonstrate that the functional diversity of marine protist communities varies in parallel to their taxonomic diversity. The coupling between functional and taxonomic diversity was conservative across different protist size-classes. However, the smallest size-fraction was characterized by larger taxonomic and functional diversity, corroborating the idea that nano and pico-plankton are part of an ocean's veil on which larger protists and metazoans might develop.

1) Introduction

Pelagic protists represent the majority of the eukaryotic diversity in the oceans (de Vargas *et al*., 2015), and fluctuations in protists community composition modulate global ecosystem processes (Worden *et al*., 2015; Guidi *et al*., 2016). Since the end of last century, a great share of protistan diversity has been unveiled by molecular methods (Caron *et al*., 2012) and recent high-throughput sequencing of genomic markers (barcodes) of complex communities (metabarcoding) has provided a greater hindsight into oceanic (de Vargas *et al*., 2015; Pernice *et al*., 2016) and coastal diversity of protists (Christaki *et al*., 2014; Massana *et al*., 2015; S. Hu *et al*., 2016). In parallel, the role of protistan ecological strategies (e.g. mixotrophy or parasitism) has been progressively recognized to be crucial in the oceans (Jephcott *et al*., 2016; Mitra *et al*., 2016; Scholz *et al*., 2016; Ward and Follows, 2016; Stoecker *et al*., 2017). Yet, only few efforts have been made to traduce molecular diversity (i.e. Molecular Operational Taxonomic Units (MOTUs or OTUs)), into functional diversity (e.g. de Vargas *et al*., 2015; Genistaris *et al*., 2015). Understanding the relation between functional and taxonomic diversity in plankton stands out as a great challenge of modern microbiology, particularly in the face of climate change and its impact on the pelagic ecosystem (Beaugrand and Kirby, 2018).

Metagenomics and metatranscriptomics analyses of marine prokaryotes showed that communities different for their taxonomic diversity could express similar functional roles by means of shared metabolic pathways (Louca *et al*., 2016; Coles *et al*., 2017; Haggerty and Dinsdale, 2017). This has led to the proposal of a new general microbial paradigm suggesting that functional and taxonomic diversity are decoupled, the functional roles being selected by the environment while the identity of the species playing the roles (i.e. taxonomic diversity) would be independent and driven by biotic interactions (Louca, 2017; Louca and Doebeli, 2017). Given the present limitations in genome analysis of micro-eukaryotes, the applicability of these hypotheses to protistan communities is difficult to test (Keeling and del Campo, 2017). Yet, functional diversity of marine protist could be studied with a biological trait approach, following the examples of functional researches on

higher metazoan benthic and pelagic communities (Mouillot *et al*., 2014; Rigolet *et al*., 2014; Benedetti *et al*., 2015). Trait-based approaches consist in using functional traits to predict performances of species within ecosystems (i.e. the fitness of species) (Violle *et al*., 2007). Functional traits represent any metric (morphology, physiology, life history, trophic strategy) that influence or relate to the fitness of an organism by impacting its reproduction, survival or resource acquisition (Violle *et al*., 2007; Litchman and Klausmeier, 2008; Litchman *et al*., 2013), that in turn inform us on their functional role (Diaz *et al*., 2013). Similar trait patterns across species highlight physiological laws, compromises and constraints (i.e. trade-offs) that inform about the nature of species ecological strategies. As for protists, the functional approaches for autotrophic species (phytoplankton) date back to the works of Margalef (1978) and Reynolds (1984) and for heterotrophs to Fenchel's studies (1982). These frameworks were successfully used to predict phytoplankton successions (Smayda and Reynolds, 2003; Alves-De-Souza *et al*., 2008; Kruk *et al*., 2011) and to describe functional responses of heterotrophic protists (Massana *et al*., 2009; Yang *et al*., 2013). Recent reviews of relevant functional traits in plankton (Litchman and Klausmeier, 2008; Litchman *et al*., 2013; Weisse *et al*., 2016), laid down the baseline for a trait-based approach of marine protistan functional diversity.

This study focuses on the relation between protistan functional and taxonomic diversity within marine coastal ecosystems. Taxonomic diversity was assessed using the deep resolution of metabarcoding, and was associated to functional diversity using a newly created trait based-approach (Violle *et al*., 2007; Diaz *et al*., 2013). Coastal ecosystems were privileged in this study because they harbor physical and hydrodynamic processes (e.g. tides, currents, upwelling, pulses of nutrients, changes in salinity, oxygen or temperature, due to seasonal cycles of freshwater inputs, and exchanges with the atmosphere or the sea bottom) that shape the taxonomic diversity of marine protists potentially corresponding to differing ecological strategies (Cloern, 1996; Barton *et al*., 2010; Telesh *et al*., 2013; Lallias *et al*., 2015; Pearman *et al*., 2017). For the first time in this study, the relation between protistan functional and taxonomic diversity is detailed across marine micro-, nano- and pico-plankton communities.

2) Results

a) Environmental characteristic of the sampled ecosystems

A total of 277 water samples were collected in a temporal and/or spatial manner across coasts of the north Atlantic Ocean (Figure 11), representing various environmental gradients (Table 1). Chemo-physical variables (temperature, salinity, and nutrients NO_3^-, NO_2^-, PO_4^{3-}, NH_4^+ and $Si(OH)^4$) were collected in all datasets, with the exception of Senegalese samples that lacked temperature and salinity measures, and the 2015 samples of the PI and PH cruises that lacked the whole environmental set. Principal Component Analysis (PCA; Figure S1) of this environmental dataset showed two major gradients. On the first PCA axis (PCA1, 39.92 % of the explained variance) samples were distributed along a gradient of nutrient concentrations. On the second axis of the PCA (PCA2, 34.14% of the explained variance) the samples were separated along a salinity-temperature gradient, distinguishing notably marine from estuarine waters.

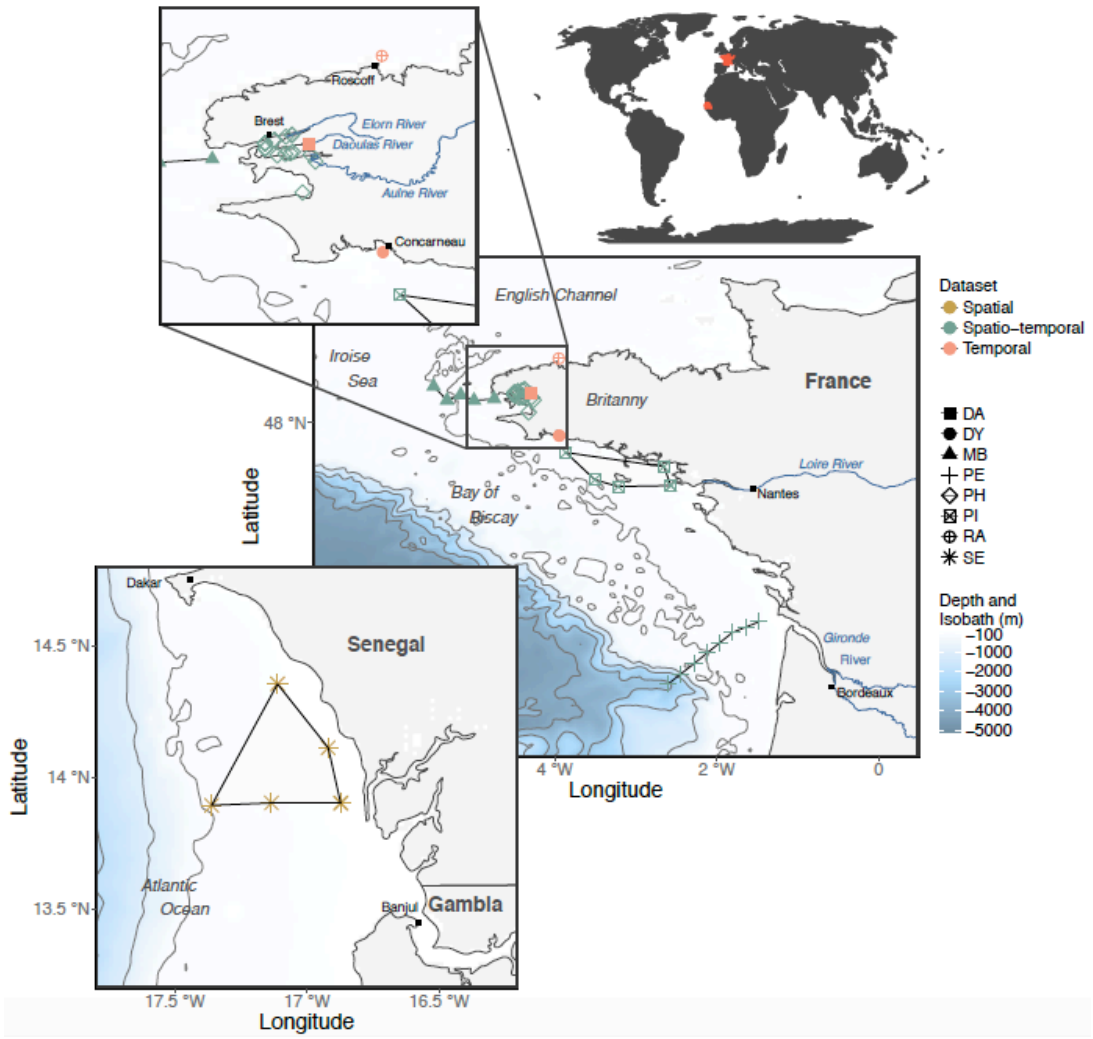

Figure 11: Map of sampling sites. Shapes and colors of dots represent respectively the geolocalisation of samples from the distinct oceanographic cruises used in this study and their sampling strategy.

Table 1: Information on the ecosystems sampled in this study.

	Sampled sites	Environmmental forcing	Depth	Year(s)	Season	Data Set type	Spatio-temporal sample frequency	Filters	Sampled Size Fractions (μm)	Replicate(s)
DA	Bay of Brest (Daoulas River)	Regular dinoflagellate bloom occuring in estuary	Surface	2013 - 2014 - 2015	spring-summer (May to August)	Temporal	Every 3 days	245	0.2-3; 3-20; >20	1 (for 3-20 and >20)
DY	South Britanny (Bay of Concarneau)	Typical mid-latitude ecosystem, influenced by the Loire plume	Surface	2012	spring-summer (March to July)	Temporal	Weekly and twice weelky during bloom	78	0.2-3; 3-20; >20	No
MB	Iroise Sea	Ushant tidal front	Surface/DCM /Mesopelagic	2014 - 2015	spring-summer (March, July and September)	Spatio-temporal	5 stations (coast to off-shore radial)	414	0.2-3; 3-10; >10	2 (all)
PE	Bay of Biscay (Gironde River plume)	Gironde plume	Surface/DCM	2012 - 2013	spring (May)	Spatio-temporal	8 stations (coast to off-shore radial)	78	0.2-3; 3-20; >20	No
PH	Bay of Brest	Tidal-influenced semi-enclosed ecosystem	Surface/DCM	2013 - 2015	summer-automn (June, September)	Spatio-temporal	16 stations	87	0.2-3; 3-20; >20	1 (for 3-20; >20 μm during 2015)
PI	Bay of Biscay (Loire River plume)	Loire plume	Surface/DCM	2013 - 2015	summer-automn (June, September)	Spatio-temporal	23 stations (north-south radial)	87	0.2-3; 3-20; >20	1 (for 3-20; >20 μm during 2015)
RA	North Britanny	Permanent water-column mixing	Surface	2009 - 2010 - 2011	all	Temporal	Twice monthly	138	0.2-3	No
SE	South-West Senegal	Upwelling ecosystem	Surface	2015	spring (November)	Spatial	6 stations (coast to off-shore radial)	26	0.2-3; 3-20; >20	1 (for 3-20 and >20)

b) Genetic diversity

Plankton samples consisted of 1145 distinct filters with different pore size ([20 or 10 μm], [3μm] and [0.2 μm]), which separated protist communities of three size-fractions here called micro-, nano- and picoplankton. DNA from the water filters was extracted and the V4 domain of 18S rDNA was amplified and sequenced. After filtration steps, sequences were clustered into OTUs with swarm2 (Mahé *et al*., 2014, 2015) and taxonomically annotated with PR^2 (Guillou *et al*., 2013). We retrieved 111 089 distinct OTUs that accounted for 3.5 x10^6 reads. Taxonomic annotation of the OTUs represented 2007 unique taxonomic references (many OTUs were annotated to the same taxa/clade). Finally, we created a taxonomic community table based on the relative abundances of each OTU in each sample.

Rarefaction curves based on the OTUs present in our dataset did not reach the theoretical asymptotic shape (Figure S2), neither when computed on separate datasets nor when performed by size fractions. Following taxonomic assignment, OTUs with low taxonomic level (i.e. annotated only at the family, supergroup or less) constituted on average 29% reads per sample (min = 0.3%; 1st quartile = 17%; 3rd quartile = 40%; max = 95%). Merging all our samples, coastal communities were mostly dominated by Dinophyta, Bacillaryophyta, Chlorophyta, the marine Alveolates (MALV) and Stramenopiles (MAST) groups, and Cryptophyta (Figure 12a). The distribution of these taxa was uneven over size-fractions. Dynophyta (32%) and Bacillaryophyta (18%) constituted most of the read number within the micro-plankton (Figure 12a). The nano-plankton was characterized by a more diversified assemblage comprising Dinophyta (24%) and Bacillariophyta (14%) but also Chlorophyta (4%), Cryptophyta (4%) and MALV (4%). Finally, the pico-plankton contained Chlorophyta (26%), MALV (10%), Dinophyta (8%), MAST (5%), Bacillariophyta (4%), Cryptophyta (4%) and Picomonadida (3%). OTUs associated to fungi and radiolarians were present in very low abundances in all samples. Ciliates ("Ciliophora") were often observed at low abundances, and across all size-fractions (micro-: 1.91%, nano-: 1.57% and pico-: 2.21%).

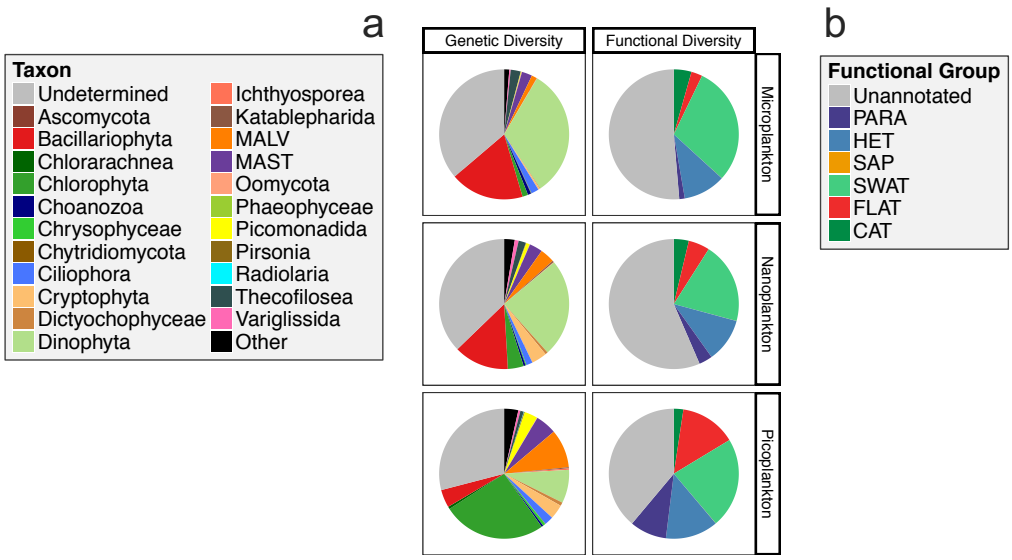

Figure 12: Coastal protist community structure in terms of a) genetic diversity (total relative read number associated to the taxa in the legend) and b) functional diversity (total relative read number associated to the 6 functional groups in the legend), across planktonic size-fractions. a) Only taxa present above 10% in at least one sample are represented, other taxa are cumulated into 'Others'. The group "Undetermined" cumulates the relative abundance of OTUs with low taxonomic affiliation (in a) and unresolved functional annotation (in b). b) Functional groups identified are named with acronyms: PARA: Parasites, HET: Strict Heterotrophs, SAP: Saprobes, SWAT: Swimmer photo-autotrophs, FLAT: Floater photo-autotrophs, CAT: non-swimmer, strict-photoautotrophs, colony-forming photo-autotrophs.

c) Functional diversity

A conceptual framework of 30 biological traits distinguishing the morphology, trophic strategy, physiology, and mode of life of both photoautotrophic and heterotrophic protists was created (Figure 13a, see Supplementary Material 1 for the ecological relevancy of each trait) (http://doi.org/10.17882/51662). As far as possible, we annotated each OTUs according to their 2007 unique taxonomic references (taxonomic annotation) with our 30 functional traits. For each taxonomic reference, we searched in the literature if a modality to each trait could be assigned in regard of their biological description (Figure 13a). Trait annotations were inferred from 717 diverse literature sources, ranging from general protistology handbooks to specialized papers, as well as from websites (bibliography was annotated for each taxonomic reference). The final annotated table represents the first functional annotation of marine protists, it is public and still improvable (http://doi.org/10.17882/51662). Despite a thorough analysis of bibliographic data, the functional annotation was not achieved for all reference taxa (Figure 13b). This

was either due to the lack of information available or to the low taxonomic level of some taxonomic references. All physiological traits and many traits related to life cycle (benthic phase, longevity, ploidy and genome size) or trophic strategies (prey, symbiont or host specialization, symbiont location, mutualistic host) could be annotated for only few protistan taxonomic references. Those under-annotated traits were discarded from our analyses. We also discarded the trait "behavior" for which no information was found for 582 references. The 13 well annotated, retained traits were: SizeMin, SizeMax, Cell Cover, Cell Shape, Presence of Spicule, Cell Symmetry, Cell Polarity, Coloniality, Motility, Chloroplast Origin, Ingestion method, Symbiosis type and Resting Stage during the life cycle. Those traits were inferred for 1669 of the 2007 taxonomic references (83%) and constituted the biological trait table used to study trade-offs and to build functional groups in this study.

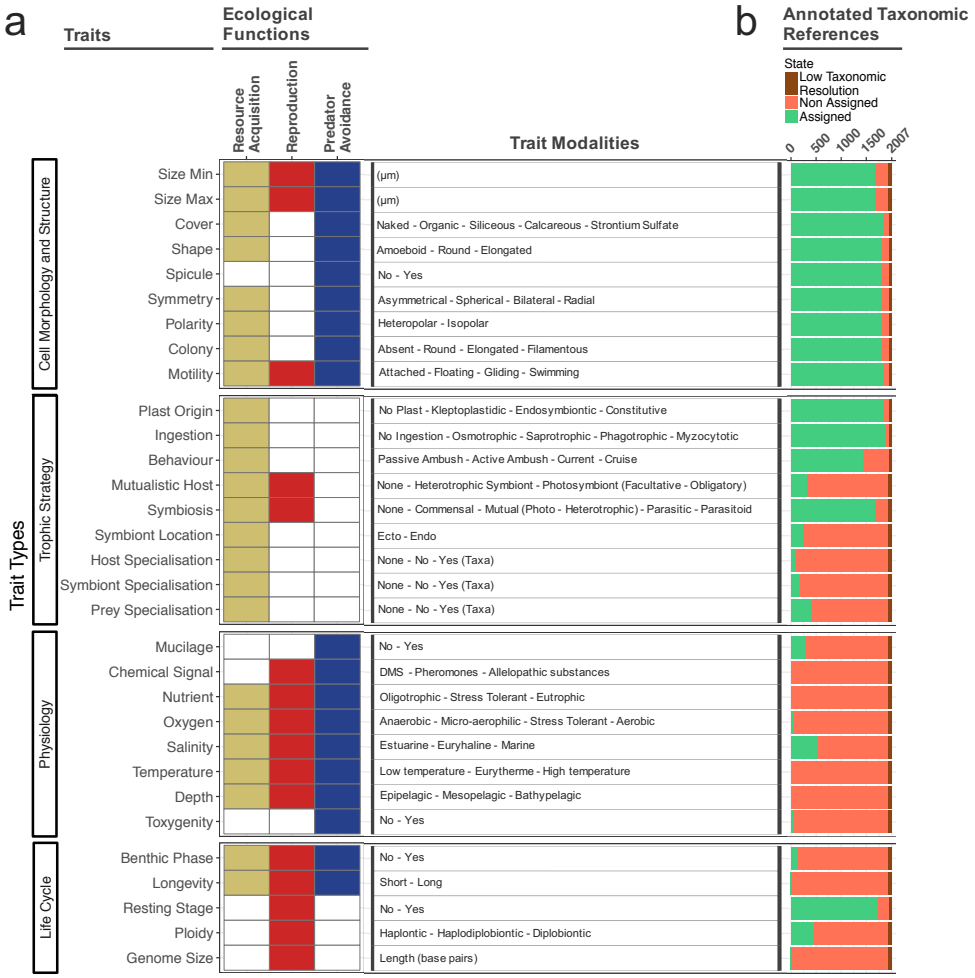

Figure 13: a) Theoretical framework of traits used to describe marine protists functional ecology and b) quality of the functional annotation for each of the 2007 taxonomic references associated to the OTUs of this study. a) The 30 traits chosen are ordered by trait type (Cell Morphology and Structure, Trophic Strategy, Physiology and Life Cycle) and associated to ecological/survival functions (Resources Acquisition, Reproduction, Predator Avoidance). Each trait is associated to different modalities. b) The proportion of the 2007 isolated taxonomic reference that have been assigned to the respective trait is represented in green. The references for which the chosen functional traits are not annotated are represented in red. References with "Low Taxonomic Resolution" corresponding to badly determined OTUs not assignable functionally (i.e. super-group or family level) are represented in dark brown.

The 1669 annotated taxonomic references corresponded to 52 180 OTUs, a considerably reduced portion (47%) of the original taxonomic community table (111 089). The reliability of the reduced dataset was tested by comparing biodiversity patterns between the reduced and the complete dataset. Briefly, two square-matrix based on the Bray-Curtis distance (Ramette 2007; Buttigieg & Ramette 2014) were

computed on the basis of the complete and the reduced dataset, the square-matrices were then compared with the Mantel test, a generalized regression approach based on permutation (Mantel, 1967). The correlation was high (Mantel's test observation = 0.91; p-value 0.0001). In addition, two diversity proxies, OTUs richness and Shannon Index (Piélou, 1966) calculated for the two datasets were also highly correlated (R^2 = 0.88 for OTUs Richness, R^2 = 0.75 for H'; Figure S3). Given these strong positive correlations, the reduced dataset was considered to be reliable, and to carry the same cross-sample biodiversity patterns of the complete dataset.

The annotated taxonomic references (1669) and their respective OTUs (52 180) were clustered into 6 functional groups identified by unsupervised statistical methods. Briefly, based on the biological trait table (13 traits x 1669 taxonomic references), a multidimensional functional space was created with Gower distance and Principal Coordinates Analysis (Maire *et al*., 2015). The multidimensional space sorts taxonomic references in coordinates dimensions according to their traits. Trait trade-offs were considered as when the modalities of distinct traits had the same coordinates (Figure S4). Traits that were not showing any trade-offs with other traits were discarded (Figure S5), most notably the traits of cell shape, size, spicules and the existence of a resting stage were isolated and did not correlate with other traits (see Experimental Procedures for details). Functional groups were created by the best partitioning of taxonomic references according to their trait coordinates (Figure S6), the clusters used were considered as functional groups and using their taxonomic references, OTUs were sorted into functional groups. A functional community table based on the functional group read abundances was created by cumulating the read abundances of the taxonomic reference belonging to each functional group. The whole methodological process is resumed in Figure 14.

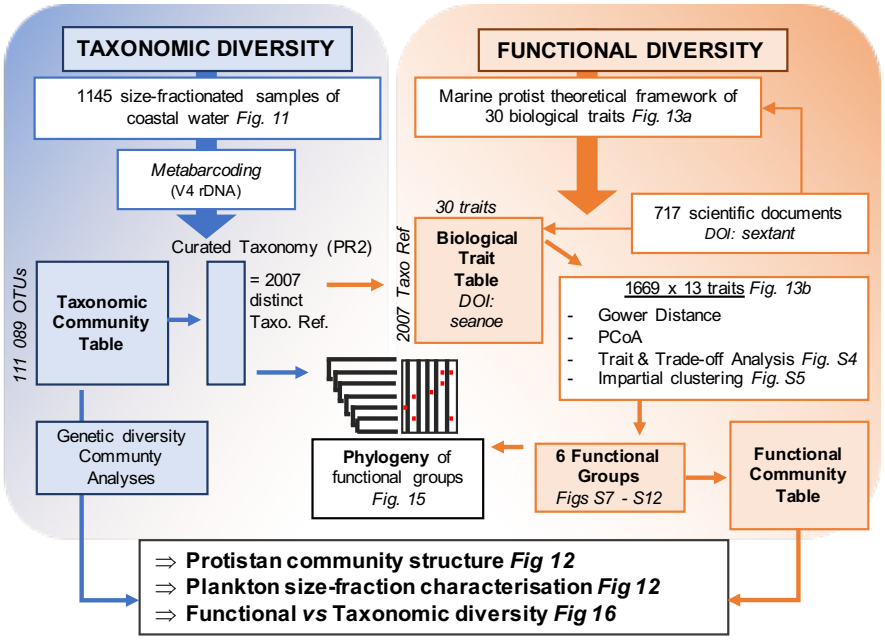

Figure 14: Explanatory scheme of the workflow methodology used in this study.

The 6 functional groups were characterized by traits and modalities of traits (Figure S7-S12) that allowed the distinction of ecological strategies. The functional groups were named accordingly: 1) PARA (PARAsites): characterized by their type of feeding, their symbiosis type, their host-attached life strategy and mostly naked cell surface; 2) HET (strict HETerotrophs): characterized by their type of feeding and the absence of plastids throughout their life cycle; 3) SAP (SAProbes) characterized by their feeding behavior; 4) SWAT (SWimmer photoAutoTrophs): also characterized by dominantly organic cover and mixotrophic trophic tendencies; 5) FLAT (FLoater photoAutoTrophs) also characterized by dominantly siliceous cover and mixotrophic trophic tendencies; 6) CAT (Colony forming photoAutoTrophs): characterized by non-swimmer, strict-photoautotrophs and ability to form colonies (CAT). Those groups contained different numbers of OTUs (PARA: 8 366, HET: 19 582, SAP: 332, SWAT: 14 333, FLAT: 4 813, and CAT: 4 754) and that were associated disproportionately to distinct taxa.

In order to infer the phylogenetic diversity of the 6 functional groups, groups taxonomic composition was studied and the relative abundance of phyla/families or generic groups that they contained was calculated (Figure 15). Most groups (5 out of 6) proved to be paraphyletic. PARA were mostly composed of MALV and Apicomplexans. HET was dominated by Marine Stramenopiles (MAST), Cilliophora, Picomonadida and Dinophyta. SAP clustered organisms from

Ascomycota, Basidiomycota, Bicoceae and Labyrinthulae. SWAT was dominated by Dinophyta, Cryptophyta and Chlorophyta. While FLAT was composed of Bacillariophyta (invariably from family and orders), Acantharea and Chlorophyta. CATs were only associated to Bacillariophyta (invariably from the family and orders).

Figure 15: Phylogenetic composition of the 6 functional groups. A simplified phylogenetic reconstruction among taxonomic groups was built inspired by a selected bibliography (Schüßler et al., 2001; Gómez et al., 2009; Burki et al., 2010; Howe et al., 2011; Lin et al., 2012; Berney et al., 2013; Yabuki et al., 2013; Keeling et al., 2014; Worden et al., 2015; Aleoshin et al., 2016). The OTUs of each functional group were associated with a classified taxon. The relative contribution (number of OTUs on total OTUs number in the functional group) of the distinct taxa to the pool of OTU from each functional group has been represented by colors (from grey to red to represent low to high contribution).

To characterize the protistan functional diversity of coastal ecosystems, the relative abundance of each functional group was calculated across the whole dataset

(277 samples) for each planktonic size-fraction (1145 filters) (Figure 12b). The relative abundance of functionally unannotated OTUs was high in all size-fractions, but lower within the pico-plankton (51%, 56.5%, 38.5% for micro-, nano- and picoplankton, respectively). The contribution of the functional groups varied across the size-fractions, in parallel to a change in taxonomic composition (Figure 12a and 12b). Micro-plankton was dominated by SWAT and HET which together accounted for more than 40% of the annotated OTUs (30% and 10.5% respectively), whereas within nano- and pico-plankton the relative composition of the functional groups was more equilibrated. FLAT and PARA were more important in the picoplankton (14% and 9%, respectively) than in the higher size fractions (micro-plankton FLAT: 3%, PARA: 1.5%; nano-plankton and FLAT: 5%, PARA: 3.5%). In contrast, CAT relative abundance was higher in micro- and nano-plankton (respectively 4.5%, 4%) and lower within the pico-plankton (2.5%). The relative abundance of SAP was very low across all size fractions (on average < 0.05% in the micro-, nano- and pico-plankton) and more generally among all samples.

d) Functional vs. taxonomical diversity of marine protists

The relationship between environmental variables, taxonomic and functional diversity, among pico-, nano- and micro-plankton communities was studied by the RV statistical coefficient of co-inertia, a multivariate generalization of the Pearson correlation coefficient (Borcard *et al*., 2011; Legendre and Legendre, 2012; Husson *et al*., 2018). Correlations (RV coefficient) between the taxonomical community table and environment variables were low but significant across all size-fraction (value for micro: 0.45, nano: 0.22 and picoplankton: 0.19, with p-value < 0.0001). Similarly, correlations between the functional community table and environmental variables were also low but significant (value for micro: 0.34, nano: 0.16 and picoplankton: 0.10, with p-value < 0.0001). For every size fraction, the correlations (RV coefficient) between the functional and the taxonomical community table were high and significant (values for micro-: 0.71, nano-: 0.46 and pico-plankton: 0.75, with p-value < 0.0001) meaning that taxonomic and functional diversity of marine protists were tightly coupled.

In order to study if protist communities different for their taxonomic composition were characterized by similar composition of our 6 functional groups, we computed a Non-metric Multi-Dimensional Scaling (NMDS) ordination separately for samples of micro-, nano- and pico-plankton on the basis of their OTUs composition. On each NMDS, samples were clustered together by an unsupervised best partitioning of samples using a k-mean method and a simple structure index (ssi) criterion. The relative abundances of the 6 functional groups within those samples and clusters were calculated. The overall aim was to compare the functional diversity across protistan communities distinct for their taxonomic composition (Figure 16). To study whether there was an effect of the environment on taxonomic and functional composition, environmental variables were projected as vectors onto each NMDS ordination space.

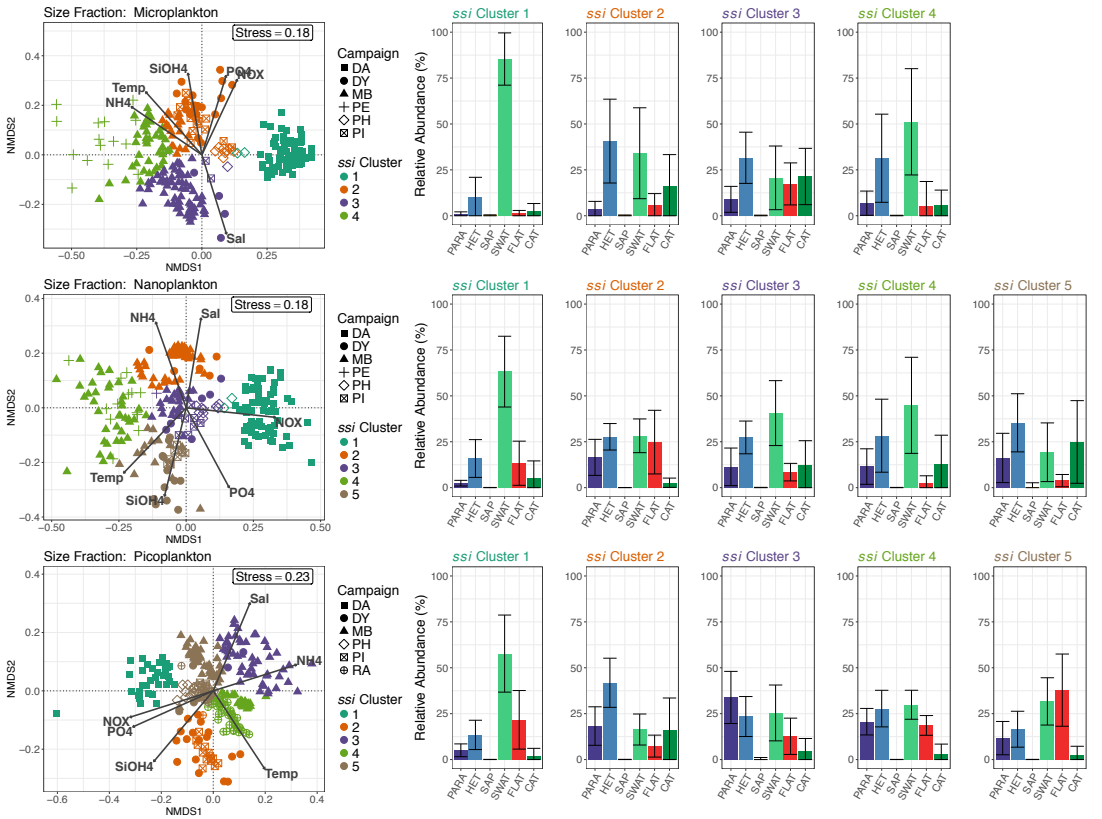

Figure 16: Taxonomic gradients across samples and size-fractions, with associated functional group composition. At the left, and from top to bottom, Non-Metric Multidimensional Scaling analyses (NMDS with Bray-Curtis distance) based on the genetic diversity (OTUs) of each sample, are represented for micro-, nano-, and pico-plankton. Dot shapes identifies the sample's dataset. Stress values of each NMDS plot, represented at top-right, indicates that two axes were sufficient to represent community dissimilarity between samples. Arrows represent environmental variables fitted onto the two NMDS axes with function envfit() of R package "vegan" (Osaksen et. al., 2016). Samples clustering (color of the dots) was calculated impartially through the kmean partitioning of samples in different number of clusters, followed by computation of the simple structure index (ssi) to select the best partitioning. Environmental variables were fitted onto NMDS. At the right are represented the average relative abundance of each functional group (PARA, HET, SAP, SWAT, FLAT, CAT) and standard deviations (error bars) within each cluster of samples in each size fraction.

In the NMDS built from taxonomic tables independently for each size-fraction (Figure 16), plankton samples clustered in 4, 5 and 5 homogeneous groups, respectively for micro-, nano- and pico-plankton. Across all size-fractions, samples from the estuarine DA campaign were markedly isolated on the first ordination axis (simple structure index, ssi, cluster 1; Figure 16), implying that this set of samples had a community structure markedly distinct from the others. The functional diversity structure of DA samples showed a strong domination of the SWAT group. This estuarine group of samples was usually opposed on the same axis with samples retrieved in the most off-shore areas, (PE in the Bay of Biscay and/or MB in the

Iroise Sea; ssi cluster 4, 4 and 3 for respectively micro-, nano- and pico-plankton; Figure 16). These clusters were dominated by the SWAT and HET groups in the micro- and nano-plankton, and characterized by a more diversified assemblage in the pico-plankton, especially with a higher importance of PARA, compared to the other size fractions. Only within pico-plankton, this set of samples was well correlated with ammonium concentration (NH_4^+) and salinity (Sal) (Figure 16). The second axis of all three NMDSs, separated a set of typical coastal waters samples (DY, PH, PI, RA and the coastal stations of MB; into ssi cluster [2 and 3], [2,3 and 5] and [2,4 and 5] for respectively micro-, nano- and pico-plankton; Figure 16). These samples correlated well with a gradient opposing salinity and nutrient concentrations (most notably $Si(OH)_4$), implying a separation between communities of winter/early spring (present in enriched and fresher-waters) and summer/productive communities (present in saltier depleted waters). Across size-fraction, winter samples were dominated by the HET group (ssi cluster 2, 5 and 2 for respectively micro-, nano- and pico-plankton. Conversely, summer/depleted conditions coincided with equilibrated functional composition with notably the phototrophic groups SWAT, FLAT and CAT in higher abundances across all size fractions (ssi clusters [3], [2] and [4 and 5] for respectively micro-, nano- and pico-plankton). Overall, variations in taxonomy (distinct OTU clusters) corresponded to changes in the relative composition and abundance of the functional groups. Indeed, clusters of samples obtained from distinct OTUs assemblages corresponded to significantly distinct functional assemblages (pvalue = 0.0001, R^2 for micro-: 0.45, nano-: 0.35 and pico-: 0.36).

Interestingly, across size-fractions functional groups seemed more evenly distributed in the smaller size fractions, while micro-plankton samples were mostly dominated by HET and SWAT (Figure 16). To investigate functional groups distribution across size-fractions, the Shannon index of equitability (Piélou, 1966), was calculated on the basis of functional groups relative abundance in each sample (Figure 17a). Kruskall-Wallis test (one-way analysis of variance) indicated that the equitability of functional diversity was significantly higher and less variable within nano- and pico-plankton samples than for micro-plankton (p-value < 0.001; Figure 17a). Taxonomic equitability (calculated on the relative of abundances of OTUs) and richness (number of OTUs by sample) showed similar patterns (Figure 17b & 17c), with significantly lower values in micro-plankton (p-value < 0.001).

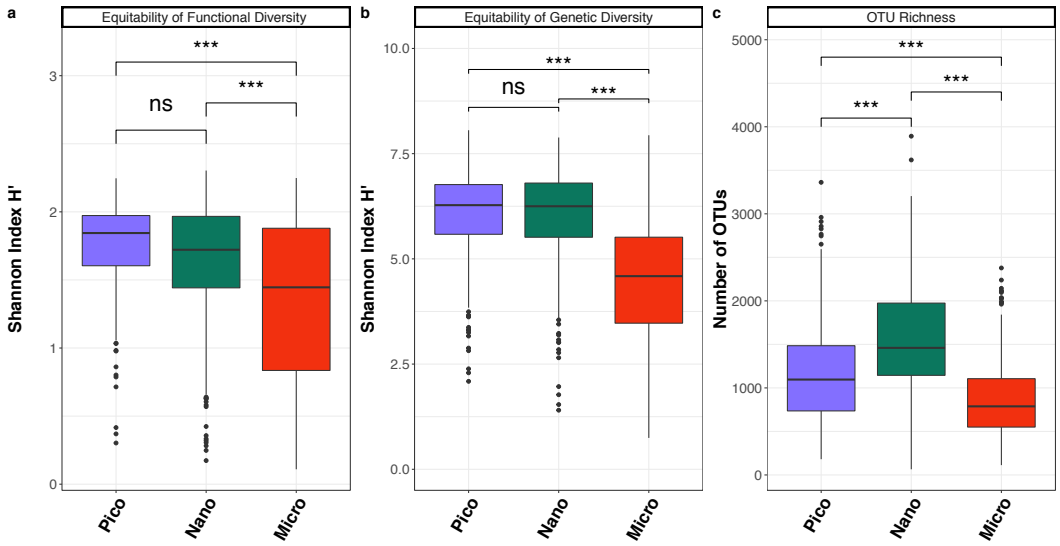

Figure 17: Boxplots comparing 3 metrics calculated for all samples of micro-, nano- and pico-plankton: a) Shannon index H' calculated on the relative abundances of the 6 functional groups and b) relative OTU abundance; c) OTUs richness with micro-plankton containing a total of 56 655 OTUs, nano-plankton 85 373 and pico-plankton 64 404. The Significance of the differences in metric values (Kruskall-Wallis test) between each size-fractions is shown above the boxplots (ns: non-significant; ***: significant with p.value < 0.001).

3) Discussion

By means of a taxonomic diversity analysis obtained by metabarcoding of the V4-18sr-DNA and a trait based approach, we were able to 1) detail patterns of protistan functional diversity and 2) to compare patterns of taxonomic and functional diversity of marine protists. Our trait-based approach allowed the construction of 6 functional groups that represented relevant ecological strategies but also functional roles of marine protists. Most functional groups were paraphyletic, being composed of phylogenetically distant group of protists. Patterns of taxonomic and functional diversity of coastal protist communities across various environments were described. Both functional and taxonomic diversity appeared more evenly distributed in the smaller size-fractions, while micro-plankton was more prompt to domination of few OTUs and functional groups. Finally, a coupling between protistan taxonomic and functional diversity was highlighted.

a) Patterns of genetic diversity of coastal protist communities

A metabarcoding approach was adopted in this study for metabarcoding's proven efficiency in analyzing protistan taxonomic diversity (de Vargas *et al*., 2015). Yet, this approach induces analytic problems due to methodological limits. In our study, water-filter clogging resulted in a relatively low sequencing depth, which prevented us from getting the entire picture of protistan diversity from the considered coastal waters (Figure S2; see other e.g. Pernice *et al*., 2015). Water filtration also contributes to contamination across size-fractions. DNA from cell-breakage and small-sized gamete or resting stages of typically large organisms are often found in marine metabarcoding surveys (Massana *et al*., 2004, 2015; Le Bescot *et al*., 2015) and might partially contaminate smaller size-fractions. The high number of OTUs identified (111 089) was likely attributed to the relatively high clustering performances allowed by swarm2 (Logares *et al*., 2015; Mahé *et al*., 2015). This number was not directly comparable to those retrieved in previous multiple sites studies, due to either the use of a different DNA marker (V9 in de Vargas *et al*., 2015 = ~110 000 OTUs), clustering method (clustering thresholds at 97%, Massana *et al*., 2015 = 15 295 OTUs; Pernice *et al*., 2016 = 2 481 OTUs), or simply because of the type of ecosystems analyzed (Neotropical rainforests, Mahé *et al*., 2017 = 26 860 OTUs). The taxonomical annotation was also imperfect as 30% of environmental reads were annotated to low taxonomic levels (i.e. kingdom, class, family). More samples and the taxonomic descriptions of rare protitsts are needed to decrease the unresolved proportion of reads and fully describe the nature of this microbial compartment (Caron *et al*., 2012; Guillou *et al*., 2013; Keeling and del Campo, 2017).

Despite those limits, our dataset still present a valuable DNA sampling of marine coastal waters (273 water samples, 1145 water filters) and the genetic diversity analyzed in this study can be considered as representative of the most abundant species of the sampled coastal protistan community.

The taxa retrieved in each size-class during this study were indeed coherent with other coastal DNA-based surveys (Christaki *et al*., 2014; Genitsaris *et al*., 2015; Massana *et al*., 2015; S. K. Hu *et al*., 2016). Dinophyta (dinoflagellates) and Bacillariophyta (diatoms) were markedly dominant in the micro-plankton. Those two groups co-occurred with Chlorophyta, Cryptophyta, Picomonadida, MALV and

MAST within the nano-plankton and pico-plankton, the latter dominated by Chlorophyta. Contrary to ocean-based sampling (de Vargas *et al*., 2015; Massana *et al*., 2015; Pernice *et al*., 2016; Biard *et al*., 2017), our coastal ecosystems were markedly lacking radiolarians. In accordance with previous studies that stressed correlation of Radiolarians diversity with water-depth and distance from the coast (Decelle *et al*., 2013; Biard *et al*., 2017), our strongest signal was found at the DCM of an offshore point within the Bay of Biscay. Haptophytes, other dominantly off-shore organisms (Massana, 2011), were in equally low numbers in our study. This underestimation could result from the selected barcode and primers (V4 18rDNA as in Stoeck *et al*., 2010) which has been acknowledged to overlook this group of protists (Liu *et al*., 2009; Massana, 2011; Bittner *et al*., 2013; Egge *et al*., 2013). Fungi were also in far lower proportions in our study than in two other studies from the coasts of the East-English-Channel (Christaki *et al*., 2014; Genitsaris *et al*., 2015). Those studies were based on the amplification of the V2 and V3 regions of eukaryotic DNA which might be more taxonomically informative for fungi than the V4 marker (Massana *et al*., 2015; Richards *et al*., 2015).

b) From a genetic to a functional diversity approach in protists: limits and potential development

Out of the 30 theoretical biological traits proposed to describe the ecological strategies of marine protists, 13 could be annotated for 83% of our taxonomic references (1669 out of the 2007). As demonstrated by statistical tests, OTUs with a functional annotation represented a reduced (ca. 50%), but representative share of our complete taxonomic table (52 180 out of 111 089 OTUs). Well annotated traits mostly concerned the trait types of morphology and trophic strategy. Within the life cycle trait type, only the production of resting-stage was relatively well annotated. Physiological and resource acquisition trait types were scarcely annotated since those kinds of information exist for few cultivated species and cannot be generalised to taxonomic references at low taxonomic levels (genus, families). This limit was also identified in another functional annotation of OTUs (de Vargas *et al*., 2015). In future research, the combination of physiological traits and phylogenetic approaches could likely help to bypass this limit by summarizing values to larger taxonomic

levels (e.g. family, genus) and in a non-putative way (Bruggeman, 2011). Other biological traits could have been included in our functional analysis, since group-specific functional analysis can rely on more specific traits than those used in this study (e.g. in Fenchel, 1980; Hansen *et al*., 1997; Weisse *et al*., 2016). In this first attempt to study the functional diversity of marine protist communities, we selected biological traits which were the most integrative and that could be generalized to the largest extent of marine protist species. Surely, this functional representation of protist diversity will be improved over time by inputs of different protist specialists. In addition, we applied this theoretical framework exclusively to coastal communities, the study of its relevancy among other ecosystems (off-shore, artic, deep-sea) remains an interesting path for future research.

Trade-offs and functional groups were defined through impartial statistical methods. These analyses selected 8 out of 13 well-annotated, and correlated with each other traits that represented common ecological strategies likely resulting from cellular constraints and/or selection processes. Consequently, 5 traits were excluded, although considered to be descriptors of protistan ecology ("resting-stage" in Litchman and Klausmeier, 2008 and Lange *et al*., 2015; "size" in Litchman and Klausmeier, 2008, Litchman *et al*., 2013 and Weisse *et al*., 2016; "spicules" in Hamm, 2005; "cell-shape" in Pahlow *et al*., 1997). In fact, the excluded traits probably form trade-offs with traits absent from our functional table. As an example, cell shape and size usually correlates with growth rate, resource requirement and uptake through allometric laws (Grover, 1989; Nielsen and Sandjensen, 1990; Edwards *et al*., 2012; Litchman *et al*., 2013); while resting stages involves strong investment on the life-cycle, longevity, stress-resistance and probably benthic-coupling (Marcus and Boero, 1998; Litchman and Klausmeier, 2008). The addition of physiological type of trait in our functional framework and the annotation of such traits will likely generate other trade-offs delineating further ecological strategies and functional groups. Despite those methodology constraints we still consider that our 6 functional groups are good candidates for resuming the functional diversity of marine protistan communities.

c) Patterns of functional diversity of coastal protist communities

Except for CAT, all the functional groups were paraphyletic (Figure 15), including various protistan phylogenetic branches. The paraphyly of our functional groups supposes that similar functional traits emerged along different lineages of protists. Biological traits of higher plants were found to be tightly structured along phylogeny, to a point where authors have supposed that phylogeny might be a proxy of functional diversity (Flynn *et al*., 2011). For freshwater phytoplankton (i.e. phototrophic protists) results are contrasted. Bruggeman (2011) showed that phylogeny was a good predictor for several morphological and physiological traits while Kruk *et al*. (2010, 2011) sorted 700 species into morphological groups without phylogenetic correlation. As for marine protists, it is likely that similar evolutionary events (competition and selection processes) might have favored the adoption of similar ecological strategy along distant phylogenetic branches (Webb *et al*., 2002; Caron *et al*., 2012). For example, parasitic protists (e.g. in MAST, MALV or gregarines) are part of distant lineages (Stramenopiles, Alveolates and Apicomplexans, respectively). Bruggeman (2011) also found that growth rates of phytoplankton species correlated well with phylogeny, supporting again the idea that the inclusion of physiological traits in the construction of functional groups could likely enhance the correlation between functional diversity and phylogeny.

The 6 functional groups identified in this study define various protistan ecological strategies, which are acknowledged to play key-roles in the structuring of pelagic communities (Worden et al., 2015): phototrophs (SWAT, FLAT and CAT), heterotrophs (HET), parasites (PARA) and saprotrophs (SAP). The phototrophic groups were discernible more by morphological adaptations, with some carrying mixotrophic potential (in SWAT and FLAT), while the three heterotrophic groups were coherently distinguished on the basis on their ingestion methods. Groups of phototrophs echoed morphological groups and life-forms proposed by Margalef (1978) and Reynolds *et al*. (1983). Their work supposed that phytoplankton adapted their shape and morphology principally to counter sedimentation, resource scarcity and predation. Our functional groups distinguished swimmers (SWAT), floaters

(FLAT) and colonial (CAT) species, which clearly represent adaptations to sedimentation (i.e. swimming can counterbalance sedimentation and colonies increase the cell buoyancy, e.g. in Pahlow *et al.*, 1997; Ploug *et al.*, 1999) and predation (swimmers have the possibility to avoid predation while colonies may discourage predators; Margalef, 1978). Heterotrophs were divided into three distinct life-strategies distinguished according to their prey and/or food preferences (SAP: dissolve and detrital matter, HET: preys; PARA: host type). By applying specific functional traits, the HET group could likely be further subdivided with addition of phagotrophic related traits, like functional and numerical response (Yang *et al.*, 2013; Weisse *et al.*, 2016), feeding mechanisms (Kiørboe, 2011) or maximal size of engulfment apparatus.

Functional groups distribution across size-fraction was uneven (Figure 12b). Within the micro-plankton, SWAT and HET were generally the dominant functional groups, while in the nano- and pico-planktonic size fractions, four groups, PARA, FLAT, SWAT and HET co-existed in comparable relative abundances (Figure 12b). Across the size classes, SAP and CAT were the less abundant functional groups. The coexistence of both phototrophic and heterotrophic functional groups in the smallest plankton can likely be explained by their functional adaptations. The persistence of phototrophic organisms within the smaller size-fractions is indeed probably due to their competitiveness in oligotrophic environments (Grover, 1989; Edwards *et al.*, 2012). If outcompeted by bigger species during repleted conditions (Agawin *et al.*, 2000), small phototrophs can indeed maintain high growth rates and thrive under very depleted conditions (Worden *et al.*, 2004). Recent researches have also highlighted mixotrophic behaviors and low light optima within pico-eukaryotes (Sanders and Gast, 2012; McKie-Krisberg and Sanders, 2014) that would further explain their survival and widespread distribution in various ecosystems. The high abundance and diversification of HET within pico-plankton might be explained by omnipresence of prey for small bacterivorous phagotrophs (Logares *et al.*, 2012; Pernice *et al.*, 2015). The persistence of PARA could be explained by the release of numerous small-size spores from hosts (Park *et al.*, 2004; Guillou *et al.*, 2008), that can also transform into dormant stage resistant along time (Gleason *et al.*, 2014; Scholz *et al.*, 2016). However, their abundance might be overestimated by a high number of copy by cell compared to other organisms in this size-fraction (Massana, 2011).

This constant signal of functional groups within the smaller size-fractions shaped distinct ecological patterns across size-fractions (Figure 17). Functional groups relative contribution was indeed significantly more equitable and less variable in pico-nano-plankton than in micro-plankton (Figure 17a). This pattern followed that of genetic diversity, where pico-nano-planktonic taxonomic communities were characterized by OTU's richness and equitability significantly higher and less variable than in micro-plankton (Figure 17). The stability of phylogenetic richness as well as a higher OTUs richness in samples of the smallest size-fractions have been already highlighted in the coastal ecosystem (Massana *et al*., 2004; Romari and Vaulot, 2004; Logares *et al*., 2014; de Vargas *et al*., 2015), here we evidence that this stability is also expressed in terms of functional diversity. Overall these results imply distinct ecological patterns across size-fractions. Protistan communities in the micro-plankton appear to be mostly dominated by successions of assemblage with low taxonomic and functional diversity, while protistan communities in the nano-pico-plankton consists of more diversified assemblages in which the dominance of the different taxonomic and functional units fluctuates little across space and time. This corroborates the idea that nano and pico-plankton are part of an ocean's veil on which larger protists and metazoans might develop (Smetacek, 2002; Fenchel and Finlay, 2004; Massana, 2011), as well as larger ecological theories on the distribution of size and species richness (Hutchinson and MacArthur, 1959).

d) Coupling between functional roles and taxonomy among marine protistan communities

The environmental variables used in this study to characterize the sampled ecosystems allowed the identification of classical environmental gradients found in the coastal environments; i.e. re- and depleted nutrient conditions and marine vs estuarine gradients (Figure S1). Functional and taxonomic community diversity were shown to vary along those gradients (Figure 16). Plankton composition is indeed known to be strongly structured by the salinity gradient (Khemakhem *et al*., 2010; Telesh *et al*., 2013), and to differentiate freshwater and saline communities at an evolutionary time-scale (Logares *et al*., 2009). Heterotrophs (via the HET and PARA groups) coincided well with offshore and winter conditions, that classically

highlighted the greater influence of microbial loop processes in these environments (Azam *et al*., 1983; Legendre and Rassoulzadegan, 1995). Phototrophs were more abundant in depleted samples coherent with the typical cycle of phytoplankton uptake (Cloern, 1996; Cloern and Jassby, 2008). However, the functional groups constructed on the basis of our genetic database corresponded to ecological strategies which could be dependent from other environmental variable not measured in this study. For instance, water-mixing and grazing pressure could have been correlated with floater (FLAT) and colony (CAT) forming functional groups (Landeira *et al*., 2014). Oxygen concentrations, prokaryotic abundances, particulate and dissolved organic matter concentration were also shown to correlate with the large-scale distribution of heterotrophic protist (Pernice *et al*., 2015) and myco-plankton dynamics (Taylor and Cunliffe, 2016). The low correlation between functional group composition and the environmental variables found in this study could also be enhanced by integrating the history of water masses conditions. A delay between changes in the environment and its effect on planktonic communities is indeed often observed (Wallenstein and Hall, 2012; Ward *et al*., 2014).

Our study showed that, in protistan coastal communities, changes in taxonomic composition corresponded to variations in the relative importance functional groups corresponding to different ecological strategies and functional roles. Conversely, studies on prokaryotic communities showed a decoupling between functional roles and the taxonomic composition. As showed by a global ocean survey of bacterial and archaean diversity, communities that were taxonomically different were characterized by similar functional groups. The environmental conditions strongly influenced the distribution of functional groups by shaping metabolic niches, but only weakly influenced taxonomic composition within individual functional groups (Louca *et al*., 2016; Louca and Doebeli, 2017). The contrast between protists and prokaryotes can be explained by their distinct evolutionary and selection processes. Different prokaryotes developed multiple cooperating enzymes that evolved in parallel with biogeochemical cycles (Falkowski *et al*., 2008). As a consequence, the functional roles of prokaryotes in their environment is explained at the molecular and metabolic level. Furthermore, among prokaryotes horizontal gene transfer is a main process of evolution (Cohan, 2002). Two distinct prokaryotic phyla can thus exchange genes (Koonin *et al*., 2001), and their functional roles might be

coded by few genes easily exchangeable by horizontal transfer. This process is supposed to create a community-wide functional redundancy among prokaryotes, that explains the decoupling between their functional and taxonomic diversity (Allison and Martiny, 2008; Falkowski *et al.*, 2008; Louca *et al.*, 2016). Micro-eukaryotes manifest their functional role at the cellular level, having developed various behaviors, specialized morphology, adaptations and strategies (Massana and Logares, 2013). This functional complexity is coded by multi-gene patterns (Burns *et al.*, 2018), that are more difficult to exchange by horizontal transfer across species (Massana and Logares, 2013; Keeling and del Campo, 2017). Protist functional diversity can therefore be explained by specific morphology and trophic behaviors that separately evolved across micro-eukaryotes, which justify the tight link between taxonomic and functional diversity of their environmental communities.

4) Conclusions

The description of morphological characters and feeding behaviors of protists was enough informative to describe functional community patterns of coastal micro-eukaryotic assemblages. A tight coupling between the taxonomic and functional diversity of coastal protistan communities was evidenced in this study. This contrasts with prokaryotic oceanic communities where functional roles are mostly played at the molecular level and are easily exchangeable, blurring the limits between taxonomy and functions. Each species of protist developed its own particular blend of morphological and behavioral specificities, which constitutes hardly exchangeable functional roles, favoring a strong coupling between taxonomy and function. We also showed that functional diversity patterns were distinct between large and small protistan communities. Indeed, micro-plankton seemed more prone to domination of one or few functional groups while the smallest size-fraction maintained the coexistence of various phototrophs and heterotrophs in a sample. This hypothesis needs to be tested across larger experimental frameworks and beyond coastal ecosystems. Our innovative analysis, developed with a trade-off approach and based on information gathered from the literature, is perfectible. Indeed, the dearth of crucial information on protists, especially, concerning biological and physiological

traits is one of the results of our analysis. This only proves the everlasting need for more observations to better understand protistan community structure; whether being *in-situ* or *in-vitro*, comprising e.g. microscopy, taxonomic abundances or ecological fluxes. Studying the genomic basis of functional roles is also a great prospect in functional ecology of protists, it still remains a difficult task because of the sequencing limits presented by protists large genomes. The more protistan genomes will be accessible the more we will be able to predict phenotypic information, ecological strategies and functional roles based on DNA sequences.

5) Experimental Procedures

a) Sampling strategy

A total number of 277 water samples were collected with a temporal and/or spatial strategies across coastal ecosystems of France and Senegal (Figure 11). Samples were collected at surface water with comparable procedures (0-5m depths). For some sites, additional samples were collected at the depth of the Deep Chlorophyll Maximum (DCM) and at mesopelagic level, identified by CTD profiles. Water replicates (one or two) were sampled during most cruises (Table 1).

Seawater samples were collected with Niskin bottles and progressively filtered onto polycarbonate membrane filters of 47 mm in diameter and 20 (or 10), 3 and 0.2 μm of pore size. Particles of the two first size fractions (>3 μm) were separated by means of a peristaltic water pump and swinnex filter supports. For the last size-fraction, 0.22 µm polyethersulfone sterivex filters were used for the samples of MB and RA at the end of the pumping circuit, for other cruises 0.5 to 1 L of the residual filtrate from the higher size classes was filtered onto 0.2 μm filters. This size fractionated sampling yielded a total number of 1145 filters allowing the study of plankton size classes. For convenience, here we define as micro-plankton (>10 or 20μm), nano- (3-20 or 10 μm) and pico- (0.22-3 μm) our size fractions, using a slightly different definition of the one commonly used for plankton studies (micro >20 μm, nano 20-2 μm and pico 2-0.2 μm as proposed by Sieburth *et al.*, 1978). Sampled water was filtered until filter clogging, which yielded a variable filtered

water volume ranging from 0.5 to 10 L across the different samples. After filtration, filters were immediately frozen in liquid nitrogen and then stored at -80 °C until genetic procedures, RA's filters were added with the lysis buffer before freezing. To estimate the environmental characteristic sampled, temperature and salinity were measured by CTD, and nutrient concentrations (NO_3^-, NO_2^-, PO_4^{3-}, NH_4^+ and $Si(OH)^4$) were measured by a Seal Analytical AA3 HR automatic analyser following procedures described by Aminot & Kérouel (2007).

b) Genetic procedures

A metabarcoding approach was adopted to characterize the taxonomic diversity of the sampled communities. The hyper-variable V4 domain of the 18S rDNA region was chosen as a barcode for its conservative character within the eukaryotic microbial community and its relatively high length (230-520bp; Nickrent & Sargent 1991) which allows a relatively good genetic distinction of marine protists (Stoeck *et al*., 2010; Behnke *et al*., 2011; Dunthorn *et al*., 2012). Genetic methods were the same for all cruises, except for the RA dataset (Roscoff-Astan). Genomic DNA was extracted following the DNA extraction kit Nucleospin Plant II (Macherey-Nagel, Hoerdt, France). DNA from RA filters was extracted with two buffers, one lysis buffer containing lyzozyme (45min at 37°C), and one composed of proteinase K and SDS (1h at 55°C). The extract was treated with phenol:chloroform:isoamyl alcohol and then processed with the Nucleospin extraction kit. Blank extractions with nuclease-free water were carried out as negative controls for contamination during the process. The quality and concentration of extracted DNA was measured using a BioTek FLx800 spectrofluorophotometer and a Quant-iT PicoGreen ds DNA quantification kit (Invitrogen, Carlsbad, CA), respectively, following the manufacturer's instructions. Final DNA concentration of all extracts was normalized to 5-10 ng/μL. PCR was then ran with V4 markers assembled with the GeT-PlaGe adapters of the sequencing platform Genotoul (http://get.genotoul.fr/ ; Forward : V4f_PlaGe 5'CTT-TCC-CTA-CAC-GAC-GCT-CTT-CCG-ATC-TCC-AGC-A(C/G)C-(C/T)GC-GGT-AAT-TCC'3, Reverse : V4f_PlaGe 5'GGA-GTT-CAG-ACG-TGT-GCT-CTT-CCG-ATC-TAC-TTT-CGT-TCT-TGA-T(C/T)(A/G)-A'3) and a taq polymerase (Phusion High-Fidelity PCR Master Mix with GC Buffer). The process of PCR amplification was carried out three times for each DNA extract

(representing a unique filter). The amplification protocol consisted of a denaturation step at 98°C for 30s, followed by two set of cycles 1) 12 x [98°C (10s), 53°C (30s), 74°C (30s)] and 2) 18 x [98°C (10s), 48°C (30s), 74°C (30s)]. The cycles were followed by a final elongation at 72°C for 10 min. Amplification results were verified by gel electrophoresis, triplicate reactions were pooled and purified using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Hoerdt, France). Purified products were diluted to obtain equimolar concentrations before library construction at Genotoul for Illumina MiSeq (2x250 bp) sequencing. Six libraries were constructed, keeping samples from different cruises and locations separated. Sequence data are available at sextant.ifremer.fr/record/16bc16ef-588a-47e2-803e-03b4acb85dca/.

c) Sequence data cleaning, filtering and clustering into OTUs and taxa

Sequenced data were submitted to quality checking by built-in modules of the USEARCH (Edgar *et al*., 2011) program comprising 1) removal of reads with biased nucleotide (according to Phred score < 1%), 2) elimination of reads with incomplete or wrong primer sequence, and 3) chimera removal. In order to eliminate PCR errors and read-sample cross contaminations a strict data cut-off has been applied to the cleaned dataset. Singletons and sequences present in less than two samples and having a total number of less than three reads over the whole data-set have been removed (de Vargas et al., 2015). Details of both treatment across sequencing run can be found in Supplementary Table 1. Taxonomic assignment of sequences was processed with the V4 reference database PR^2 (Guillou *et al*., 2013). All sequences with percentage of identity to the reference database ≤ 80% were removed (Stoeck *et al*., 2010; de Vargas *et al*., 2015; Mahé *et al*., 2017) considering that values under this threshold lead to unreliable taxonomic assignment. Reads annotated to "Metazoa" and to multi-cellular plants were also removed from the data base, however annotated fungi were kept. Metabarcodes were then clustered into Operational Taxonomic Units (OTUs) by the agglomerative, unsupervised single-linkage-clustering algorithm Swarm 2 (Mahé *et al*., 2014, 2015), with a default clustering threshold of d = 1 (Mahé *et al*., 2015). Final clustering of those sequences

allowed the creation of 111 089 OTUs cumulating into 3.5 x10^6 reads. Each of those OTUs was given the taxonomic reference of its most abundant metabarcode, resulting into 2007 distinct taxonomic reference. Sampling quality was evaluated by rarefaction curves (reads vs. OTUs numbers) calculated with the *rarecurve*() function of R package "vegan" (Osaksen *et. al.*, 2016; Figure S2).

d) Functional approach

Functionally annotated OTUs represented a reduced portion of the complete dataset (52 180/ 111 089 OTUs). Correlation between both datasets was calculated with the Mantel-test (Mantel, 1967). The method consists in the comparison of two dissimilarity matrices sharing the same samples but based on differing variables. Here, dissimilarity matrices of the complete and the reduced dataset were calculated with the Bray-Curtis distance, a distance adapted to datasets affected by the "double-zero problem" (Ramette, 2007; Legendre and Legendre, 2012; Buttigieg and Ramette, 2014). Mantel-tests were calculated with 9999 permutations. Shannon and OTUs richness were also compared by means of a linear regression (Figure S3).

To seek for trade-offs across traits, a functional multi-dimensional space was constructed. Gower distance was computed on the biological trait table to calculate a distance between the selected taxonomic references (1669) according to their traits (13); this distance was then analyzed by Principal Coordinate Analysis (PCoA). Gower distance is generally used in functional studies because it can deal with different sorts of traits (e.g. numeric and categorical traits ; Legendre and Legendre, 2012; Maire *et al.*, 2015). When Gower distance and PCoA are associated, some dimensions can carry "non-euclidean" information with negative eigen-values (Legendre and Legendre, 2012). Euclidean dimensions were thus selected according to a neutral statistical method (Maire *et al.*, 2015). The PCoA axes then selected resumed information about each of our 13 traits. We considered that when the information of distinct traits was explained by the same PCoA axis, the modalities of those distinct traits were part of a trade-off that delineated one or more ecological strategy. The correlation between traits and PCoA axes was studied with a Spearman Rank test (Figure S5). The first 2 PCoA axis showed correlations with numerous traits, indicating trade-offs between those traits. PCoA axes, 3, 4 and 5 correlated

with few and isolated traits (i.e. those traits observed few to no trade-offs), they were excluded from functional group construction.

We used the scores of each taxonomic reference on the PCoA axis informing on trade-offs to build functional group (i.e. ecological strategy). The Simple Structure Index (SSI) method of k-mean was used as an impartial criterion to select the best partitioning of our taxonomic references in functional groups (Laliberté *et al.*, 2015; Borcard *et al.*, 2011; Figure S6). The annotated taxonomic references (1669) were then associated to a functional group. Finally, OTUs read abundances were cumulated into their respective functional group, the sum of reads was used to calculate a relative abundance in each sample. In this way, a functional community table was build. The whole methodological process is resumed in Figure 14.

e) Statistical Analyses

A Principal Component Analysis (PCA), computed on temperature, salinity and nutrients measures (NOx = NO_3^- + NO_2^-, PO_4^{3-} and $Si(OH)^4$) was performed (Figure S1). This analysis allowed the characterization of environmental gradients and highlighted differences among the sampling cruises. Unfortunately, those environmental characteristics were not available for some samples, those samples were thus absent from the PCA. In particular, Senegalese samples were lacking temperature and salinity measures, while the whole set of environmental variables was completely absent for the 2015 samples of the PI and PH cruises.

Relationship between environmental variables, taxonomic and functional diversity, among pico, nano- and micro-plankton communities, were tested with the RV statistical coefficient of co-inertia, a multivariate generalization of the Pearson correlation coefficient (Borcard *et al.*, 2011; Legendre and Legendre, 2012; Husson *et al.*, 2018). The test was run on the taxonomic community table, the functional community table and the environmental dataset (same variables that were used in the PCA) of micro-, nano- and pico-plankton samples. Datasets without environmental measures (same as in the PCA analysis) were discarded of this analysis, tests were conducted with 256, 254 and 271 samples respectively for micro-, nano- and pico-plankton. For deeper investigations, the same dataset was used and a Non-Metric Multidimensional Scaling Analysis (NMDS) was calculated on the complete

taxonomic table (with Bray Curtis distance) to ordinate samples of each size fraction on the basis of their OTU composition. Environmental values were fitted as explicative vectors on the two axes of the NMDS with the *envfit* function of package "vegan" (Oksanen *et al.*, 2016). Clusters of samples were constructed on the basis of the SSI method of k-mean partitioning. The relative importance of each functional group was calculated within each cluster of each size fractions. The *Adonis* test, a non-parametric multivariate analysis of variance (Oksanen *et al.*, 2016; with 9999 permutations), was used to determine whether if the functional groups showed distinctive distribution along clusters of each size-fraction.

Finally, Shannon index (Piélou, 1966) was calculated on the basis of the functional and taxonomic community tables, as well as OTUs richness. These metrics were compared across size-fractions with a Kruskal-Wallis test (a non-parametric one-way ANOVA test).

All Statistical analysis were performed with R software (R Core Team Development, 2015), in particular community analyses were performed with the "vegan" and "FactoMineR" packages (Oksanen *et al*., 2016; Husson *et al*., 2018).

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6) Supplementary Material

Supplementary Material 1: Ecological relevancy of the 30 traits proposed to describe the functional diversity of marine protists

Marine protists live in a multi-variable world where both the environment and species interactions have shaped distinct ecological strategies (Worden *et al*., 2015). Here we propose 30 functional traits that describe those strategies, and explain their survival in the environment (Violle *et al*., 2007). This text is companion of Figure 13. Traits were annotated only when mentioned in bibliography and generalizable to the taxonomic reference of Operational Taxonomic Units. By choosing to work in this non-speculative manner, mixotrophy, supposed to be widespread (Selosse *et al*., 2016), was probably under-estimated.

Morphological traits

- *Cell Size (Minimum and maximum)*: defined as a key trait for phytoplankton in Litchman & Klausmeier (2008). Involved in growth and metabolic rates (Litchman *et al*., 2007), sinking rates (Smayda, 1969), grazer resistance (Thingstad *et al*., 2005) and resource acquisition for phototrophs, (Grover, 1989; Yoshiyama and Klausmeier, 2008) heterotrophs (Hansen *et al*., 1994; Naustvoll, 2000) and parasites (Lafferty and Kuris, 2002). As illustrated in the work of Reynolds, Margalef and Fenchel, size already distinguishes strategies with distinct functional roles (e.g. C and S vs. R strategy, or prey optima for heterotrophic protists).

- *Cell Cover*: diminishes palatability for predators (Reynolds, 2006). Can involve and additional nutrient requirement for siliceous, calcite, strontium-sulfate covers. The constituents of the cell cover have also a role in global biogeochemical cycles (Le Quere *et al*., 2005).

- *Cell Shape*: elongation decreases palatability, and shape is also involved in resource acquisition for phototrophs by modifying the surface/volume ratio of the cell (Grover, 1989; Pahlow *et al*., 1997; Litchman *et al*., 2010).

- *Spicule(s)*: diminishes palatability for predators (Hamm, 2005).

- *Symmetry* and *Polarity*: proxies of investment in cell structure, complexity. They also influence *Cell Shape*. Proposed in Litchman *et al*. (2010).

- *Colony*: the colonial mode of life was proposed to play both the roles of predator avoidance (increasing in size and complexity of the structure) and improvement of resource acquisition by increasing water renewal around the cell (Margalef, 1978). It could also increase buoyancy, which is useful in order to avoid sinking (Margalef, 1978; Ploug *et al*., 1999).

- *Motility*: plays a role in survival (predator avoidance), reproduction (mating), and resource acquisition (prey search and capture), even for phototrophic species by increasing renewal of nutrient-replete water around the cell (Karp-Boss *et al*., 1996; Ginger *et al*., 2008; Kiørboe, 2011; Nielsen and Kiørboe, 2015). When motility varied during the life-cycle, the motility was annotated according to the trophic stage.

Trophic Strategy

- *Plastid Origin*: plastids are organelles allowing the phototrophic strategy, i.e. creation of organic matter using energy issued from light and carbon dioxide (McFadden, 2014). Plastid can be synthesized by the cell but also originate from kleptoplastidy or endosymbiosis (Mitra *et al*., 2016).

- *Ingestion*: highlights the heterotrophic strategy, i.e. creation of new organic matter thanks to the catabolism of organic matter (Sherr and Sherr, 2000). It is proposed here that the method of ingestion informs on the nature of preys available for the heterotroph (i.e. osmotrophic: dissolve organic matter; saprotrophic: dissolved, dead and detrital matter; phagotrophic: smaller size or similar size than the predator; myzocitosic: all living organism) (Gleason *et al*., 2008; Jeong *et al*., 2010; Worden *et al*., 2015). Both *Plastid Origin* and *Ingestion* method were used distinctly to detail the possibility of mixotrophic behavior.

Investment in these two traits represents the functional role of marine protists within the pelagic food-web (Worden *et al*., 2015).

- *Behavior*: describes the feeding processes of the organism (encounter and interception of the resource) (Kiørboe, 2011), it is linked to cell motility.

- *Mutualistic hosts*: hosting of any other organisms, and details on the type and the need of the symbiont for the hosts to thrive in the environment (Stachowicz, 2001; Decelle *et al*., 2015; Stal and Silvia, 2016).

- *Symbiosis*: whether the organism is engaged (is a guest) in a symbiosis and the effects it has on its hosts (Stachowicz, 2001; Decelle *et al*., 2015; Stal and Silvia, 2016). Parasitoids were distinguished from parasites as they could have further impact on the host population (Lafferty and Kuris, 2002).

- *Symbiont Location*: endo- or ecto—symbionts have different impact on the holobionts. It explains distinct parasitic patterns and affect *Specialisation* (see below).

- *Specialization*: indicates any specialization on the relationship with another species (predation, guest or host symbiosis). Generalists and specialists have distinct effects on the fitness of the other organisms population and possibly on ecosystem dynamics (Lafferty *et al*., 2008).

Physiological traits

- *Mucilage*: when synthesized, it influences negatively grazing, allows buffering of osmo-regulation and is involved in the size of mucilaginous colonies (Margalef, 1978; Grattepanche *et al*., 2011).
- *Chemical Signal*: an information on allelopathic, mating and osmolytic composites produced by the species and that could help it to thrive in the environment (Wolfe, 2000; Schwartz *et al*., 2016).
- *Niche related traits*: preferences and tolerance range for influential environmental metrics (i.e. nutrient, dissolved oxygen concentration, depth, light,

temperature and salinity) (Brun *et al*., 2015). Typical performance traits and contrary to the principle of functional trait (Violle *et al*., 2007), but useful to test the known environmental preferences and to explain species distribution.

- *Toxygeny*: Synthesis of toxins harmful at the ecosystemic scale (other organisms' communities) (Heisler *et al*., 2008; Gu *et al*., 2013). A performance trait linked to *Chemical signal*.

Life Cycle

- *Benthic Phase*: if occurring during the life cycle, the complete species fitness would be influenced by resuspension and hydro-dynamism (Ohtsuka *et al*., 2015).

- *Longevity*: could help highlights stress-tolerant species present at low nutrient concentrations (Grime, 1974; Reynolds C.S., 2003).

- *Resting Stage*: represents a competitive advantage during unfavorable environmental conditions (Litchman and Klausmeier, 2008; Lange *et al*., 2015). Could also be linked with a benthic phase.

- *Ploidy*: the capacity to reproduce allows genetic variations, genetic flexibility could be an adaptive advantage against ecological pressures (Litchman and Klausmeier, 2008).

- *Genome size*: by reducing genome size, cells can reduce needs for growth-limiting elements, cells could present an adaptation to resource scarcity (Pommier *et al*., 2007; Litchman *et al*., 2010; Raven *et al*., 2013).

Environmental dataset

As a first step in our analysis, a PCA was computed on all environmental measures collected (Figure S1). This analysis showed two main gradients, the first axis fitted well with salinity and distinguished, at the left, the DA samples that were taken at the mouth of an estuary from more marine samples, at the right (Figure S1). The second axis opposed nutrients (bottom, NOx and PO_4) with temperature (top) (Figure S1), we hypothesized that this gradient represented samples taken in winter (lower temperature and higher nutrient concentration due to limitations in phytoplankton's uptake) from samples taken in summer (warmer waters and nutrients depleted by phytoplankton' uptake). Our samples spread along this two gradients implying that a continuum of environmental conditions was sampled.

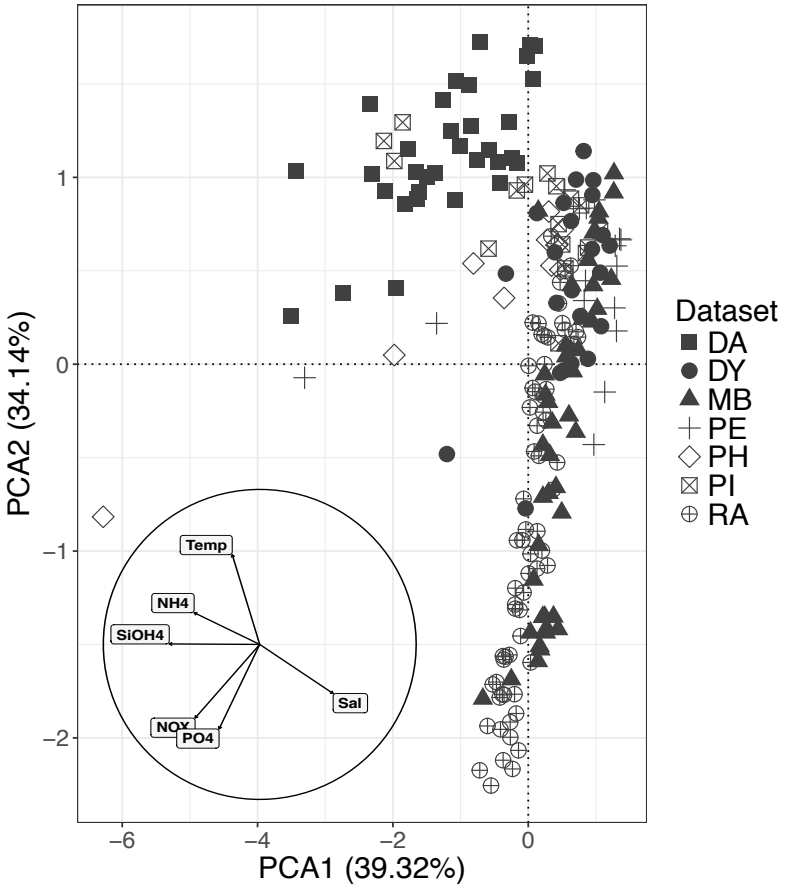

Figure S 1: Biplot of a Principal Component Analysis (PCA) based on the physical-chemical variables analyzed in all our samples with their correlation with the PCA axis (circle). Dot positions represent the gradients of the physical-chemical variables and the shapes indicate the different datasets. The correlation circle represents the correlation between environmental variables and the two axes of the PCA. Percentage of variance explained on total variance is presented next to axis names.

Biodiversity saturation

The next step was to investigate the protistan community found in these samples. This was carried by metabarcoding with a sequencing of environmental DNA, and to test if more samples would have brought more distinct OTUs we computed rarefaction curves (Figure S2). As the rarefaction curves did not reach an asymptotic plateau it was considered that the biodiversity of marine protists was not saturated and more samples would have brought more OTUs.

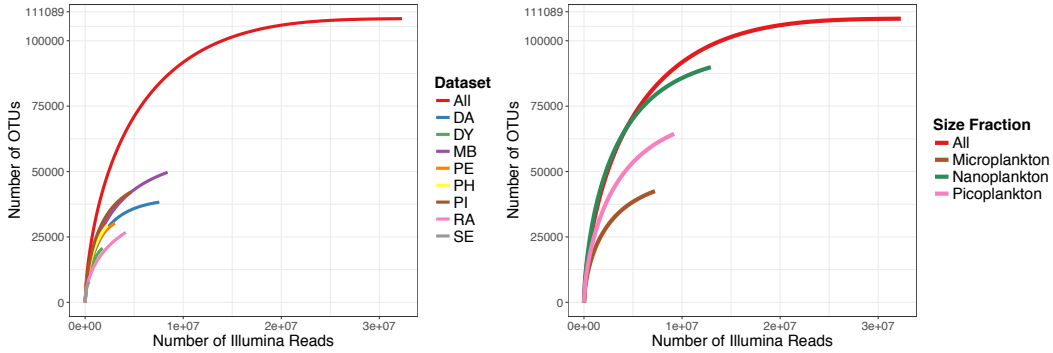

Figure S 2: Rarefaction curves constructed cumulating the samples of each sampling cruise (left) and for each size fractions (right), cumulating all samples available. The sampling effort is represented by the number of reads in relation to the species richness as the number OTUs. The function [rarecurve() function of "vegan" (Osaksen et al., 2016)] samples an increasing number of reads with a rate of 100 000 reads/sample and without replacement.

Functional Approach

The annotation of functional traits to the OTUs from the metabarcoding was perfectible, only 52 180 of the 111 089 total OTUs could be sorted into a functional group. To test if the reduced dataset still showed diversity patterns comparable to the complete dataset we studied the linear regression in between two diversity metrics estimated on both datasets, Species Richness (SR, here OTUs) and the Shannon Index H' (Figure S3). This analysis showed a good fit in between the two datasets across the two metrics (Figure S3, R^2 = 0.88 and 0.75 respectively for the SR and H'), implying that the reduced dataset still represented most of the diversity patterns of the original dataset. The reduced dataset was later used to estimate functional diversity patterns.

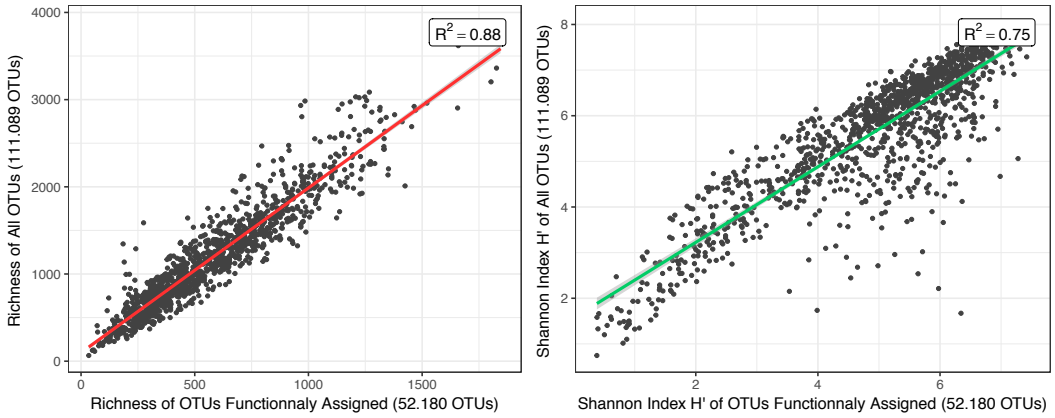

Figure S 3: Correlation between two diversity indexes (left: OTU richness and right: Shannon Index H') calculated on the complete community table (111 089 OTUs x 1 145 sampling sites) and a table with only the OTUs concerned with the functional annotation (52 180 OTUs x 1 145 sampling sites). Lines represent results from a fitted linear model of the data, the R^2 represent the fraction of variance (between 0 and 1) explained by the fitted linear model.

Our 52 180 OTUs were annotated to 1669 distinct taxonomic references to which 13 traits could be inferred. We wanted to 1/ study the trade-offs in between traits and 2/ to cluster together organisms that had similar strategies, representing functional groups.

The 1669 taxonomic references were plotted on a multidimensional space according to the similarity in their traits, this space was calculated with the Gower distance and a Principal Coordinate Analysis (PCoA) (Figure S4). PCoA computes as much dimensions as there are taxonomic references (i.e. 1669), we thus used a statistical method to select the dimensions that were useful to represent the initial trait table (here 5, according to Maire *et al*., 2015) (Figure S4). Then to study trade-offs we study traits correlation within the multidimensional space (Figure S5). Traits that were correlated to the same dimensions represented a compromise between traits, that was considered as a trade-off highlighting distinct strategies (Figure S5). Trade-offs were visible on Axis 1 and Axis 2 of the PCoA by highlighting clear correlations between: Cell Cover, Cell Symmetry, Cell Polarity, Coloniality, Motility, Plast Origin, Ingestion method and Symbiosis type (Figure S5). Conversely, Axis 3, 4 and 5 were dominated by one or two of the remaining traits (Resting Stages, Size Min, Size Max, Shape (Figure S5).

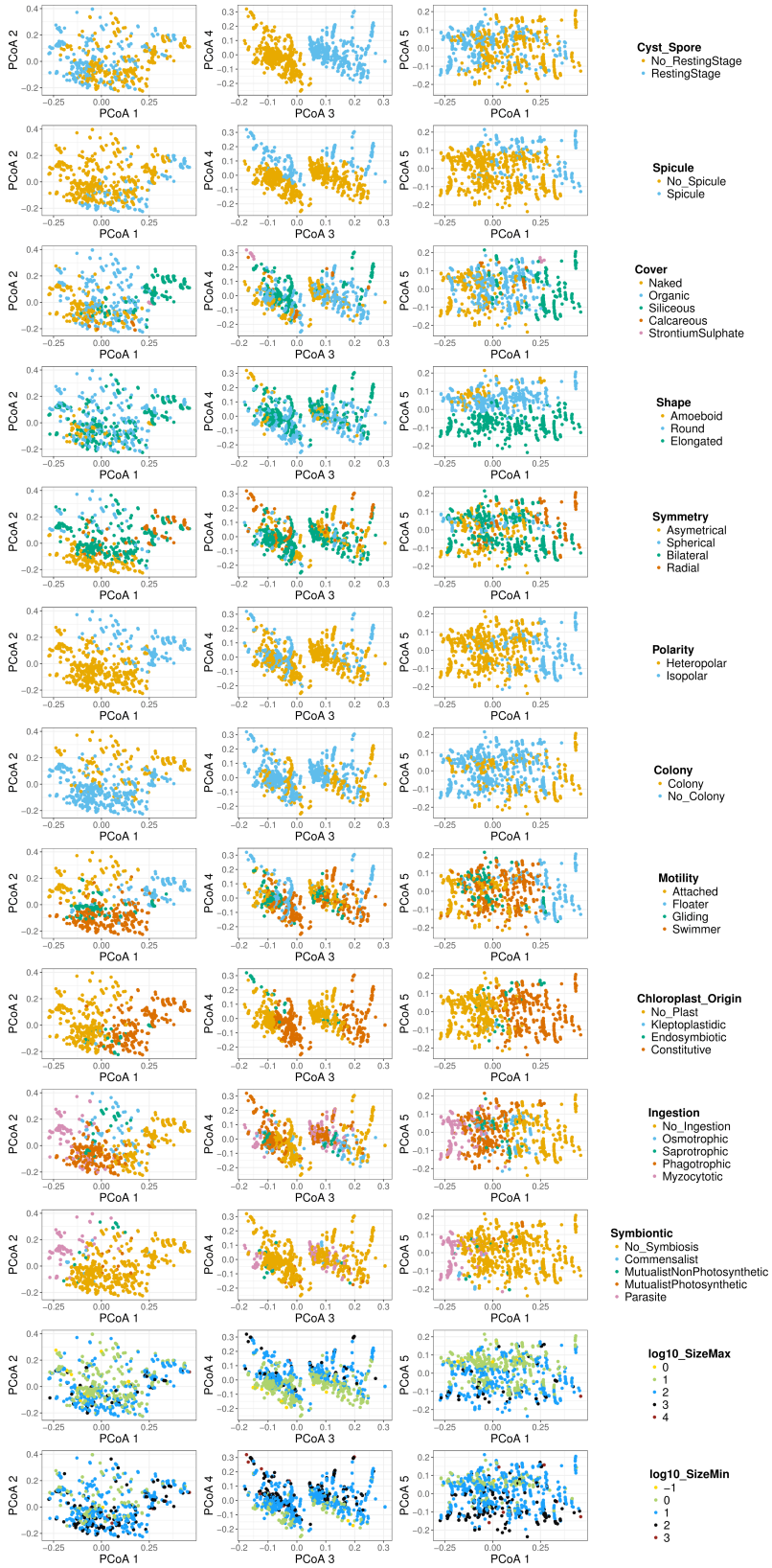

Figure S 4: Functional space analysis built through a Principal Coordinate Analysis (PCoA) using the Gower distance and our trait table (13 traits, 1669 taxonomic references).

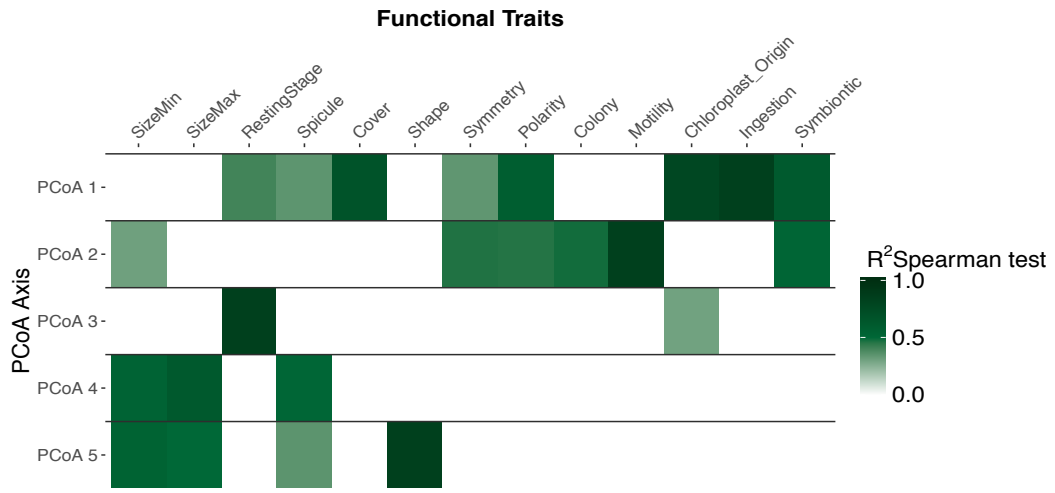

Figure S 5: Identification of Trade-offs between traits. The correlation of distinct traits on the same PCoA axis highlights a trade-off between traits. The correlation is characterised by the R^2 from the Spearman rank correlation. For clarity, correlations with R2 values < 0.3 and with a p-value > 0.05 were discarded, only the strongest correlations remain.

As a consequence of the trade-off analysis, we used the first two dimensions (that carried trade-offs) to create functional groups. We used the coordinates of our 1669 taxonomic references on the two first axis of the PCoA, and computed an unsupervised clustering based on a k-mean method and a statistical criterion of best partitioning (Figure S6). On the basis of the ssi criterion we used the partitioning in 6 functional groups (Figure S6).

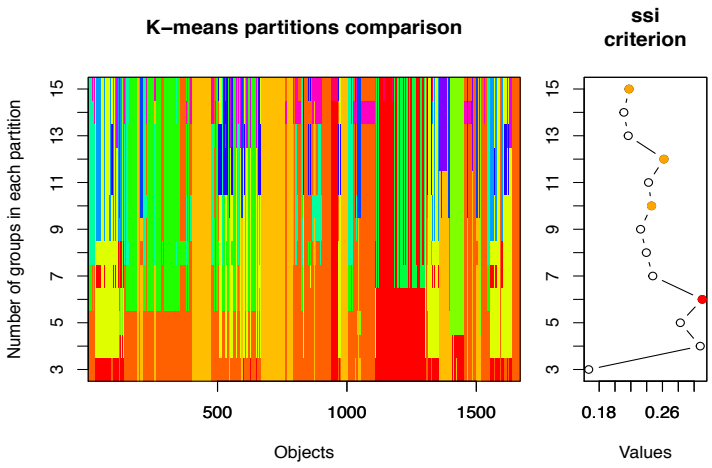

Figure S 6: Best partitioning resulting from the Simple Structure Index (SSI) based on Axis 1 and 2 of the PCoA. This graph is the results of the cascadeKM() from R package vegan (Osaksen et al., 2016). a) The principal graph represents the distribution of the 1669 taxonomic references (objects) within partitioning (y axis) of increasing number of divisions (Number of groups in each division). b) results from the SSI criterion, the highest value indicated with a red dot shows the number of groups with the best partitioning of the functional traits.

Finally, to study the ecological strategies represented by our six functional groups, we studied the dominant trait modalities of the taxonomic references within each group (Figure S7-S13). We identified: parasites (PARA, 1), phagotrophic protists (HET, 2), saprotrophic protists (SAP, 3), swimmer and phototrophic protists (SWAT, 4), non-swimmer and phototrophic protists (FLAT, 5), and colonial phototrophic protists (CAT, 6) (Figure S7-S13)

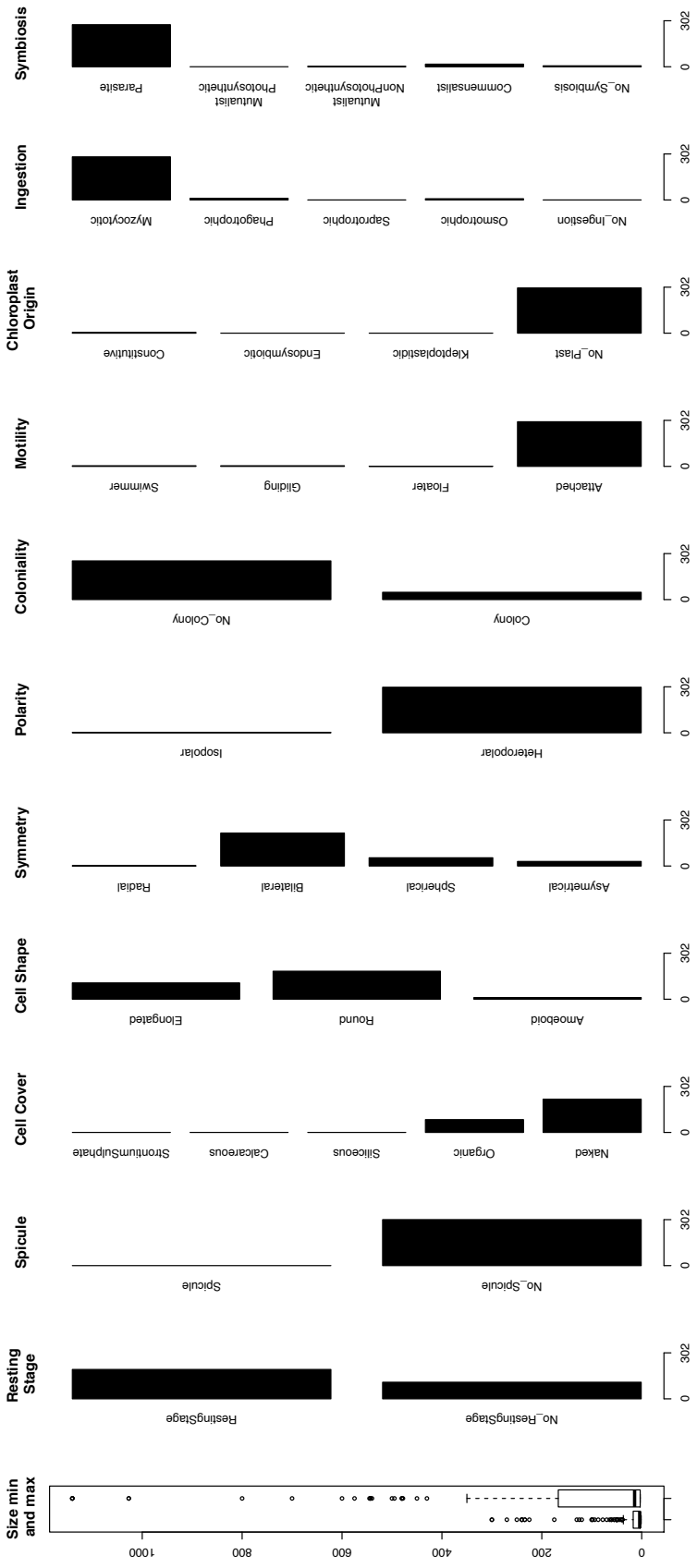

Figure S 7: Traits composition within functional group 1 (PARA : 302 taxonomic references). Barplots represent the number of taxonomic references annotated with a trait (x axis) within a trait modality (y axis).

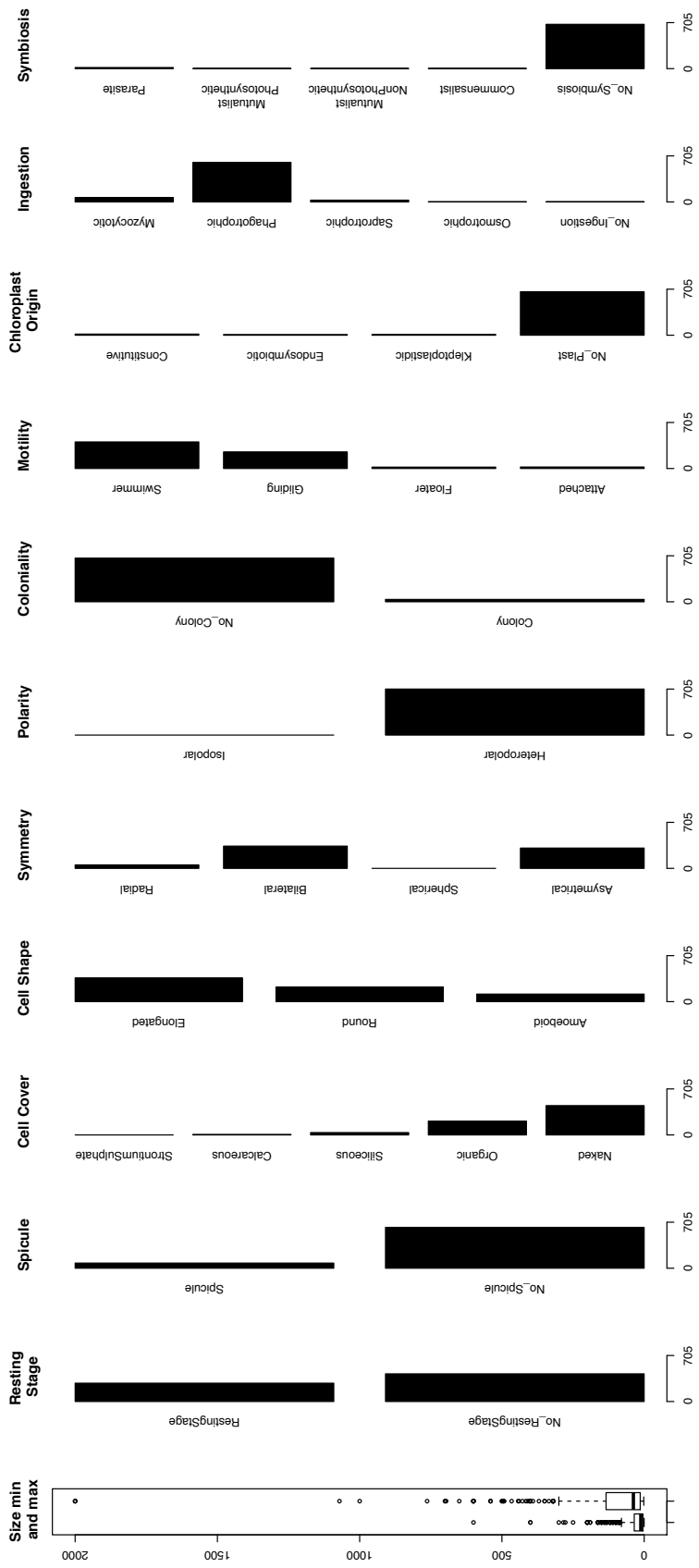

Figure S 8: Traits composition within functional group 2 (HET: 705 taxonomic references). Barplots represents the number of taxonomic references annoted with a trait (x axis) within a trait category (y axis).

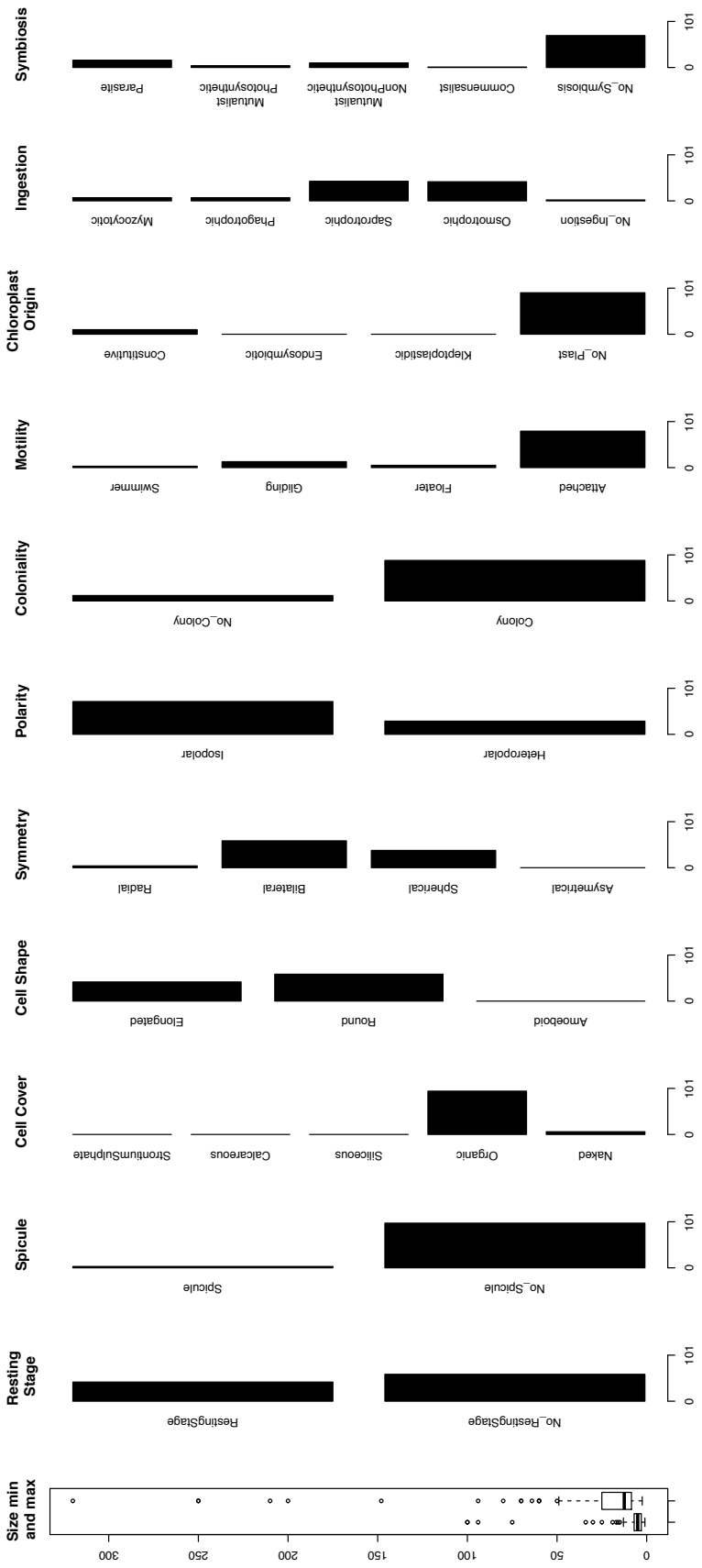

Figure S 9: Traits composition within functional group 3 (SAP: 101 taxonomic references). Barplots represents the number of taxonomic references annoted with a trait (x axis) within a trait category (y axis).

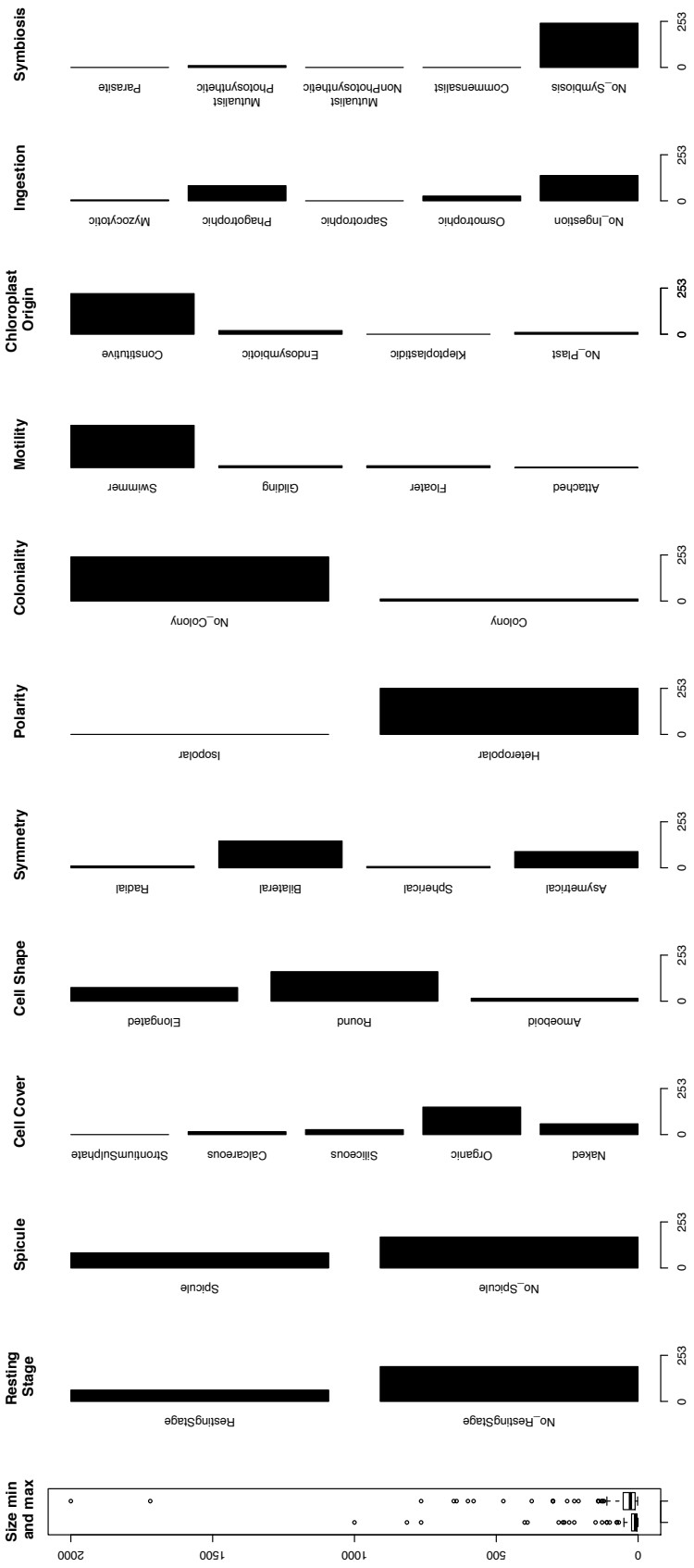

Figure S 10: Traits composition within functional group 4 (SWAT: 253 taxonomic references). Barplots represents the number of taxonomic references annoted with a trait (x axis) within a trait category (y axis).

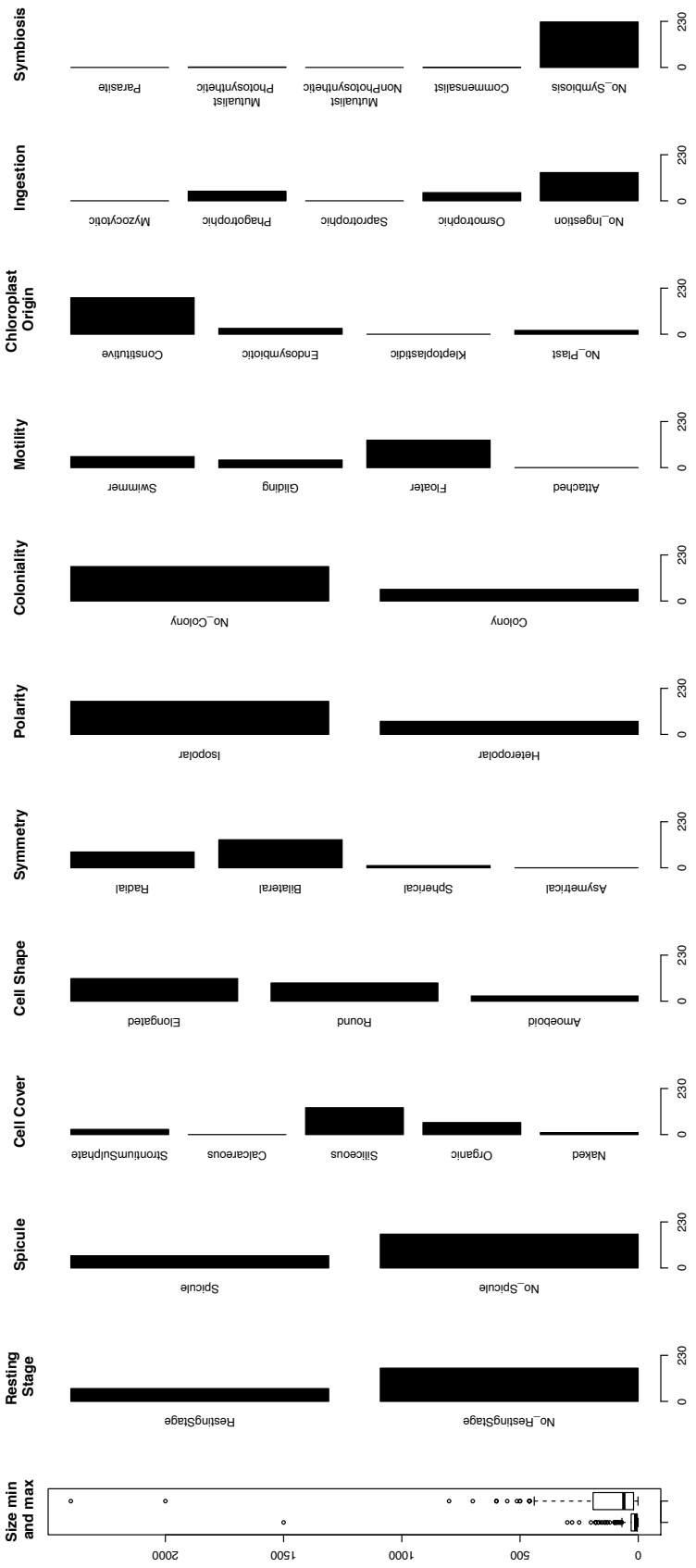

Figure S 11: Traits composition within functional group 5 (FLAT: 230 taxonomic references). Barplots represents the number of taxonomic references annoted with a trait (x axis) within a trait category (y axis).

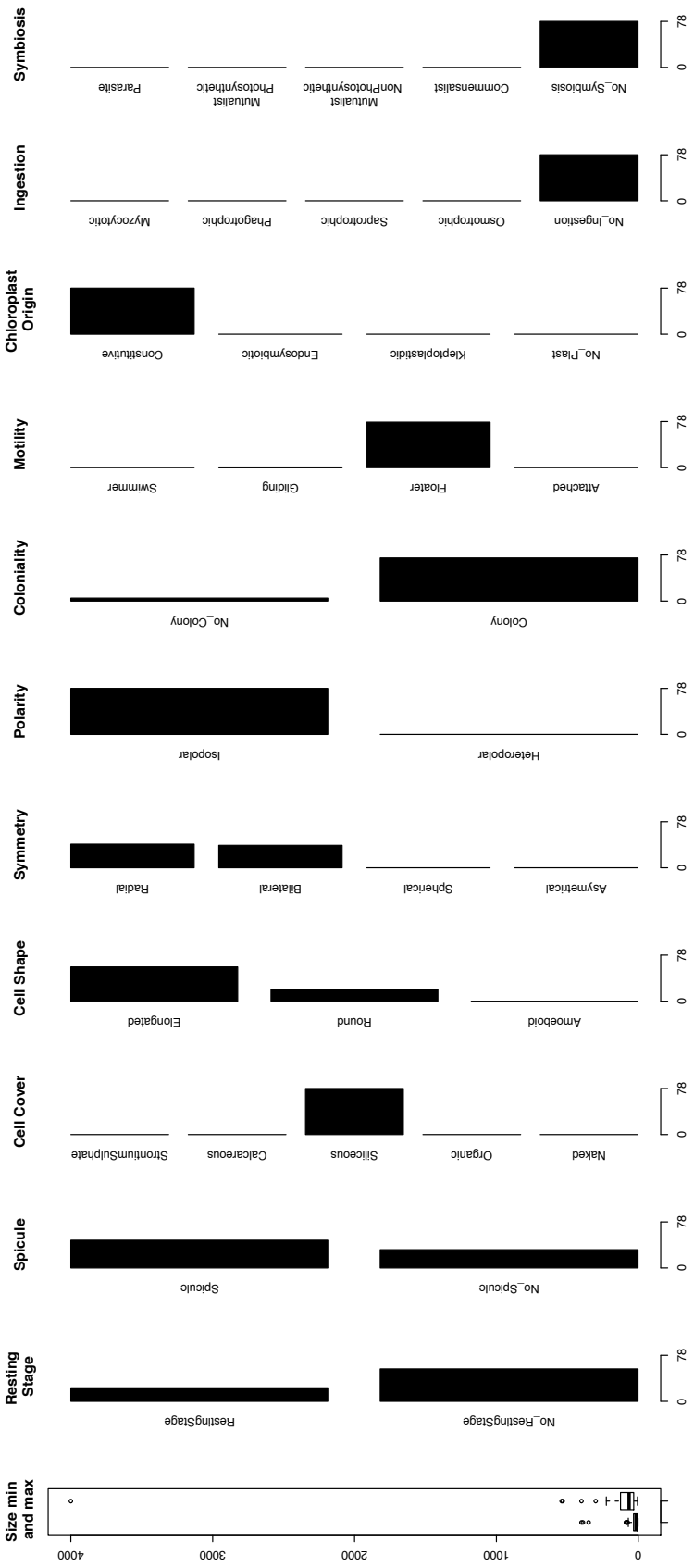

Figure S 12: Traits composition within functional group 6 (CAT: 78 taxonomic references). Barplots represents the number of taxonomic references annoted with a trait (x axis) within a trait category (y axis).

CHAPTER II: PATTERNS OF PROTISTAN DIVERSITY OVER A COASTAL TIDAL FRONT

Résumé (en français)

Dans cette partie nous avons cherché à comprendre comment l'environnement pouvait structurer la diversité taxonomique et fonctionnelle des protistes marins. Cette problématique a été appliquée à un front de marée apparaissant dans la mer d'Iroise lors de la période estivale. Les fronts de marées représentent la frontière entre 1/ les masses d'eau peu profondes, où la marée mélange verticalement l'ensemble de la colonne d'eau, et 2/ les masses d'eau plus profonde, où la marée n'arrive pas à mélanger l'ensemble de la colonne d'eau et où une stratification peu s'établir en été du au réchauffement des eaux de surface. En été, à cause du bloom printanier de phytoplancton, les eaux côtières (peu profondes) et du large (plus profondes) sont globalement épuisées en nutriment. Toutefois, au niveau du front, la marée permet un mélange local entre les eaux de surface et les eaux du fond enrichies en nutriments. Ce phénomène permet le maintien d'une forte production primaire au cours de la période estivale. Afin d'étudier comment ces phénomènes pouvaient structurer la diversité des protistes marins, 5 stations réparties sur le front de la mer d'Iroise ont été échantillonnées en Mars, Juillet et Septembre 2015. Nous nous sommes servis de notre approche de traits pour distinguer les protistes phototrophes, représentant le phytoplancton eucaryote, des protistes hétérotrophes. Ces deux communautés ont été analysées dans deux sous-sections présentées dans ce chapitre. Brièvement, les protistes phototrophes présentent un maximum de diversité taxonomique et fonctionnelle au niveau du front, ce maximum de diversité est constitué a) d'un mélange des stratégie écologiques développées autour du front et favorisées de manière cyclique et b) d'espèces dont la croissance est maintenue par les pulses de nutriments. Inversement, la diversité des protistes hétérotrophes est peu structurée par l'environnement, nous faisons l'hypothèse que ce compartiment est plus influencé par l'abondance de leur ressource et nous recommandons l'inclusion de données quantitatives sur leur proies potentielles (e.g. protistes, procaryotes, matière organique).

Context

Interested in how the environment could shape the taxonomic and functional diversity of marine protists, we studied patterns of protistan diversity over a coastal tidal front in the Iroise Sea (West Brittany, France). We used our functional approach to distinguish the phototrophic protists, representative of eukaryotic phytoplankton, from the heterotrophic protists. These two communities were analyzed separately in two subsections within this chapter. The first subsection focuses on eukaryotic phytoplankton, we highlight that the front strongly shaped the diversity of phototrophic protists by influencing the availability of resources necessary to its growth and by the mixing of distinct ecological strategies developed in the Iroise Sea. Reversely, the front influenced less heterotrophic protists and our functional traits were unhelpful to understand their dynamic. We argue that the diversity of these protists might be more influenced by the abundance and the type of preys found in the environment. Such variables should be measured in the future to understand and predict the dynamic of heterotrophic protists.

Author Contributions

The samples used in this chapter have been retrieved by staff members of the LEMAR and Ifremer lab, as well as crew members from the ship 'Albert-Lucas'. I took part in all the genetic procedures with the help of Sophie Schmitt (a beloved technician of the Ifremer de Brest). All samples were sequenced by the Genotoul platform and bioinformatics were carried out by Stéphane Audic (SBR). Environmental variables were measured by staff members of the LEMAR and Ifremer. Mathilde Cadier, Clarisse Lemonier, Louis Marié and Laurent Memery all participated in discussions that have structured this paper. A joint dataset of the prokaryotic community will be introduced at the end of this paper, this dataset was constituted by Clarisse Lemonier (PhD), under the supervision of Lois Maignien and Christine Paillard. I have carried out all the analysis presented in this chapter. I have written this manuscript under the supervision of Marc Sourisseau and Raffaele Siano. The first section of this chapter will soon be submitted to Frontiers in Microbiology.

A. Patterns of phytoplankton diversity over a coastal tidal front

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Abstract

Understanding patterns of phytoplankton production and diversity across marine ecosystems remains a difficult task due to the high variability of the physical environment at the sub-mesoscale. Here we use typical oceanographic measure (Chlorophyll *a*, temperature, and nutrients), the metabarcoding of marine protists and a functional approach to estimate patterns of phytoplankton over a marine tidal front in the Iroise Sea. Across three sampling campaigns in 2015, we observed an increase of resource limitation over the zone in summer. Despite this process, vertical mixing over the continental shelf allowed to maintain high nutrient concentrations, high primary production and a peak of eukaryotic phytoplankton diversity in the vicinity of the front. The peak of eukaryotic phytoplankton at the front was influenced by 1) the local mixing of the distinct communities found on both sides of the front, 2) a decrease in competitive exclusion and 3) intermediate disturbances favoring the maintenance of various ecological strategies.

1) Introduction

Oceanic communities of photosynthetic organisms (i.e. phytoplankton) with various ecological strategies are responsible for about 50% of the earth's primary production (Field *et al*., 1998), that fuels the biomass of larger organisms (Legendre, 1990; Brander, 2007) and shape global biogeochemical cycles (Falkowski *et al*., 1998; Worden *et al*., 2015). The diversity of phytoplankton is of prime importance in these processes (Cardinale, 2011; do Rosario Gomes *et al*., 2014), and is a complex retroactive function of its environment (Falkowski *et al*., 1998; Barton *et al*., 2010). Understanding the dynamics in the taxonomic and functional composition of this bulk of organisms is thus a great challenge. The factors brought forward to explain patterns of phytoplankton diversity involve: 1) advection and dispersal (e.g. water currents, mixing) (Chust *et al*., 2013; Lévy *et al*., 2015), 2) resource limitations and availability (here light and nutrients) (Hutchinson, 1961; Barton *et al*., 2010), both associated with competitive exclusion (Hardin, 1960) and ecological specializations (Smayda and Reynolds, 2001), and 3) biotic interactions, whether trophic (e.g. predation) or symbiotic (e.g. mutualism, parasitism) (Dodson and Brooks, 1965; Decelle *et al*., 2012; Kazamia *et al*., 2016). The amplitude of those factors but also their periodicity are major drivers of phytoplankton diversity (Reynolds *et al*., 1993; Huisman, 2010). It has been recently hypothesized that the highly variable physical processes apparent at the submesoscale of the ocean could strongly influence phytoplankton diversity (Clayton *et al*., 2013; Lévy *et al*., 2015).

Tidal fronts forming over continental margins are submesocale processes that are zones of high primary production (Simpson and Hunter, 1974; Holligan, 1981; Sharples *et al*., 2009). They are the frontier in between 1) the coastal shallow zones over which the turbulence of tides (bottom friction) mixes uniformly the whole water column and 2) the offshore deeper zones where this turbulence cannot spread over the whole water column and break the summer stratification (Franks, 1992). As a consequence, fronts can be easily targeted as the regions where the offshore stratification is abruptly reduced (Simpson, 1981; Le Fèvre *et al*., 1983). Usually as summer progresses, the isolated upper layer of the offshore regions becomes

increasingly depleted in nutrients, indeed in this area, nutrients originate from the bottom layer but are consummated during the spring bloom (Sverdrup, 1953; Martinez *et al*., 2011). At the coast, nutrients inputs by river-runoffs also become scarcer and this area become depleted too (Morin *et al.*, 1985; Cloern, 1987). However, in the intermediate depth of fronts, tides can erode and break the upper stratification. This results in a local mixing between the nutrient-rich bottom waters and the euphotic surface (Simpson and Hunter, 1974; Mariette and Le Cann, 1985; Sharples *et al.*, 2007) that causes local outbursts of primary production (Sharples, 2008; Maguer *et al*., 2015; Cadier *et al*., 2017a) supporting large food-webs at a regional scale (Le Fèvre, 1986; Ayata *et al*., 2011; Schultes *et al*., 2013). The effect of vertical mixing at fronts is also strongly regulated by the spring/neap tide cycle (Cadier, Gorgues, LHelguen, *et al.*, 2017). Indeed, during the more turbulent spring-tides nutrients inputs in the surface are strong, however it is only during the weaker neap-tides, when stratification forms again at the front, that phytoplanktonic cells can remain in the enriched euphotic layer and grow better (Sharples, 2008; Maguer *et al*., 2015; Cadier *et al*., 2017a). In addition to these repeated cycles of production, it has been recently hypothesized that tidal fronts could represent hotspots of diversity for phytoplankton (Cadier, Sourisseau, *et al*., 2017), based on two hypotheses: 1/ the local mixing of ecological strategies adapted to the distinct biotopes surrounding the front (i.e. an ecotone; Maarel, 1990) and 2/ the local decrease in competitive exclusion due to better resource availability (e.g. in Cardinale *et al*., 2009; Huisman, 2010). Here, we propose to test these two previous hypotheses in order to better understand the drivers of phytoplankton community structure at front.

The coupling between phytoplankton diversity and sub-mesoscale physics has already been studied by expert identification under microscope (Le Fèvre and Grall, 1970; Le Corre *et al*., 1993; Mousing *et al*., 2016) and/or trait-based modeling (Clayton *et al*., 2013; Lévy *et al*., 2015; Cadier, Sourisseau, *et al*., 2017). However, microscopic identification cannot account for the diversity of small phytoplankton cells (Li, 1994, 2002), while models are constructed with strong assumptions, such as the omission of mortality factors and symbiotic interactions, and thus need observations for validation (Shimoda and Arhonditsis, 2016). Recently, high-throughput sequencing of genetic markers, i.e. metabarcoding (Stoeck *et al*., 2010), have highlighted an unsuspected diversity of marine protists (i.e. unicellular

eukaryotes) (de Vargas *et al*., 2015) that are key members of phytoplankton (Worden *et al*., 2015). Furthermore, a recent functional approach has annotated Operational Taxonomic Units (OTUs), issued from metabarcoding, with biological traits (Ramond *et al*., submitted) in order to study the link in between taxonomic and functional diversity in marine protists. Combined with the taxonomic depth of metabarcoding, this tool can give us great insights into the interaction between eukaryotic phytoplankton and the complex physicochemical environment that represents tidal fronts.

In this study, we sampled a tidal front that forms seasonally within the Iroise Sea (Mariette and Le Cann, 1985). With the depth of metabarcoding, a biological trait approach, and typical oceanographic measures, we aimed to explain the dynamic of phytoplankton diversity across this tidal front.

2) Material and methods

a) Oceanographic context and sampling strategy

The Ushant tidal front forms in the Iroise Sea (Atlantic, Western France), lasts from May to October (Morin *et al*., 1985), and, as other fronts, it is proven to be highly productive throughout summer (Le Boyer *et al*., 2009). The physicochemical conditions leading to the front formation have been extensively studied in this area (Mariette and Le Cann, 1985; Morin *et al*., 1985; Le Boyer *et al*., 2009; Chevallier *et al*., 2014), as well as their effects on planktonic communities (Le Fèvre and Grall, 1970; Le Corre *et al*., 1993; Schultes *et al*., 2013; Landeira *et al*., 2014; Cadier, Sourisseau, *et al*., 2017), which makes the Iroise Sea an area of interest for oceanographic surveys. A strip of cold water extending from the Ushant Island (and above) to the entrance of the bay of Brest contrasts with the warmer offshore waters and is observed when measuring the Sea Surface Temperature of the Iroise Sea in summer (Figure 18; Le Boyer *et al.*, 2009; Muller *et al.*, 2010). The frontier between the two water masses is sharp but highly dynamic due to winds, tidal cycles and density driven currents (Muller *et al.*, 2010; Pasquet *et al.*, 2012). Nutrient inputs at this frontier are often observed and are strongly regulated by the spring-neap tide

cycle (Le Boyer *et al.*, 2009; Landeira *et al.*, 2014; Cadier, Gorgues, LHelguen, *et al.*, 2017). Nutrients can also be advected towards the western stratified zone due to baroclinic instabilities (Pasquet *et al.*, 2012). Accordingly, the phytoplankton uptake and growth are strongly influenced by the spring-neap tide cycle (Cadier, Gorgues, LHelguen, *et al.*, 2017) and the maximal phytoplankton biomass is usually found slightly westward to the front, in the stratified offshore waters, where phytoplankton growth is made easier due to better light availability (Le Boyer *et al.*, 2009).

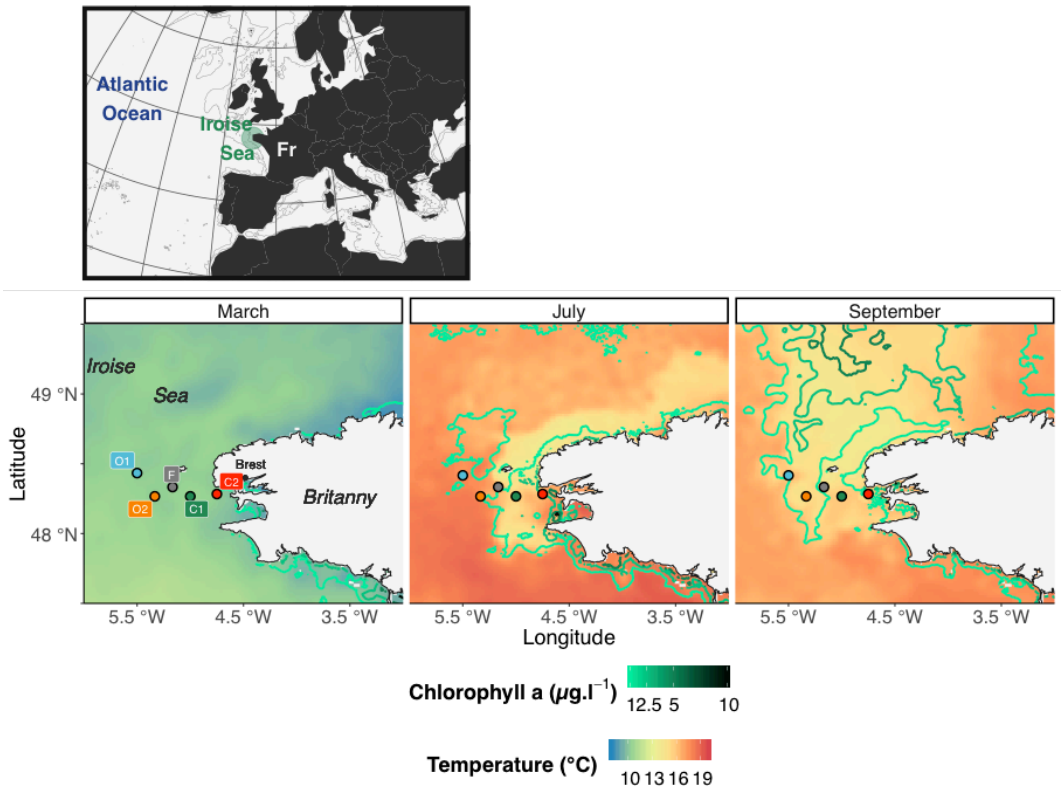

Figure 18: Hydrological conditions in the Iroise Sea during our three sampling campaigns. The sampling sites (dots and names) are superimposed on the corresponding temperature (background color) and chlorophyll a (isolign) estimated with satellite (MODIS-Aqua Ocean Color Data, 2014).

Five stations distributed across the Iroise Sea (respectively from the open-ocean to the coast: O1, O2, F, C1, C2; see their geo-localization onto Figure 18) were sampled three times during 2015, representing three seasonal configurations with different resources limitations for phytoplankton: "early spring" (10-12 March), "early summer" (1-3 July) and "end of summer" (8-10 September). Sampling was carried out between spring and neap tide in March, and slightly after neap tides in July and September (Figure S13). A sampling rosette equipped with Niskin bottles

(10L), a conductivity-temperature-depth probe (CTD) and a fluorescence sensor were used for profiling water stratification and Chlorophyll *a* concentration over the water column. Vertical profiles of temperature and fluorescence of each sample are represented in Figure S14. Water samples were collected at surface and at the bottom for all stations. In addition, when present, the deep chlorophyll maximum (DCM) was sampled and identified by fluorescence profile during CTD deployment. The water samples were triplicated by repeated cast at the same geographic position, here, we present results from 63 distinct samples comprising only surface and DCM.

Seawater was sampled throughout a sequential filtration approach used in order to separate communities of micro-, nano- and pico-plankton (respectively > 10, 10-3 and 3-0.2 μm). Carbonate membrane filters of 47 mm in diameter were used for pore sizes of 10 and 3 μm, while polyether-sulfone sterivex were chosen for the pore size of 0.2 μm. For each sample seawater was filtered until filter clogging, which yielded variable filtered volumes ranging from 2.7 to 5.6 L. The filters were frozen onboard into liquid nitrogen and later stored at -80 °C until DNA extraction. Macronutrients concentrations (here, NO_3^-, NO_2^-) were analyzed with a Seal Analytical AA3 HR automatic analyser following procedures described by Aminot & Kérouel (2007). Pigments concentrations (notably chlorophyll *a*) were analyzed by High-Performance Liquid Chromatography (HPLC) (Figure S15).

b) Genetic procedures

Environmental DNA was isolated and identified with a metabarcoding approach to characterize the genetic and taxonomic diversity of protistan communities of the Iroise Sea. The hyper-variable V4 domain of the 18S rDNA region was chosen as a barcode for its conservative character within the eukaryotic microbial community and its relatively high length (230-520bp; Nickrent & Sargent 1991) which allows a good genetic distinction of marine protists (Stoeck *et al*., 2010; Behnke *et al*., 2011; Dunthorn *et al*., 2012). Genomic DNA, issued from cells collected on water filters, was isolated following the protocol of DNA extraction kit Nucleospin Plant II (Macherey-Nagel, Hoerdt, France). In parallel, some blank extractions (Millipore filtered water) were carried out to check and validate the extraction procedure. DNA quality (proteins/DNA absorbance: A260/A280) and concentration of purified products were respectively measured using a BioTek FLX 80

spectrofluorophotometer and a Quant-iT PicoGreen dsDNA quantification kit (Invitrogen, Cralsbad, CA, USA) following the manufacturer's instructions. Final DNA concentration of all extracts was normalized to 5-10 ng/μL. PCR was then ran with V4 markers assembled with the GeT-PlaGe adapters of the sequencing platform Genotoul (http://get.genotoul.fr/ ; Forward: V4f_PlaGe: 5'CTT-TCC-CTA-CAC-GAC-GCT-CTT-CCG-ATC-TCC-AGC-A(C/G)C-(C/T)GC-GGT-AAT-TCC'3, Reverse: V4f_PlaGe 5'GGA-GTT-CAG-ACG-TGT-GCT-CTT-CCG-ATC-TAC-TTT-CGT-TCT-TGA-T(C/T)(A/G)-A'3). The process of PCR amplification was carried out three times for each DNA extract (representing a unique filter). The amplification protocol consisted of a denaturation step at 98°C for 30s, followed by two set of cycles 1) 12 x [98°C (10s), 53°C (30s), 74°C (30s)] and 2) 18 x [98°C (10s), 48°C (30s), 74°C (30s)]. The cycles were followed by a final elongation at 72°C for 10 min. Amplification results were verified by gel electrophoresis, triplicate reactions were pooled and purified using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Hoerdt, France). Purified products were diluted to obtain equimolar concentrations before library construction at Genotoul for Illumina MISeq (2x250) sequencing. Two libraries were assembled, one library contained samples from other datasets. Sequencing results are available at (doi.org/10.12770/16bc16ef-588a-47e2-803e-03b4acb85dca).

c) Bioinformatics analyses

Bioinformatics were carried out on a larger sequencing dataset (a total of 7 libraries, see Chapter I) to increase the number of sequence which allows a refined OTU construction and error detection. Sequenced data were submitted to quality checking by built-in modules of the USEARCH program (Edgar *et al.*, 2011), comprising 1) removal of reads with biased nucleotide (according to Phred score < 1%), 2) elimination of reads with incomplete or wrong primer sequence, and 3) chimera removal. Singletons and sequences present in less than two samples and having a total number of less than three reads over the whole data-set have been removed to eliminate PCR errors and read-sample cross contaminations (following de Vargas *et al.*, 2015). Taxonomic assignation of sequences was processed with the V4 reference database (Guillou *et al.*, 2013). All sequences with percentage of identity to the reference database ≤ 80% were removed, considering that values under this threshold

lead to unreliable taxonomic assignment (Stoeck *et al.*, 2010; de Vargas *et al.*, 2015; Mahé *et al.*, 2017). Reads annotated to "Metazoa" and to multi-cellular plants were also removed from the data base, however annotated fungi were kept. Metabarcodes were then clustered into Operational Taxonomic Units (OTUs) by the agglomerative, unsupervised single-linkage-clustering algorithm Swarm 2 (Mahé *et al.*, 2014, 2015), with a default clustering threshold of d = 1 (see Mahé *et al.*, 2015). Final clustering of those sequences allowed the creation of 111 089 OTUs. Each of those OTUs was given the taxonomic reference of its most abundant metabarcode. The final dataset analyzed here (63 samples) contains 33 060 OTUs, annotated to 1028 distinct taxonomic references and cumulating into 3.5 x 10^6 reads. Sampling quality was evaluated by rarefaction curves (reads vs. OTUs numbers) calculated with the *rarecurve*() function of R package "vegan" (Osaksen *et. al.*, 2016; Figure S16). A taxonomic community table based of the relative abundances of each OTU in each sample was created and used for community analyses. To present the complete metabarcoding dataset, each OTU was annotated with a simplified taxonomic rank. The difference in community, i.e. OTUs, composition between surface and DCM samples was tested with a Permutational Multivariate Analysis of Variance (PERMANOVA; *adonis()* function of R package "vegan").

d) Phytoplankton Diversity analyses

Selection of OTUs with photoautotrophic capacities was carried out with a trait-based approach previously developed (see Chapter I; doi.org/10.17882/51662). Briefly, using their taxonomic references and an extended bibliography, our OTUs were annotated with 13 biological traits (SizeMin, SizeMax, Cell Cover, Cell Shape, Presence of Spicule, Cell Symmetry, Cell Polarity, Coloniality, Motility, Plastid Origin, Ingestion method, Symbiosis type and Resting Stage during the life cycle). Because of the low taxonomic resolution of some OTUs (i.e. assigned only at the family level, class or domain) and/or the lack of scientific information, these traits could only be annotated to a subset of 803 out of the 1028 distinct taxonomic references (corresponding to 14 704 of the 33 060 total OTUs) retrieved in our dataset. Eukaryotic phytoplankton was selected as OTUs with inherent capabilities to

photo-autotrophy, considered as constitutive phototrophic protists (with inherent capabilities to photoautotrophy; see Mitra *et al.*, 2016).

To summarize phytoplankton diversity across our dataset, phytoplankton richness was calculated for each season (3), station (5), depth (2 when the DCM was sampled) and planktonic size-fraction (3), corresponding to 183 distinct samples. To account for this great number of measures, the variability of phytoplankton richness was first studied using boxplot of values across seasons, stations and size-fractions. Secondly, phytoplankton OTUs from distinct replicates and depth were united to represent the total phytoplankton richness across seasons, stations and size-fractions.

To understand the spatial structuration of total phytoplankton richness, OTUs were flagged as 'ubiquitous' if they were shared by at least two stations of the same season, and as 'Specifics to Station X' if they were retrieved only at station X of the same season. To test two hypotheses, we considered that a station with a higher number of 'ubiquitous' OTUs represented an ecotone, and a station with a higher number of 'specific' OTUs represented a zone of low competitive exclusion. Differences in phytoplankton richness across stations were tested with the Kruskall-Wallis test.

Phytoplankton richness was also studied according to its abundance. With this aim, rank abundance curves were built by calculating the total read number of OTUs, OTUs were then sorted according to their abundance across each season. The distribution of 'Ubiquitous' and 'Specifics' OTUs was studied across three communities divided by arbitrary abundance thresholds; the 'abundant', 'low' and 'very low' community; composed of OTUs with respectively a read number > 0.1%, between 0.1-0.001%, and < 0.001% of the total read number by season. The number of OTUs part of the 'abundant community' was further studied to consider the most successful OTUs in each location.

Finally, to study the "temporal" stability of the spatial structuration, the number of OTUs shared between stations and across the three seasons was calculated by means of a connectivity network (number of shared OTUs, e.g. in Villar *et al.*, 2015). To account for potential seasonal structuration of phytoplankton richness across depth, surface and DCM samples were distinguished in this analysis. Most importantly, given the distinct total phytoplankton richness across seasons (due to filtration and sequencing issues), we compared the connectivity patterns observed in the dataset with those calculated in a subdataset composed of a curated number of

OTUs by season. The subdataset was created by means of an OTU number normalization (see the experimental process inspired by Gobet *et al.*, (2010) in Figure S17), the correlation between the connectivity matrices of the original and the curated dataset were studied with the Spearman rank correlation. Further analyses of the connectivity network can be found in Figure S19.

e) Functional diversity analyses

The most successful ecological strategies of phytoplankton in each location were studied by investigating the traits and modalities of the 'abundant community' (OTUs with an abundance > 0.1% of the total read number in each season). A focus was made on the stations sampled in September because this period corresponds the strongest effects of the front on the phytoplankton community (Cadier, Gorgues, Sourisseau, *et al.*, 2017). Inspired by other biological trait analyses (Bremner *et al.*, 2006; Legendre and Legendre, 2012), we computed a co-inertia analysis (Dray et al., 2003) in between: 1/ a presence-absence table of the abundant OTUs found across the 5 stations, and 2/ a table composed of 13 traits describing these OTUs (more details can be found in Figure S19). The traits that well explained diversity patterns within the abundant community in September were further investigated.

In a second approach, we focused on all the ecological strategies of phytoplankton found across stations and seasons. To measure the number of distinct ecological strategies found in each location we calculated functional richness by following protocols described for other functional diversity analyses (Villéger *et al.*, 2008; Laliberte *et al.*, 2010; Maire *et al.*, 2015). Briefly, this method uses all the functional units in a community to build a multidimensional functional space, the species found in a sample represent a subset of this multidimensional space, and functional richness measures the volume of the subset community on the total volume of the multidimensional space (values oscillates in between 0 and 1). Functional richness is non-weighted and was computed with a built-in R function (available at villeger.sebastien.free.fr/). Here, we selected all the taxonomic references of phytoplankton OTUs found in our dataset to which 13 traits and modalities were annotated, and built a multidimensional space using the Gower distance and Principal Coordinate Analysis (PCoA). The number of PCoA dimensions necessary to represent the initial biological trait table, 3 in our study, was

chosen with rigorous statistics (Maire *et al.*, 2015). As a first step, functional richness was calculated on the presence-absence table of phytoplankton taxonomic references (284 taxonomic references, 6756 OTUs) found in each distinct sample, at this stage taxonomic references from the three size-fractions were merged. In a second step, functional richness was computed with all taxonomic references from the union of distinct replicates and depth to represent the total phytoplankton richness across seasons and stations. Differences in the distribution and the variance of functional richness were tested respectively with the Kruskall-Wallis and the Bartlett tests.

3) Results

a) Oceanographic Context

The position of the Ushant Tidal front being highly dynamic, the sampling stations need to be placed in their oceanographic context. Here, the position and effect of the front were inferred by Sea Surface Temperature (SST) estimated by satellite data (Figure 18; MODIS-Aqua Ocean Color Data, 2014) and in-situ oceanographic measures (Figure S14 & S15). Briefly, in March water was homogenous around the Iroise Sea, both vertically and horizontally (ca 9 °C), conditions for the front were not met (Figure 18 & S14). Phytoplankton biomass was low (0.5 μg/L) and nutrients high (up to 12 μM for NOx = nitrate + nitrite) indicating that sampling occurred slightly before the spring bloom (Figure S15) and that phytoplankton's growth was limited by the low light availability. Reversely, in July and September, the most offshore stations (O1 and O2) presented a thermocline (up to 18°C in surface to 12°C at depth) while the more coastal (C1 and C2) were weakly stratified (ca 15°C, Figure S14). In transition between these two water masses, the station F presented a moderate thermocline (13-16 °C). Phytoplankton biomass (Figure S15) increased throughout these two seasons, and DCM were observed and sampled in stratified stations (i.e. O1, O2 and in a lesser extent F). The maxima in phytoplankton biomass were observed at the DCM of station O2 (from a maximum of 1.5 $\mu g.L^{-1}$ in July up to 5.5 $\mu g.L^{-1}$ in September). In July, the spring bloom had already depleted nutrients

all over the zone (0 μM) except at the DCM of stratified stations and below and in coastal waters where intermediate values (2 μM) indicated the delayed/limited phytoplankton's uptake and new inputs from rivers in the coast. In September, nutrients were fully depleted everywhere in surface waters (0 μM) except at station F, where nutrients were observed up to the surface (2 μM).

In summary, the front was fully established in July and September. Because it was moderately stratified and presented nutrient inputs up to the surface in September (typical from the front), the station F was the station the closest to the front both in July and September. Differences in nutrient inputs between July and September might be explained by a stronger spring tide preceding the September campaign compared with July (Figure S13). Competition for nutrients and light thus globally increased in the Iroise Sea throughout summer, supposedly increasing competitive exclusion for phytoplankton, at the exception of station F and O2 where nutrient inputs and production were still observed.

b) Metabarcoding of the Protistan Community

Despite the high number of OTUs retrieved (33 060), rarefaction curves built on the metabarcoding dataset indicated that the diversity of protists sampled was not fully saturated (Figure S16). The increase in primary production in the Iroise Sea throughout summer (Figure S15), coincided with a decreasing in the total number of OTUs by season from March to September (respectively from 17 089 to 11 245 OTUs) with however a similar sequencing effort (from 1 to 1.2 $x10^6$ reads by season).

The total read abundance in our dataset was dominated by OTUs with well annotated taxonomy (66% at least annotated to the family level). Abundant taxa were: Dinophyta (i.e. dinoflagellates, 25% of the total read abundance), Bacillaryophyta (i.e. Diatoms, 14%), Thecofilosea (2%), Cryptophyta (2%) and Ciliophora (i.e. ciliates, 1.5%) that dominated micro- and nano-plankton; while Chlorophyta (10%) and Marine Alveolates and Stramenopiles (MALV: 5% and MAST: 2%) dominated pico-plankton. Due to cells-breakage, DNA from ciliates, diatoms or dinoflagellates, organisms with a usual cell diameter higher than 10 μm, was found across nano- and pico-plankton (Figure 19). This is a classical artefact of sequencing surveys (Massana, 2011). Among replicates, the same clades dominated

but there existed small changes in the relative abundance due to replicate corresponding to repeated rosette dives on the same geographic location. Across stratified waters in July and September, no significant difference was found between the OTU composition at surface and at the DCM (PERMANOVA, R^2: 0.03 with 9999 permutations). Across functionally annotated OTUs (66% of total read number), constitutive phototrophs were overall dominating (42% of total read number) but heterotrophs were significantly present (13%). The functionally unannotated reads (44% of total read), were composed mostly of the OTUs annotated only at the family or at a higher taxonomic level (e.g. Order, Class, Division), for which functional traits could not be generalized. Here, we detail patterns of phototrophic clades across our monitoring, the dynamic of heterotrophic protists across our samples will be further studied in a companion section.

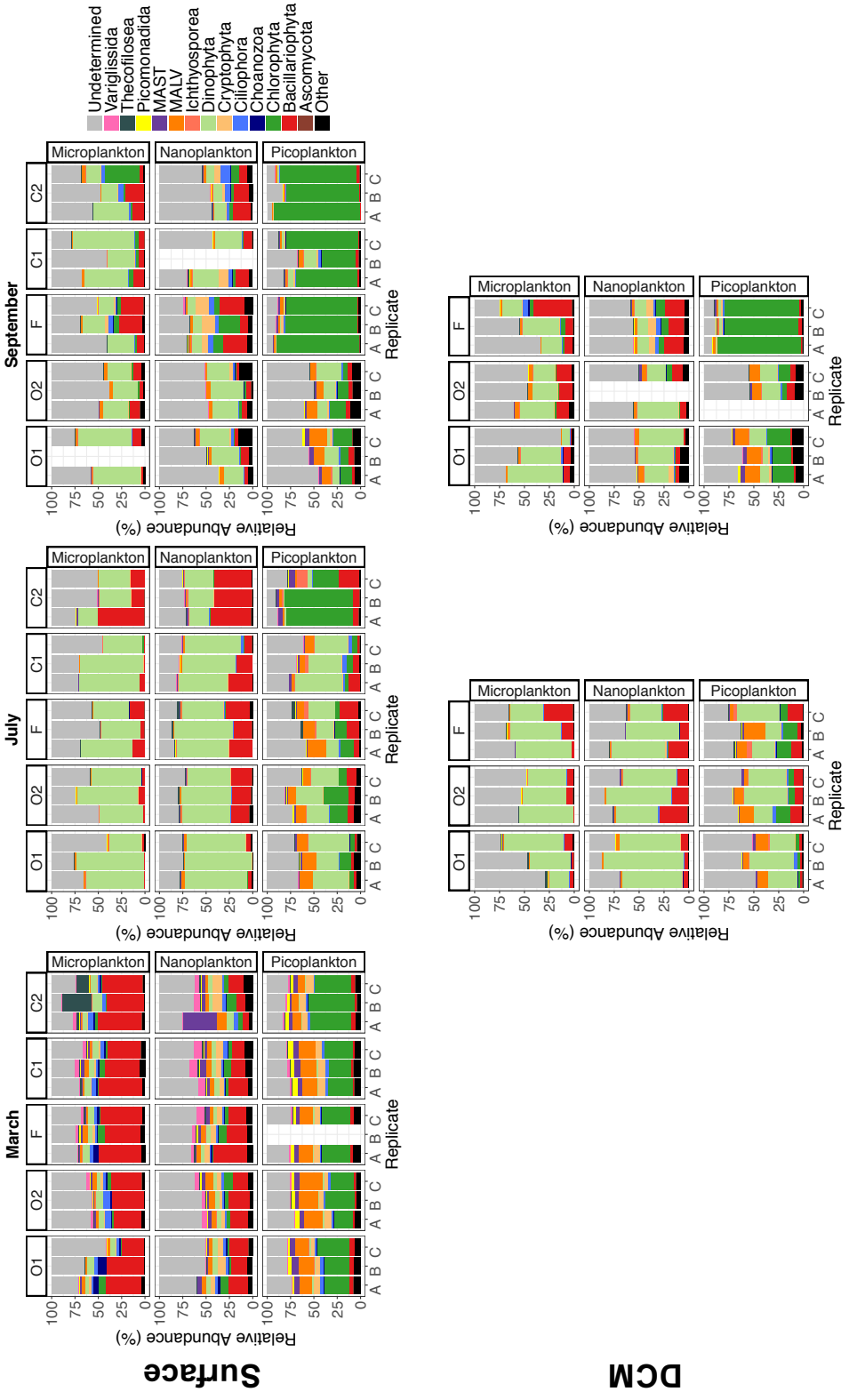

Figure 19: Distribution of the distinct protistan taxa estimated by metabarcoding in the Iroise Sea in March, July and September 2015. Samples are organized by replicates, size-fractions, sampling stations (from the open-ocean to the coast, left to right), depth and season. Relative abundance was calculated based on the number of reads of OTUs corresponding to the shown taxa, 'Other' represented the read number of taxonomic ranks with a relative abundance < 10%, 'Undetermined' represented the read number of OTUs with a low taxonomic level.

In March, phytoplankton was dominated by diatoms in the micro- and nano-plankton (respectively around 40 and 20 % by sample) while Chlorophyta dominated pico-plankton (around 30 by sample, Figure 19). The relative abundance of each phytoplankton taxa was homogenous all across the Iroise Sea.

In July, diatoms were less abundant and replaced by dinoflagellates in the higher size-fractions. The diatoms/dinoflagellates ratio particularly decreased from the coast to the offshore waters (Figure 19). Among pico-plankton, Chlorophyta still represented the most important phototrophic group, but dinoflagellates and diatoms appeared also consistently in this size-fraction. Pico-plankton was relatively homogenous except for the most coastal station (C2) that showed a strong domination of Chlorophyta.

In September, micro- and nano-plankton showed patterns in the dinoflagellates/diatoms ratio that were similar to July, with the coastal and frontal stations presenting more diatoms than the off-shore stations (Figure 19). The frontal and coastal stations (F, C1 and C2) also showed noticeable abundances of Cryptophyta in the nano-plankton. Across pico-plankton, stations were divided into two groups, one with the coastal and frontal stations (F, C1 and C2) where Chlorophyta strongly dominated and one with the offshore stations (O1 and O2) where the signal was more equilibrated between Chlorophyta, diatoms and dinoflagellates (Figure 19).

c) Phytoplankton Diversity Patterns

Phototrophic OTUs were selected and phytoplankton richness was studied with various criteria.

Across size-fractions (Figure 20a), micro-plankton samples presented the highest richness, values ranged from 970 to 65 OTUs by sample. Nano-plankton, 729 to 28 OTUs, and pico-plankton, 504 to 45 OTUs by sample, presented lower values. Along seasons, and at the same time that the average chlorophyll *a* biomass increased (Figure S15), richness declined in all size fractions (Figure 20a). However, the decline for the micro- (from a maximum of 970 in March down to 467 in July and 381 in September) was strongest than for nano- and pico-plankton (with richness maxima of 729 and 504 OTUs in March, to 529 and 270 OTUs in September, respectively for nano- and pico-plankton). Statistical tests indicated that there existed

no significant difference in phytoplankton richness in between DCM and surface samples (Kruskall-Wallis test, p-value = 0.1).

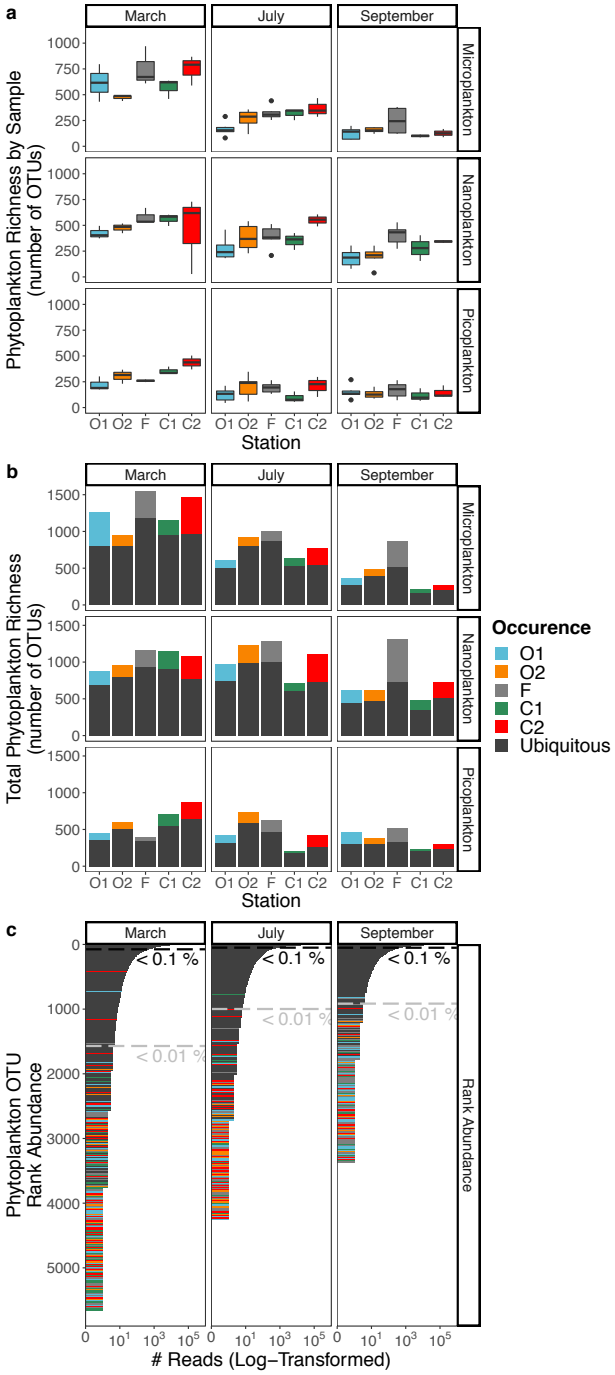

Figure 20: Eukaryotic phytoplankton OTUs richness in the Iroise Sea in March, July and September 2015. a) Phytoplankton OTUs richness was calculated in each of our 184 samples and the variation of richness is presented as boxplots across station, season and size-fraction. b) Total phytoplankton richness when cumulating the OTUs retrieved in each station, season and size-fraction. c) Phytoplankton OTU rank abundance curves in each season with abundance thresholds separating the OTUs part of the 'abundant' (> 0.1% of the total read number by season), 'low' (0.1-0.001%) and 'very low' (< 0.001 %) communities. OTUs were colored according to their occurrence in two stations of a same season ('ubiquitous', black) or in one unique station in the same season ('specific', colored).

Phytoplankton richness increased when we calculated the total phytoplankton richness across stations, seasons and size-fractions (maximum of 1544 OTUs; Figure 20b). This indicated that replicates were useful into discovering new OTUs at single location and thus in the sampling effort. Over stratified stations DCM and surface samples were also cumulated in order to calculate total phytoplankton richness. In accordance with the results of the PERMANOVA, this did not add many OTUs to stations sampled at two depth; as an example, during productive periods phytoplankton richness in the coastal stations (C1 and C2, sampled only at surface) was not statistically different that the richness of more offshore stations (O1 and O2) (Kruskall-Wallis test, p-value = 0.3; Figure 20b). Throughout the summer increase in production (Figure S15), total phytoplankton richness also declined (Figure 20b). However, across stations there existed discrepancies to this pattern. During July, when the front was strongly marked in temperature profiles (Figure S14), the stations nearest from the front (F and O2) were richer than the others (above 900, 1200, 600 OTUs in micro, nano and pico-plankton compared with values markedly under in other stations); while later during September the unique environmental configuration witnessed close to the tidal front (station F) coincided also with a higher phytoplankton richness compared to all stations (821, 1303 514 phytoplankton OTUs respectively in micro, nano and pico-plankton, compared to values markedly lower in other stations). Consequently, phytoplankton richness was significantly higher at station F compared with the other stations during productive periods (Kruskall-Wallis test, p-value = 0.019).

Phytoplankton richness within the 'abundant' community (> 0.1% of the total read number by season) followed similar patterns (Table 1). The number of abundant OTUs decreased from March (ranging in between 15 and 21 OTUs) to the productive periods in all stations (ranging in between 9 and 13 OTUs) at the exception of the stations the closest to the front, i.e. station F and O2 in July, and station F in September (with values ranging in between 18 and 21). The conditions found at the front thus helped to maintain a higher number of successful OTUs in summer. "Ubiquitous" OTUs, i.e. OTUs present in at least two stations within a same season, constituted the larger part (601 out of 603 OTUs abundant across each seasons) of the 'abundant' community (> 0.1% of the total read number) (Figure 20c). "Specific" OTUs, i.e. OTUs retrieved only at station X within a same season, were present in the 'low' community (0.1-0.001%) but dominant in the 'very low' community (<

0.001% of the total read number) (Figure 20c). This indicated logically that the most abundant species had a higher chance of dispersal and detection. In average, "specifics" OTUs represented a small relative fraction of total phytoplankton richness across all seasons (Figure 20b; 27%, 26% and 34% respectively for March, July and September), and size-fractions (23%, 24% and 23% respectively for micro, nano, and pico-plankton). In some samples, "specific" OTUs proportions were however markedly above these values. The highest proportions of "specifics" OTUs were markedly observed in September at the station F, where they represented 39 and 43% of OTUs richness respectively in the micro- and nano-plankton (Figure 20b).

Table 2: Number of abundant phytoplankton OTUs (> 0.1% of the total read number by season) by station and season, and total distinct OTUs in the abundant community by season

	O1	O2	F	C1	C2	Distinct Abundant Phytoplankton OTUs
March	18	15	17	21	20	29
July	13	18	20	9	12	23
September	11	11	21	10	12	27

Finally, the number of eukaryotic phytoplankton OTUs shared between stations across all seasons (called connectivity in the rest of the document), was investigated to highlight temporal patterns of phytoplankton diversity (Figure 21). As phytoplankton richness was variable across seasons (Figure 20b), we tested the robustness of these diversity patterns within a subdataset with a curated number of OTUs by season (Figure S17). A good fit in between the original dataset and the curated one (Spearman Rank Correlation with the original dataset = 0.99) indicated that the diversity patterns were not influenced by the heterogeneous number of OTUs retrieved in each season (Figure S17), we thus detail diversity patterns of the original dataset (Figure 21). First, stations of a same season presented a higher connectivity compared with stations across seasons (Figure 21 and Figure S18a), illustrating the high seasonal renewal in the phytoplankton community across all areas. Secondly, the connectivity between stations within a same season decreased over the year (Figure 21 and Figure S18a), highlighting the progressive separation of communities across stations throughout summer. Cross-seasonal connectivity indicated significant

patterns, 1/ in March all stations were rich and phytoplankton OTUs were widespread (high connectivity intra-season, Figure 21), 2/ in July the occurrence of these OTUs were restricted to the coastal C2 and the frontal station F (cross-season link in between March and July), indicating a strong renewal of the phytoplankton community elsewhere, 3/ in September, the OTUs maintained in July were found in a greater extent at the front (cross-seasonal link in between July and September, Figure 21), which also resulted in a tight link in between the frontal station in September and all stations in March. This indicates that there existed a strong influence of migration on the peak of phytoplankton richness found at the front during our monitoring, but also that in September this station was the sole where OTUs from March did not become extinct. As a consequence, the frontal station shared more OTUs cross-seasonally than other stations, however the frontal station also shared a higher number of OTUs within seasons (Figure 21 and S18b).

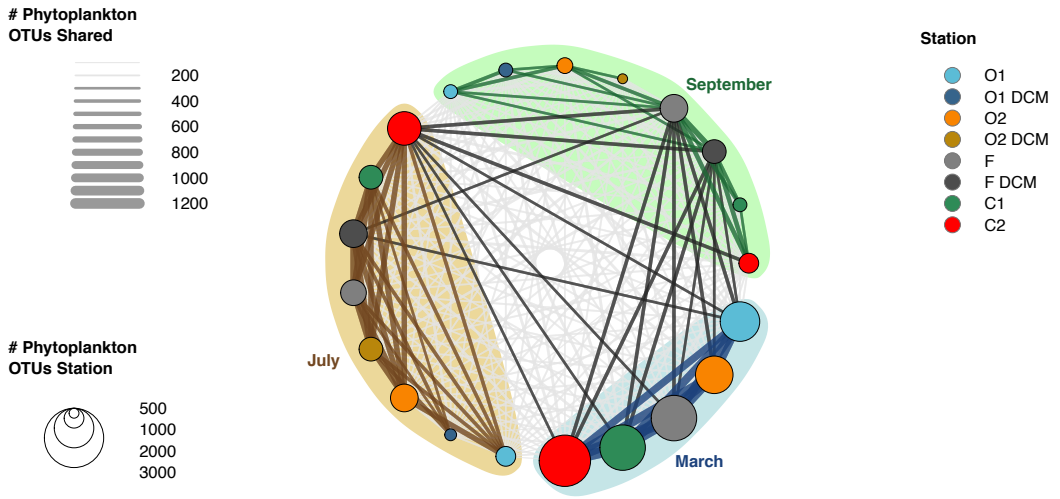

Figure 21 Connectivity network of the number of eukaryotic phytoplankton OTUs shared in the Iroise Sea in March, July and September 2015 across our 5 stations and depth (at surface and DCM). Node size represents the number of OTUs in each station (see node color) of each season; link size represents the number of OTUs shared between stations; link color represents: low connectivity (light grey in the background, < 300 OTUs shared), intra-seasonal (colored) or cross-seasonal (black) seasonal.

d) Functional Diversity

To present the ecological strategies of phytoplankton favored in vicinity of the front, a focus was made on the 'abundant' community of September when the front and gradients in community were fully established. Co-inertia analyses indicated that the OTUs present in the stations of September were well discriminated by the following traits: cell size, coloniality, cell cover, ingestion method, cell symmetry and

symbiosis type. At station F, the abundant community was constituted of a larger number of OTUs (Table 2), but also of a larger number of ecological strategies (Figure 22). The front allowed the maintaining in high abundance of three diatoms OTUs corresponding to *Thalassiosira sp*., *Skeletonema sp*. and *Leptocylindrus danicus*, that accounted for a higher proportion of colonial, siliceous, radial and large strategies (Figure 22). *Skeletonema sp*. and *Leptocylindrus danicus* were notably found only at station F. Also present at station F were four dinoflagellates, *Alexandrium sp*., *Gyrodinium impudicum*, *Amphidoma languida* and *Karenia sp*., that added-up to 1 colonial, 2 phagotrophic (mixotrophic), and 4 asymmetrical strategies (Figure 22). Only *Alexandrium sp*. was specific to the front, *Gyrodinium impudicum* was only found on the coastal side of the front (C1) while *Amphidoma sp* was only found on the offshore side (O1 and O2), altogether this highlights the mixing at the front of strategies competitive on either side of the front. Members of Chlorophyta and Cryptophyta OTUs were common to all stations but did not represented a particular strategy. In other stations, siliceous Dictyochales and an OTU associated to the endosymbiont of *Amphisolenia bidentata* were found abundant only on the most offshore sites (O1 and O2), while *Lepidodinium sp*. and *Scrippsiela sp*. were found at the coast, respectively only at station C1 and C2 (Figure 22). More information on these species can be found on our trait table (http://doi.org/10.17882/51662).

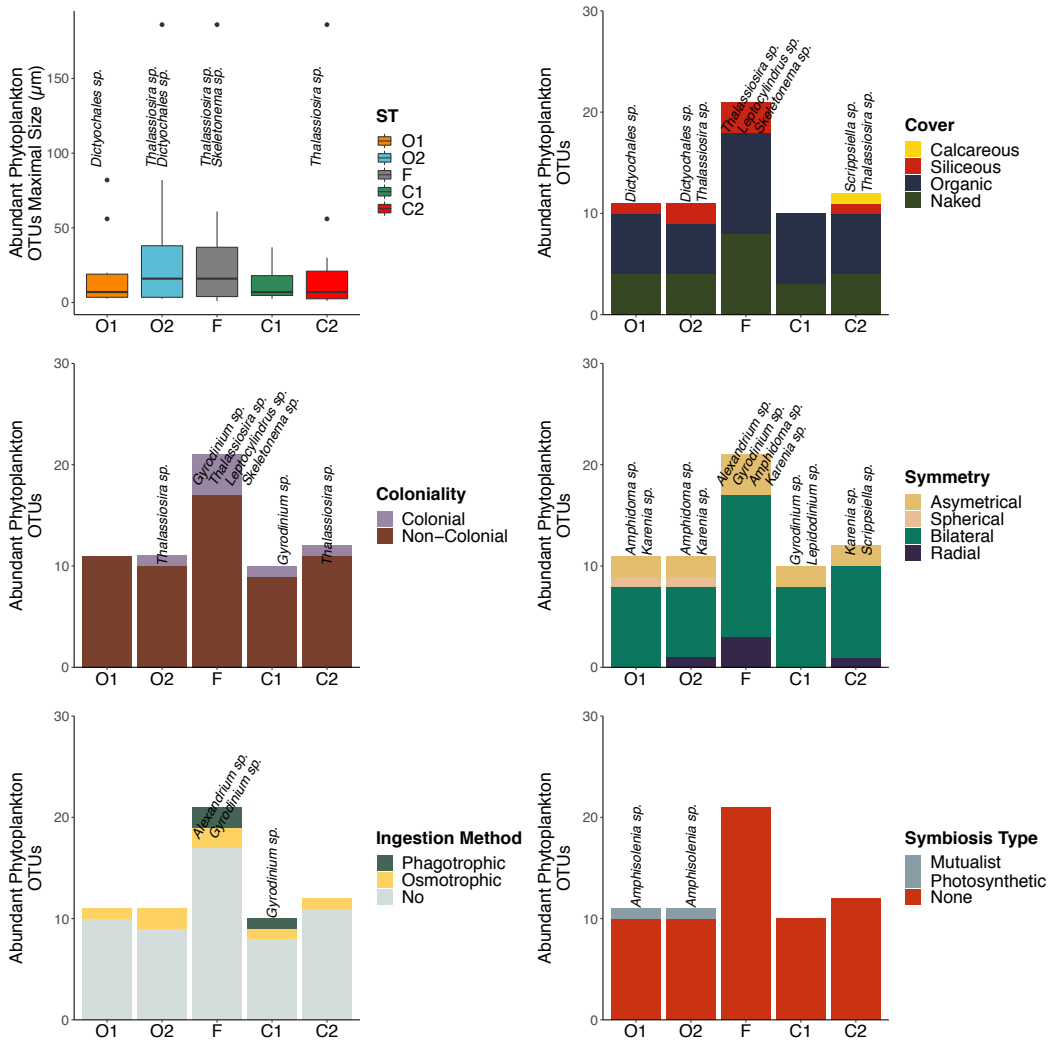

Figure 22: Traits and modalities of the phytoplankton OTUs part of the "abundant' community in September (> 0.1% of the total read number in September) across each sampling station. Relevant traits were selected with a co-inertia analysis detailed in the supplementary material. The taxonomic reference of OTUs with a particular modality in each trait were written above the modality. Traits for each OTUs were gathered from literature, an overview of this work can be found at http://doi.org/10.17882/51662.

Functional richness was calculated for each 63 sample (mixing the three size-fractions), using the taxonomic references of all phytoplankton OTUs and 13 traits (Figure 23). Functional richness of phytoplankton was significantly lower in March (between 0.68-0.81; Kruskall-Wallis test p-value < 0.05; Figure 23), when phytoplankton's growth was light limited (Figure S15). Values in the two productive periods (July and September), were higher in average and ranged between 0.55 and 0.96 (Figure 23). In July, functional richness was the highest at the three stations O1, O2 and F (> 0.8 compared to < 0.8 at the coast). In September, the same stations (O1, O2 and F) showed the highest values (> 0.8), however at station F, functional

richness was constantly higher than 0.8 while there existed variability within the replicates of O1 and O2. In September, no significant differences in values of functional richness were observed between station F and the others (Kruskall-Wallis test p-value = 0.3) but values were significantly less variable (Bartlett test, p-value > 0.001), implying that station F was the only one to maintain high functional richness across replicates. When calculating total functional richness by station and season, similar patterns were observed with the offshore and frontal stations presenting the highest values (O1, O2 and F > 0.95) while the coastal showed markedly lower values (between 0.8 and 0.85) (Figure 23). These patterns indicated that waters on the offshore side of the front presented more distinct ecological strategies than at the coast.

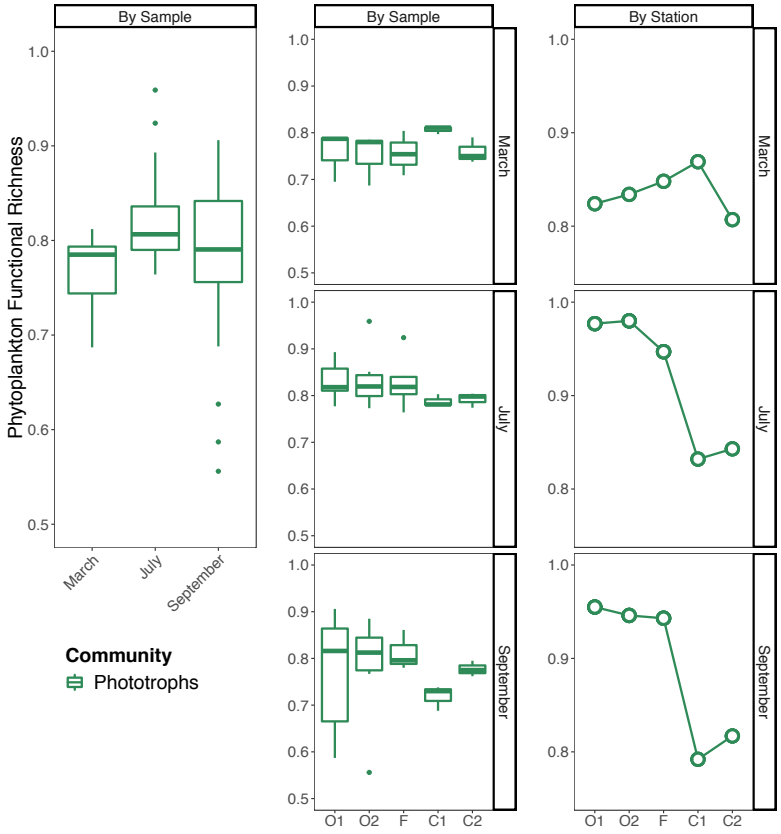

Figure 23: Functional richness of eukaryotic phytoplankton across seasons (boxplots values of 184 distinct samples, at the left) and sampling stations, when calculated by sample (184 distinct samples, in the middle) and when cumulating the total functional richness by station and season (45 distinct sample, at the right). In the 'By sample' analyses, functional richness was calculated for each replicate of each depth and station but merging all size-fractions, to present their variation, these results were presented as boxplots. In the 'By Station' analysis we calculated a single value of functional richness for the total number of OTUs found in each station and season (thus represented by a single value). Functional richness was calculated on the basis of 13 biological traits describing the OTUs in our dataset (see doi.org/10.17882/51662) and following a protocol detailed in (Maire et al., 2015).

4) Discussion

Patterns of phytoplankton diversity across a tidal front were investigated from several perspectives. Classically, inputs of nutrients and primary production were found in the vicinity of the front in September while other stations were markedly depleted. With a metabarcoding approach 1/ we detailed patterns of phytoplankton taxa across our monitoring and 2/ we demonstrated that the front corresponded to a peak in eukaryotic phytoplankton richness. A higher proportion of 'ubiquitous' OTUs was found at the front in September indicating the influence of migration on this phytoplankton diversity hotspot. There existed also a higher proportion of OTUs specific to the frontal station in September, this and connectivity patterns indicated that the front also helped to maintain locally a diversity of phytoplankton OTUs that were excluded by competition in other area. Finally, with a functional approach we detailed the most successful ecological strategies of phytoplankton across the front and explained patterns of functional richness.

a) Phytoplankton community composition

The Iroise Sea represented a patchwork of environments associated with various conditions and limitations for phytoplankton's growth. The prominent factors highlighted in our study were nutrients and light availability. In the ocean, the early production associated with the increase of light, elevated turbulence and high nutrients concentrations are dominated by diatoms (Margalef, 1978; Reynolds C.S., 2003). Diatoms are indeed the best competitors under high nutrient concentrations (Litchman *et al*., 2007). However, under lower nutrient concentration and water mixing, usually coinciding with summer conditions, diatoms lack the adaptations developed by dinoflagellates that ultimately prevail in these environments (e.g. swimming to persist in the euphotic zone when mixing is low and/or mixotrophic capabilities to compensate for low nutrient concentration) (Margalef, 1978; Thingstad, 1998; Litchman *et al*., 2007). Under light limitations, e.g. in winter or in deep DCM, the growth of either diatoms or dinoflagellates is strongly lowered, and smaller pico-phytoplankton is usually favored (Uitz *et al*., 2008; Marañón, 2015). Furthermore, the small cell size of pico-phytoplankton increases their surface per

volume ratio, favoring the uptake of pico-size phytoplankton in oligotrophic environments (Raven, 1998).

The metabarcoding approach developed in our study shows results in agreement with this general framework (Figure 19) and previous observations in the Iroise sea (Le Fèvre and Grall, 1970; Le Corre *et al*., 1993). Winter/early-spring conditions in March represented enriched and mixed waters where light only started to be more available, these waters were logically dominated by diatoms probably announcing the future spring bloom. The following summer conditions represented depleted and stratified waters, and were thus dominated by dinoflagellates. However, in summer, coastal and especially frontal waters (NOx ~ 2 μM) were markedly less depleted and stratified than open-ocean waters and these conditions helped to maintain a diatom community mixed with dinoflagellates. Chlorophytes dominated pico-phytoplankton all over the Iroise Sea in March, when light and nutrients limited phytoplankton's growth. Chlorophytes have indeed low optimal irradiance (e.g. for *Micromonas spp*. in McKie-Krisberg and Sanders, 2014), and can be better competitors than larger cells (e.g. diatoms or dinoflagellates) under light and nutrient limited environments (Marañón, 2015). This is also confirmed by the domination of chlorophytes in coastal area in summer, indeed in coastal ecosystems higher light limitations can occur due to higher vertical mixing and turbidity (Cloern, 1987; Castaing *et al.*, 1999; Schultes *et al.*, 2013), in addition to the strong nutrient depletion we observed (NOx ~ 0 µM, Figure S15). At the DCM of the most offshore sites which is another light-limited environment, Chlorophytes relative abundance was however low. This could be explained by the competition with *Synechococcus* (Worden *et al.*, 2004), a prokaryote favored under more oligotrophic environments and precluded by our approach.

Our DNA marker (V4 18S-rDNA; as in Stoeck *et al*., 2010) is known for overlooking Haptophytes (Bittner *et al*., 2013; Egge *et al*., 2015), however these organisms are usually found on more off-shore waters (Massana, 2011) and at low abundance in coastal ecosystems. Also, other markers specific to phytoplankton could have been used (Decelle *et al*., 2015), but our approach will be further used to investigate patterns of heterotrophic protists in a companion paper. However, these biases did not prevent the observation of classical patterns of eukaryotic phytoplankton driven by environmental conditions (Margalef, 1978; Marañón,

2015), that are often reported in the Iroise Sea (Le Fèvre and Grall, 1970; Landeira *et al*., 2014; Cadier, Sourisseau, *et al*., 2017).

b) Phytoplankton diversity and environmental drivers

Sub-mesoscales physics are affecting the environmental conditions and rate of resource supply involved in phytoplankton growth (Sharples *et al*., 2009; Mahadevan, 2016). Fluctuations in the environment have also been shown to influence phytoplankton diversity, through mixing, advection and modulation of exclusive competition processes (Huisman, 2010; Clayton *et al*., 2013; Vallina *et al*., 2014). These processes have rarely been tackled from the metabarcoding perspective (Villar *et al*., 2015) and even less at the submesoscale, while paradoxically this approach constitutes the new template for plankton diversity analyses (de Vargas *et al*., 2015; Chust *et al*., 2017). There are still biases inherent to this approach, notably in waters increasingly rich in particles where water filtering is being reduced by clogging despite the differential filtration. To compensate for this bias, we replicated our samples and this process allowed us indeed to retrieve more OTUs in productive periods (Figure 20a and 20b). A second bias comes from PCR, by multiplying sequences exponentially (Saiki *et al*., 1988), PCR increases the gap in abundance between abundant and rare species, and this gap is maintained after DNA sequencing (despite similar sequencing depth, Figure S16). Overall these co-factors tend to diminish OTUs diversity in productive ecosystems and we found indeed a lower OTUs diversity during the productive seasons (July and September) in comparison with our sampling in March (Figure S16). In order to tackle the subject of phytoplankton richness we thus tested whether if the patterns of phytoplankton diversity we observed were robust in a dataset with a curated number of OTUs by season (Figure S17). Given the robustness observed, our approach and results were comforted. We thus detail patterns of phytoplankton diversity at the submesoscale, for the first-time with a metabarcoding approach.

In summer, the environmental conditions of the Iroise Sea highlighted the presence of the Ushant tidal front separating two water-masses that developed phytoplankton communities with distinct ecological strategies. In summer, by distinct mechanisms, competition for resources availability was higher in both coastal and open waters (Figure S15), resulting in lower phytoplankton diversity

(Figure 20). In contrast, waters of the front maintained a higher taxonomic diversity of phytoplankton throughout summer (Figure 20 and 21), and especially in September when the front is usually the most pronounced.

In agreement with previous assumptions on the Iroise Sea (Cadier, Sourisseau, *et al*., 2017), higher proportions of "ubiquitous" OTUs were found at the front (Figure 20b), indicating the effect of an ecotone in between the coastal and the offshore area. Connectivity patterns further highlighted the effect of OTUs migration with, in September, a high number of OTUs shared between the frontal maxima and the coastal area in July and over all the stations in March (Figure 21). The impact of migration due to dispersal on marine phytoplankton diversity is frequently observed (Chust *et al*., 2013), but ecotones at the boundary of distinct water masses have seldom been reported for marine phytoplankton (but see e.g. Ribalet *et al*., 2010). The effects of migration by large water currents have also been reported at the larger scale of oceanic fronts, with long life duration and slow advection (Villar *et al*., 2015). However, in a costal tidal front, organisms are subject to strong advection (see the high connectivity intra-vs inter- season, Figure 21). Thus, in order to be detectable at the front over time, a species should have immigrated 1) recently, and in detectable proportions, or 2) earlier, and been maintained locally in detectable proportions with favorable growth conditions. Both these processes were evidenced in September, as the frontal station showed a higher connectivity 1) within seasons (recent immigration) but also 2) cross-seasonally (earlier maintained immigration) (Figure 21 & S18b). The mechanisms helping to maintain previous immigrant were probably the nutrients inputs that were observed up to the surface at the frontal station in September (Figure S15). In July, the water mass at the front was just starting to undergo a spring tide (Figure S13), this might explain that nutrients were still scarce but also that phytoplankton OTUs were less detectable, because less growing. In addition, phytoplankton diversity markedly decreased in the depleted areas surrounding the front (Figure 20 and 21), this highlighted that competitive exclusion had already settled in but that the water conditions found at the front cyclically decreased the timescale of competitive exclusion.

These processes influenced markedly more the higher size-fractions (Figure 20b). In the pico-plankton, phytoplankton taxonomic richness was lower (Figure 20), this result was in agreement with a previous observation of a lower dominance of phototrophic protists in the pico-plankton (see Chapter I). The lower fluctuations in

the taxonomic diversity of pico-plankton could further be explained by the better adaptability of these small organisms to resource limited environments (Raven, 1998; Marañón, 2015). This adaptability probably makes small organisms more suited to competitive environments, while competitive exclusion would have a stronger impact on the taxonomic diversity of the larger size-fractions.

Other methods also stressed the distinct influence of the submesoscale on the vertical gradient of phytoplankton diversity (Huisman *et al*., 2006; Cadier, Sourisseau, *et al*., 2017), however probably due to the high sampling depth of metabarcoding we found no significant structuration of phytoplankton diversity along depth (Figure 20, PERMANOVA). Fluctuation of the relative abundance of phototrophic protists along depth was however reported by other genetic-based investigations (Cabello *et al*., 2016; Dos Santos *et al*., 2017), the fluctuations observed were found in few meters, a depth resolution that could not be investigated in our survey.

In brief conclusion to this section, we found a hotspot of eukaryotic phytoplankton diversity at the Ushant tidal front. At the submesoscale and in a coastal ecosystem, we evidenced the influence of process typically influencing phytoplankton patterns at the oceanic scale, most notably 1/ the presence of an ecotone (Ribalet *et al*., 2010), fueled by dispersal and migration (Barton *et al*., 2010; Chust *et al*., 2013), and 2/ a decrease in the timescale of competitive exclusion (Clayton *et al*., 2013). In addition, variability in the front cycle (Sharples *et al*., 2007; Maguer *et al*., 2015; Cadier, Gorgues, LHelguen, *et al*., 2017) might also induce turnovers in the identity of the dominant ecological strategy, preventing the domination of only few species (Reynolds *et al*., 1993; Huisman, 2010). We propose to study the ecological strategies found at the front in September by coupling metabarcoding and a functional approach.

c) Phytoplankton ecological strategies and environmental drivers

Higher abundance of dominant OTUs (part of ‘abundant’ community) were found at the front in September, indicating that the repeated cycles of various growth

conditions acted in a timescale that allowed the survival of OTUs with potentially multiple ecological strategies (Reynolds *et al*., 1993).

In September, the dominant ecological strategies specific to the front; i.e. the diatoms *Skeletonema sp*. and *Leptocylindrus danicus*; presented a colonial behavior and passive motility (Figure 22). This is realistic in regard to the enriched and turbulent waters in the front. The ability to form colonies that are easily advected to the surface (Margalef, 1978; Pahlow *et al*., 1997), combined with the better competitive abilities of diatoms within high nutrient conditions (Litchman *et al*., 2007), seems indeed to provide an advantageous strategy. However, under less turbulent and deplete conditions, e.g. during neap-tides at the front, chain-forming diatoms are markedly less competitive (Landeira *et al*., 2014) and are supposedly replaced by a more competitive strategy (Huisman, 2010). In our dataset, such strategy could be represented by presence of the dinoflagellate OTUs *Gyrodinium spp*., *Alexandrium sp*., *Karenia sp*., or the species *Amphidoma languida* all found at the front and in more depleted areas (Figure 22). As mentioned earlier, dinoflagellates are indeed more competitive in stratified and depleted conditions (Margalef, 1978; Thingstad *et al*., 2005), notably due to their motility and mixotrophic capabilities, that were indeed evidenced by our trait approach (Figure 22). In accordance with our previous observations, there existed ecological strategies more successful in the coastal area or the offshore area, and the front presented a local mix of these organisms but also organisms maintained only locally which accounted for a higher number of successful strategies. When studying all ecological strategies (successful and less successful OTUs) with functional richness, the front displayed high values in a consistent manner across replicates (Figure 23), indicating the presence of nearly all possible strategies found in our monitoring. These phenomena acting in the vicinity of the front recall the Intermediate Disturbance Hypothesis (Connell, 1978). The hypothesis states that fluctuations in the environment maintains species coexistence by changes in the identity of the most competitive species at a higher rate than competitive exclusion (Huisman, 2010). However, it has been brought forward that fluctuations do not prevent competitive exclusion to happen on a larger timescale (Fox, 2013). Still, in an annual cycle and in snapshot observations like ours, functional and taxonomic diversity at a certain location can conceivably be higher. Later in the Iroise Sea, competitive exclusion might increase when conditions for the tidal front formation slowly decline in

autumn/winter and light becomes limiting. In these conditions only pico-phytoplankton is supposed to persist (Cadier, Gorgues, Sourisseau, *et al*., 2017). However, species with other ecological strategies could avoid competition by forming resting stages and bloom the following year (Lebret *et al*., 2012; Figueroa *et al*., 2018), this strategy was however not evidenced in our study.

Interestingly, there existed a decoupling in between the taxonomic and functional diversity of phytoplankton in the Iroise Sea. Indeed, during July and September, the taxonomic richness of the coastal and open waters showed similar values in taxonomic richness (Figure 20) but the most offshore area was markedly richer in functional diversity (Figure 23). Taxonomic and functional diversity are not necessarily coupled in the environment (Mouillot *et al*., 2013), and this indicated that there existed more functional redundancy in the most coastal area than in the open waters. In the light of the current debate on the functional redundancy of marine microbes (Louca *et al*., 2016; Galand *et al*., 2018), it seems thus relevant to compare coastal and oceanic systems. According to their higher functional richness, the offshore waters presented additional phytoplankton strategies. When studying the most successful strategies (Figure 22), open waters presented notably the trait of symbiosis, with the presence of a Pelagophyte endosymbiont of *Amphisolenia bidentata* (Daugbjerg *et al*., 2013). However, the extent to which symbioses that involve two protists might be more abundant in the open-ocean than in coastal ecosystems remains yet unknown.

5) Conclusion

In the Iroise Sea, we evidenced that submesoscale physics helped to shape a hotspot of phytoplankton diversity in the vicinity of the Ushant tidal front. If physical processes were involved in higher resource availability, the ecological processes that structured the maximum of phytoplankton diversity also involved dispersal at an ecotone and a decrease in the timescale of competitive exclusion. By studying the functional diversity of organisms across the front, we also evidenced that the front helped to maintain a higher diversity of organisms with distinct ecological strategies, supposing the influence of intermediate disturbances preventing the domination of a single ecological strategy. The influence of submesoscale physics on phytoplankton needs to be more acknowledged in the future. For example, it is well known that the primary production at fronts supports large food-web and fish production (Le Fèvre, 1986; Sharples *et al*., 2009). In addition, it is highly probable that similar processes influence the functioning of the mixed layer pump (Thomas *et al*., 2004; Dall'Olmo *et al*., 2016), which increases the oceanic storage of carbon over continental shelves. Following the co-evolution in between submesoscale processes and the organisms that depend on it remains a necessary challenge, especially in the face of the global change imposed by human activities.

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6) Supplementary Material

Environmental configuration

To characterize the water masses sampled in this study, few distinct environmental variables were used and are presented here.

Due to its strong influence on the formation and effects of the front, we first studied the sping-neap tide cycle throughout 2015. The cycle was estimated by the daily maximal water height measured in the port of Le Conquet (France, Brittany, 48°21'33"N - 4°46'51"O), located at the mouth of the Bay of Brest (Figure S13). In March, our sampling was located in between a local peak and a minimum in Maximal water height (Figure S13), indicating that the sampling occurred in a transition period between spring and neap tides. In July, our sampling occurred after a neap tide and at the very beginning of the next spring tide (Figure S13). The previous spring tide was markedly weak. Finally, in September our sampling occurred very close to a neap tide, and was preceded by a strong spring tide (Figure S13).

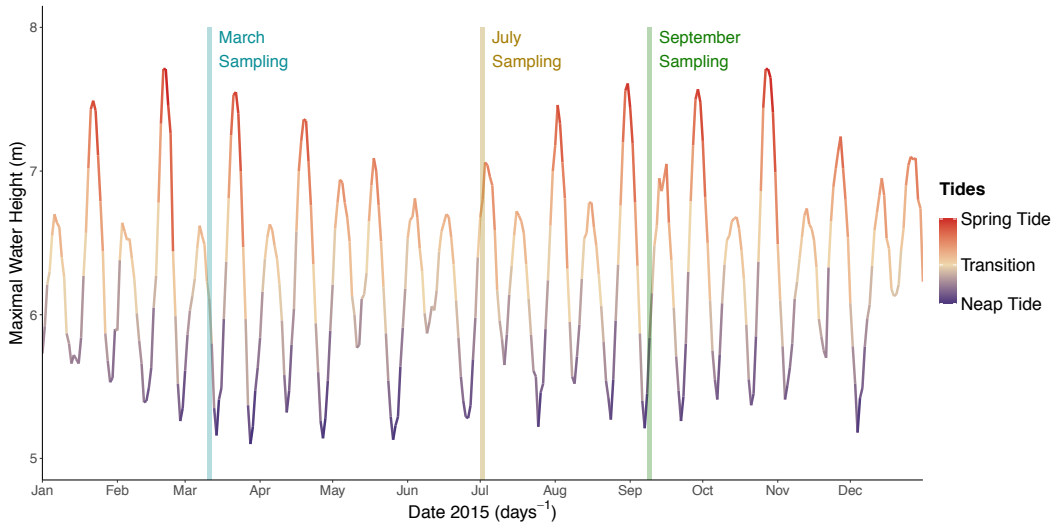

Figure S 13 : Daily Maximal Water Height (m) at Le Conquet (France, Britanny, 48°21'33"N - 4°46'51"O) in 2015 and dates of our monitoring in the Iroise Sea. Data were acquired at the SHOM website (Service hydrographique et océanographique de la Marine, maree.shom.fr).

The hydrological conditions found in each of our station across our three sampling campaigns were also studied (Figure S14 and S15). Vertical profiles of Temperature (°C) and Fluorescence indicated significant patterns (Figure S14). In

March, temperature was homogenous around the Iroise Sea (11°C), both horizontally and vertically. Fluorescence was low indicating low biomasses of phytoplankton (ca 0 μg/L) (Figure S14). The Ushant tidal front was not established during this campaign. In July, Temperature distinguished two water masses: a) the offshore stations O1 and O2 where temperature was stratified along depth (from 18°C in surface to 12°C in depth) and b) the coastal station C1 and C2 where stratification was much weaker (in surface, respectively from 17 to 15°C, and at the bottom, from 14.5 to 13) (Figure S14). Station F appeared in transition between these two water masses (from 16 to 13°C) and was supposedly the closest from the Ushant tidal front. Fluorescence profiles indicated that the phytoplankton biomass was found mostly in Deep Chlorophyll Maxima and surface at stations O1, O2 (up to 5 μg/L) and in a smaller proportion at station F (2μg/L) (Figure S14). In September, temperature profiles distinguished two water masses: a) offshore stations O1 and O2 where temperature was again stratified (from 16°C in surface to 12°C in depth) and b) the coastal station C1 and C2 where stratification was weaker (around 15°C) (Figure S14). Station F was in transition between these two water masses (from 15 to 13°C) and again supposedly the closest from the Ushant tidal front. Fluorescence profile indicated that the phytoplankton biomass was found mostly in Deep Chlorophyll Maxima and surface at stations O1, O2 (up to 8 μg/L in O2 and in average 3 μg/L in O1) and in a smaller proportion F (2μg/L) (Figure S14).

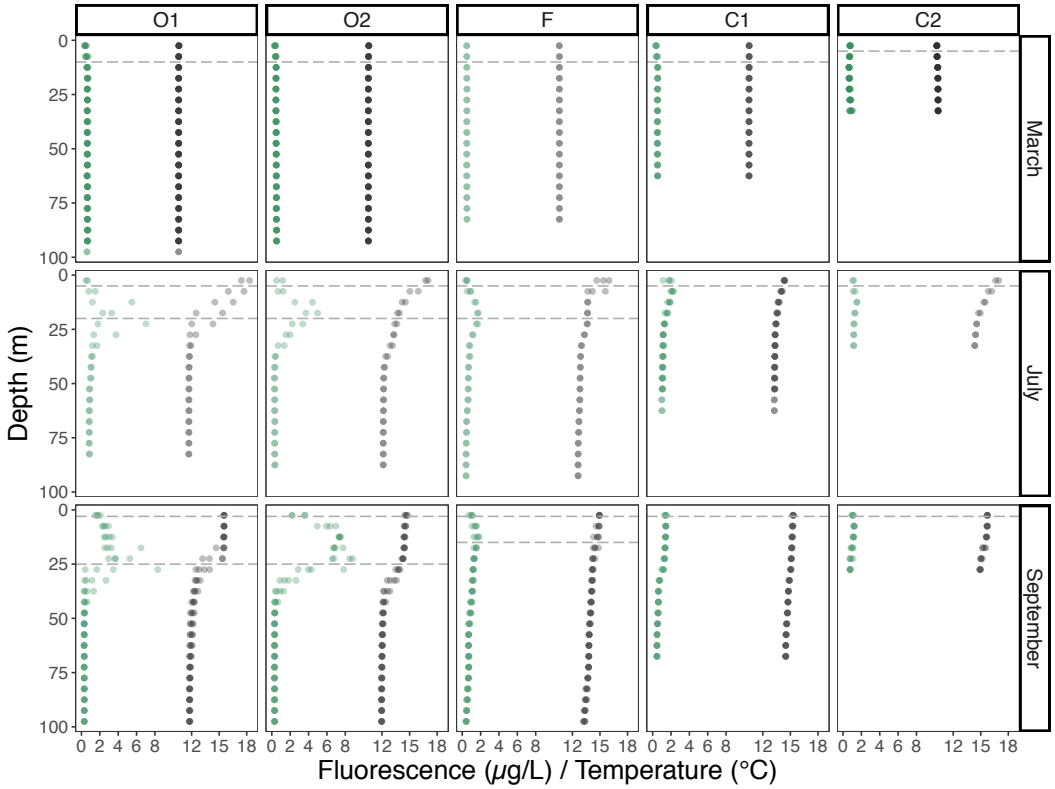

Figure S 14: Vertical profiles of fluorescence (green, µg/L) and temperature (black, °C) across the five stations (top frame) and three sampling campaigns (right frame) in 2015 within the Iroise Sea. The five stations correspond to a gradient of offshore (at the left) and coastal (to the right) locations. Fluorescence and temperature were measured with a fluorescence sensor and a CTD probe. Values of the down-cast were averaged every 5 meters. The repeated profiles correspond to the triplicate dive used for community sampling. Dashed horizontal lines present the depth of sampling for surface and, when present, DCM sampling.

Finally, we investigated the chlorophyll *a* and NOx (nitrate + nitrite) concentrations within our surface and DCM samples (Figure S15). No phytoplankton biomass was observed in March (ca 0 μg/L) and nutrients were repleted (up to 12 μM) (Figure S15). Primary production was probably still limited by light availability in this season. In July, phytoplankton biomass was higher than in March (from 0.5 to 1.5 μg/L), the maximum was observed at the DCM of station O2. NOx were generally depleted (0 μM) at the exception of moderate values in surface at C1 and C2 and at the DCM in O1 and F (ca 2μM) (Figure S15). The spring-bloom had globally depleted the nutrients in the Iroise Sea, supposedly this increased competitive exclusion for the phytoplankton community. The remaining NOx concentrations could highlight 1/ the ongoing nature of phytoplankton uptake or 2/ local light limitations to phytoplankton's uptake. Frontal conditions do not appear to increase nutrients in surface at station F. In September, phytoplankton biomass was

even higher than in July (from 0.4 μg/L up to 5.5 μg/L) (Figure S15). In this period, NOx were totally depleted (0 μM) except at the station F where inputs were observed up to the surface (2 μM). Accordingly, moderate chlorophyll*a* concentrations were observed in this station (2 μg/L), however the highest phytoplankton biomass was observed in the neighbor station O2 (5.5 μg/L). Supposedly, nutrient limitations thus occurred everywhere in the Iroise Sea except at the front where inputs were observed and where they maintained a moderate production. Phytoplankton biomass was also found in the offshore stratified side of the front (O2), this production could be issued of advected production from the front or by a local production favored by earlier nutrient inputs from the front and better light availability on this region.

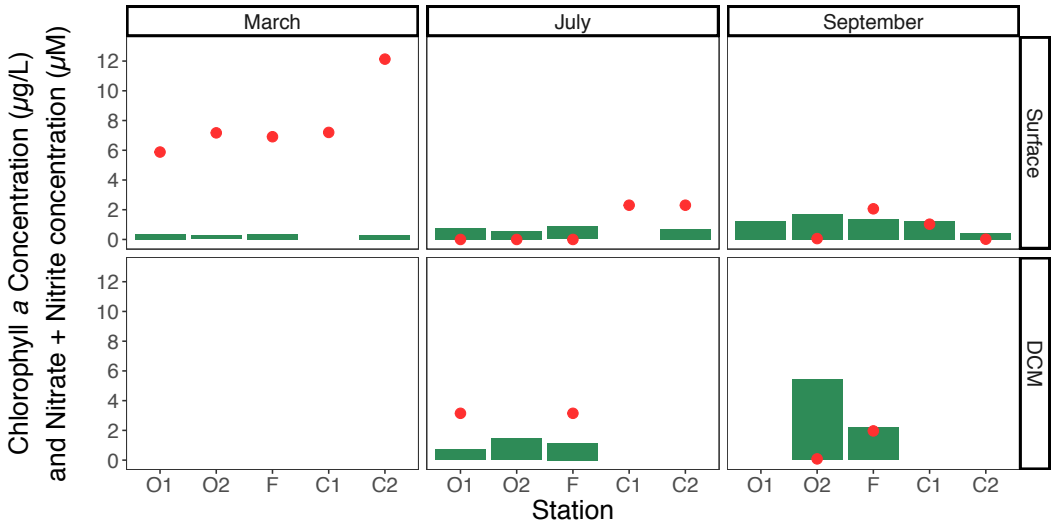

Figure S 15 : Chlorophyll a (green, µg/L) and NOx = Nitrate + Nitrite concentrations (red, µM) across five stations, two depth (Surface and DCM when observed in the vertical profiles, see Figure S1) and three sampling campaigns within the Iroise Sea. Chlorophyll a was analysed by High-Performance Liquid Chromatography (HPLC) and NOx with a Seal Analytical AA3 HR automatic analyser. The five stations correspond to a gradient of offshore (at the left) and coastal (to the right) locations. Chlorophyll a values are slightly lower than those approximated by fluorescence (see Figure S1). There existed missing values for both NOx and Chlorophyll measures.

In brief summary to this supplementary work, conditions for the front were found only in July and September, the station F appeared as the closest to the front. Accordingly, higher primary production was found in July and September, most notably at the frontal station and in the neighbor off-shore station O2. Nutrient inputs were observed up to the surface in September but not in July, this difference could be due to a weaker spring tide before the sampling of July.

Phytoplankton Biodiversity analysis

The next step was to investigate the protistan community found in these samples. This was carried by metabarcoding with a sequencing of environmental DNA, and to test if more samples would have brought more distinct OTUs we computed rarefaction curves (Figure S16). Rarefaction curves did not show an asymptote implying that biodiversity was not saturated during our campaigns. More sampling effort should increase the number of OTUs retrieved. Another relevant result is that the March campaign yielded more OTUs compared to July and September (17 000 compared to 12 and 10 000) despite a similar sequencing depth (between 1 and 1.2 x106 reads). This could be due to the higher phytoplankton biomass in the water masses of July and September (Figure S15), which would favor filter clogging.

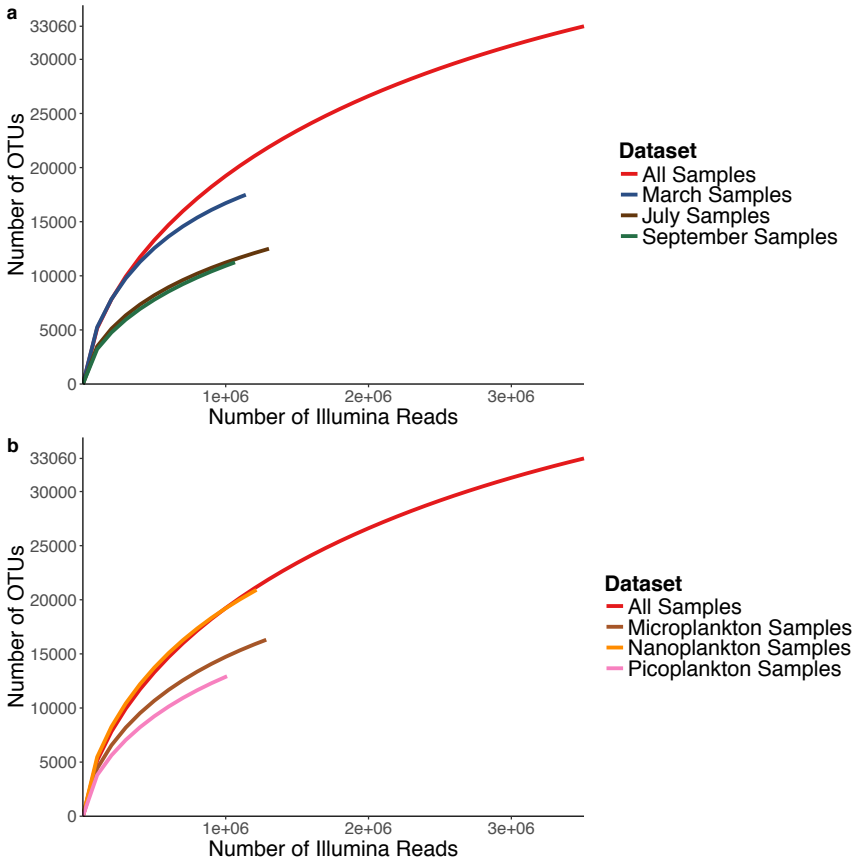

Figure S 16: Rarefaction curves constructed cumulating the samples of our monitoring in the Iroise Sea by size fraction independently and season sampled. The sequencing depth is represented by the number of reads in relation to the species richness as the number OTUs. The function [rarecurve() function of "vegan" (Osaksen et al., 2016)] samples an increasing number of reads with a rate of 100 000 reads/sample and without replacement. Rarefaction curves we constructing all samples presented in our paper, a) by sampling campaign and b) by size fractions.

Due to the supposed lower sampling effort in the most productive periods, we investigated if the phytoplankton diversity patterns presented by our dataset would be similar in a dataset with a curated number of OTUs by season (Figure S17). As a first step, we selected all the phototrophic protists OTUs in our dataset, here called phytoplankton, and sorted these OTUs according to their abundance within each season (Figure S17a). We selected a threshold of 3377 OTUs (corresponding to the total number of phytoplankton OTUs retrieved in September, the less rich season) and kept the first 3377 most abundant OTUs in each season. The effect that this removal had on the initial biodiversity patterns of the original dataset was investigated (at the right, Figure S17a). To do so we studied the pairwise correlations between the distance matrix (Bray-Curtis) of the original dataset and the distance matrix of the reduced dataset (Bray-Curtis) by both the Mantel test and the Spearman rank correlation (as in Gobet *et al.*, 2010). The threshold selected (3377 final OTUs) represented distinct proportion of OTUs removed in each dataset (March: 40%, July: 21% and September: 0%), but did not impact the diversity patterns of the original dataset (Mantel and Spearman r coefficient > 0.9, Figure S17a).

As a second step, we built a connectivity network of phytoplankton OTUs across our dataset (by station, depth and season) with the same methodology as developed in Figure 21 (Figure S17b). Most importantly we compared the connectivity matrix of the original dataset and the normalized one (Figure S17c), more precisely we compared the number of phytoplankton OTUs shared in each pairwise association between station (links between nodes in Figure S17b). In Figure 17c, the points represent the number of OTUs by link in both the original and the normalized datasets. The good correlation (Spearman rank correlation = 0.99) indicated that connectivity patterns were robust in the original dataset despite a distinct number of OTUs by season.

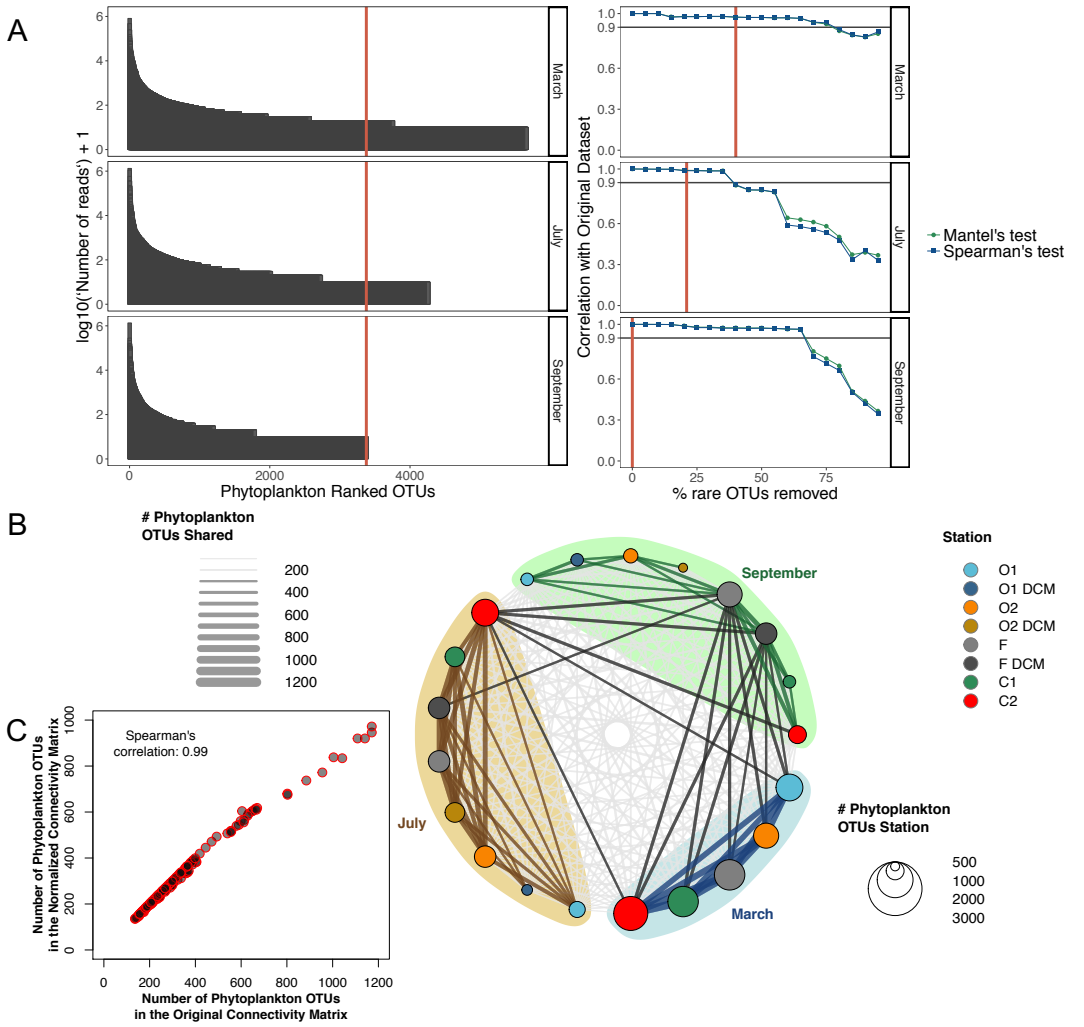

Figure S 17: Test of the robustness of the phytoplankton diversity patterns observed in the Iroise Sea. A) Normalization processes to get a similar number of phytoplankton OTUs by season, the abundance of OTUs (each bar in the plot A) was transformed (log10(x)+1) for the sake of visual representation (left). We selected a threshold of 3377 OTUs (corresponding to the total number of phytoplankton OTUs retrieved in September, the less rich season) and kept the first 3377 most abundant OTUs in each season. The effect of the removal on the original dataset (right), was estimated by the mantel test and spearman rank correlation as in Gobet et al. 2010; B) Results of the normalisation processes on the OTUs connectivity across stations and seasons. This connectivity network represents the number of eukaryotic phytoplankton OTUs shared across our sampling stations (at surface and DCM) and seasons in the curated dataset. Node size represents the number of OTUs in each station (see node color) of each season; link size represents the number of OTUs shared between stations; link color represents: low connectivity (light grey in the background, < 300 OTUs shared), intra-seasonal (colored) or cross-seasonal (black) seasonal.; C) Pairwise comparison of the connectivity matrix of the original dataset and the normalized one. The correlation between the two connectivity matrices was studied by the spearman rank correlation.

After validating our approach, we further studied the initial connectivity matrix (Figure 21). To help the understanding of Figure 21, we studied the density distribution of the links (number of shared OTUs between stations) in the connectivity matrix (Figure S18). When focusing on links shared by station across seasons (Figure S18a), the number of OTUs shared within a same season decreased from March to September, in March stations shared in between 600 and 1200 OTUs, in July they shared lower numbers of OTUs from 750 to < 100 OTUs, in September, the number of OTUs shared ranged in between 600 and < 100 OTUs. The number of OTUs shared within a season was generally higher than across season (from <100 to a 450 OTUs shared maximum, but mostly ranging under 300 OTUs). When focusing on links shared by stations (Figure S18b), the number of OTUs shared with the frontal station had a generally higher number of phytoplankton OTUs, both within a season (the most observed links ranged around 600 OTUs compared 200 to 600 OTUs for links involving other stations) and across season (for links with the frontal station the values ranges in between 0 and 500 OTUs compared with 0 to 350 for links involved with other stations).

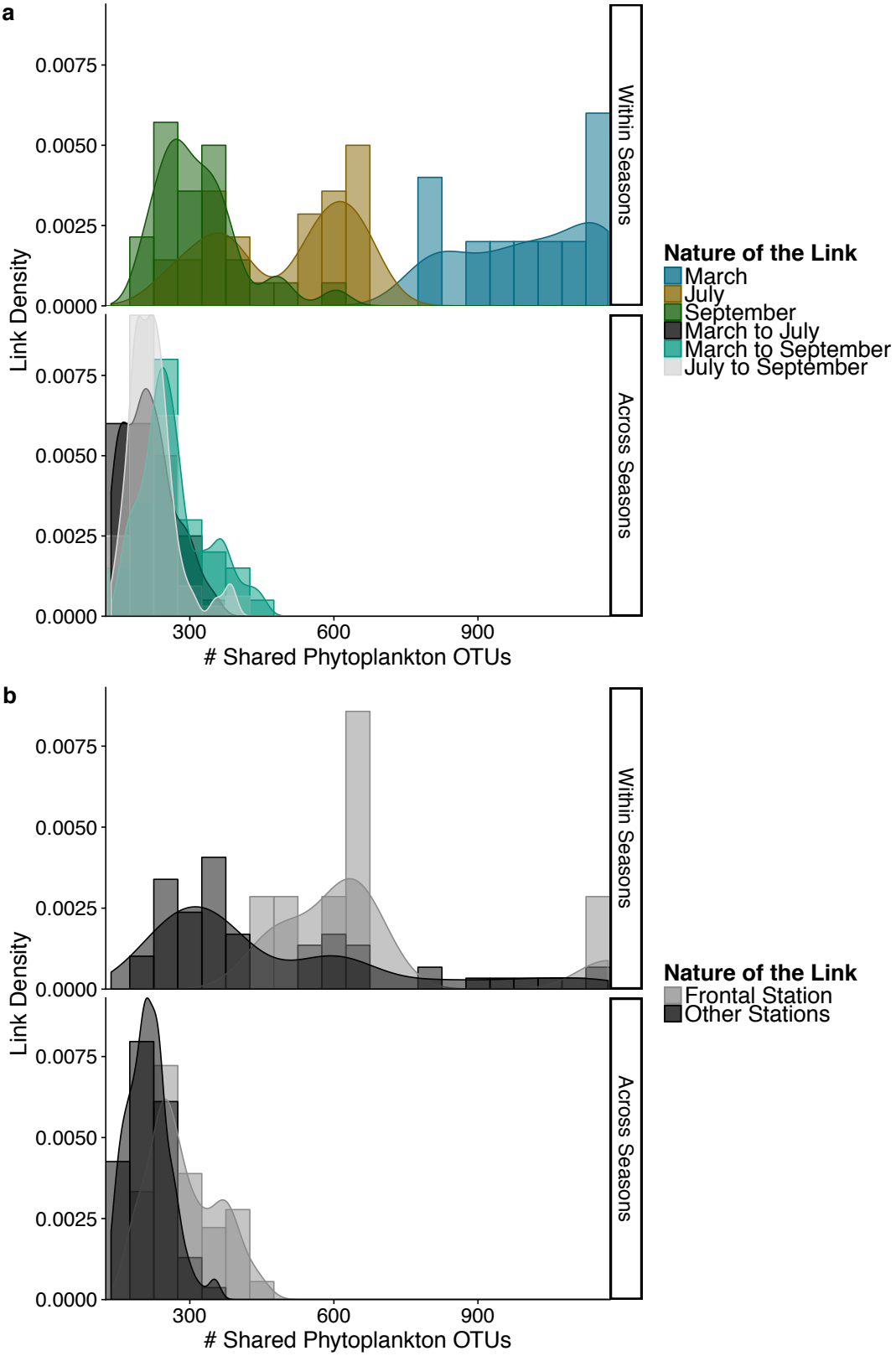

Figure S 18: Density distribution of the weight of connectivity links in the connectivity network of our monitoring of the Iroise Sea in 2015. Links represent the number of phytoplankton OTUs shared. We focused on a) links shared by sampling campaigns, b) or by sampling stations, within a same season or cross season.

Functional Diversity of phytoplankton

To study the trait the most relevant to distinguish the phytoplankton communities of each stations in September (the campaign where the front had the most influence), we computed a co-inertia analysis (Dray *et al.*, 2003). Briefly, a) a Principal Component Analysis (PCA) was calculated on a table constituted of the presence-absence of the abundant phytoplankton community in September (OTUs > 0.1% of the total read number in September) in our five stations (5 stations vs. 27 OTUs, see Table 2). b) A subset of our trait table describing only the OTUs of the abundant community (13 traits x 27 OTUs) was transformed with a Fuzzy Correspondence Analysis (FCA, conversion of categorical variables into numerical variables, see Bremner *et al.,* (2006) and references therein). c) Co-inertia was computed on the FCA and the PCA in order to estimate their co-inertia. Co-inertia is a method based on the co-structure in between two table, inertia is high when the two tables vary simultaneously, that is measured by the RV coefficient. Co-inertia also creates a single space in which traits, stations and traits can be plotted according to their co-structure (Figure S19).

The RV coefficient (0.22) indicated that the co-inertia in between the FCA and the PCA was low, and thus that traits variated only moderately with the presence-absence of OTUs across the 5 stations. This was probably due to the high number OTUs common to multiple station. We still analyzed the results based on the first (46% of total inertia explained) and the second (36%) axis of co-inertia. Only the modalities of trait that most explained station differentiation (above the absolute value of 0.05 on at least one axis) were plotted. The taxonomic reference of each OTUs that were present in one or two stations were also plotted to highlight the strongest gradients. The traits highlighted were: cell size ("Nano" represent cells in between 3-10 μm in the FCA), coloniality ("Colonial"), ingestion method ("Phagotrophic"), cell cover ("Siliceous"), symmetry ("Radial" and "Spherical"), and symbiosis type ("MutualistPhotosynthetic"). The occurrence of these trait and their modalities was further studied in the abundant phytoplankton OTUs of September in Figure 22.

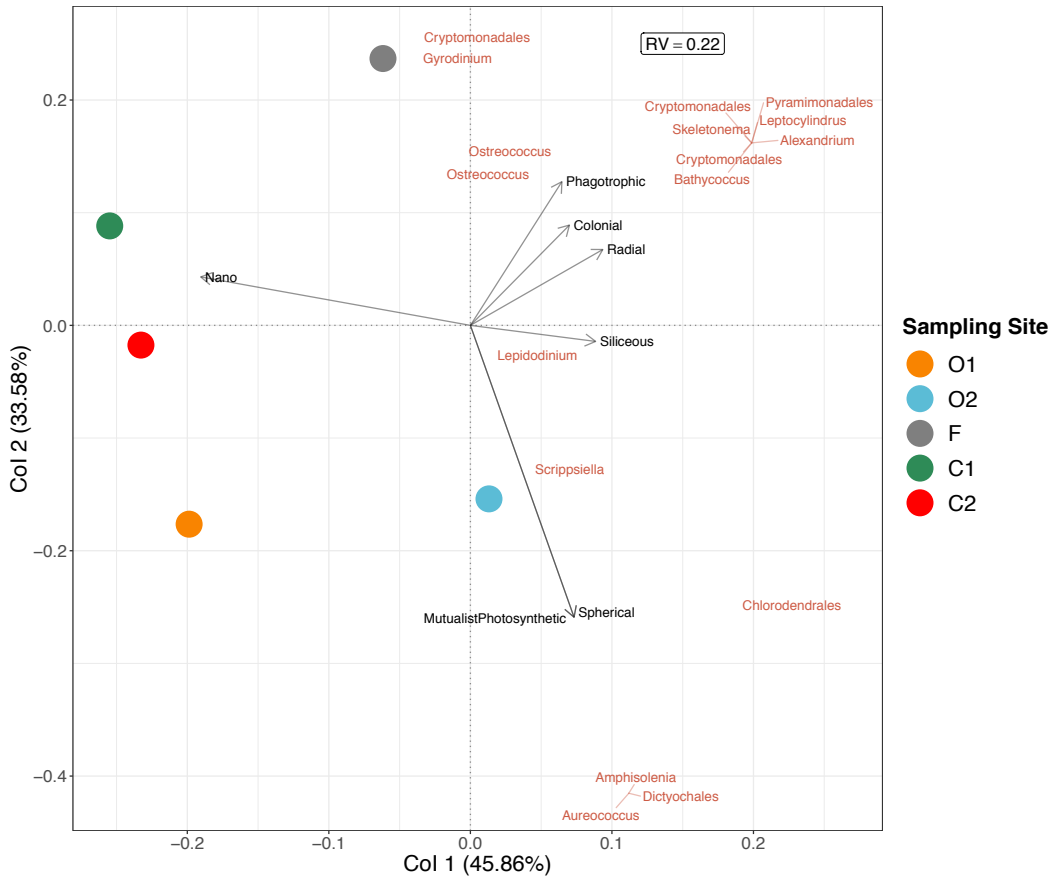

Figure S 19: Co-inertia analyses of the abundant community of phytoplankton across stations in September in our monitoring of the Iroise Sea and the dominant trait expressed. A first table composed of the presence-absence of phytoplankton OTUs from the 'Abundant' community (> 0.1% of the total read number in September) in each of the 5 stations of our monitoring in September was analysed with principal component analysis (PCA). Based on our trait table we constituted a second table corresponding to 13 traits that described the organisms found in the 'abundant' community, this table was further analysed by fuzzy correspondence analysis (FCA). Based on the PCA and the FCA, co-inertia fits each station, OTUs and modality of traits into a common constrained analysis. the RV coefficient is a measure of co-inertia (ranging in between 0 and 1).

B. Heterotrophic protists: dynamic and diversity over a coastal tidal front

1) Introduction

The phagotrophy of heterotrophic protists plays significant roles in marine ecosystems, 1/ these organisms represent indeed a high source of mortality for other member of the plankton (i.e. prokaryotes, phytoplankton and even for other heterotrophic protists) (Sherr and Sherr, 2002), 2/ they can later be used as preferential food by higher meta-zooplankton (Sherr and Sherr, 1988), and 3/ they participate to the regeneration of nutrients by the microbial loop (Azam *et al*., 1983; Ducklow, 1983). Recently, metabarcoding highlighted a wide diversity of unknown marine heterotrophic protists, whether in the deep sea (López-García *et al*., 2001; Pernice *et al*., 2015), in coastal ecosystems (Massana *et al*., 2004, 2015) or in the global ocean (de Vargas *et al*., 2015). These discoveries highlighted our poor knowledge about the taxa that constitute the micro-zooplankton as well as their ecology. The factors brought forward to explain the distribution of heterotrophic protists in marine ecosystems highlight the influence of dispersal (Dolan, 2005; Dolan *et al*., 2007), environmental preferences (Atkinson *et al*., 2003; Massana *et al*., 2006), or resource availability and associated competition (Hardin, 1960). The nature and abundance of resource necessary to the growth of heterotrophic protists distinguishes various strategies (Fenchel, 1982a, 1982b), with distinct prey-size preferences (Hansen *et al*., 1994; García-Comas *et al*., 2016), taxonomic preferences (Massana *et al*., 2009), and, overall, a wide array of functional response to natural assemblages of preys (Massana *et al*., 2009; Weisse *et al*., 2016).

Here we propose to investigate the heterotrophic protists coincident with the eukaryotic phytoplankton community of the Iroise Sea in 2015. First, the factors that can explain changes in the heterotrophic-phototrophic ratio of the marine protistan community were studied. Then the major clades of heterotrophic taxa found in our metabarcoding survey are detailed and we studied whether if functional traits could explain these patterns. Finally, we propose a perspective work on trophic ecology

that could be coupled with a joint survey of prokaryotic communities to better understand the effect of heterotrophic protists on the pelagic ecosystem.

2) Material and methods

The sampling strategy, genetic and bioinformatics procedures, functional annotation work and metabarcoding dataset were the same than in the previous section A. When distinct analyses were carried out, the specific methodological aspects are detailed in a short paragraph before presenting the results.

3) Results

a) The heterotrophs/phototrophs ratio

OTUs were annotated functionally thanks to our trait table (see Ramond et al., *submitted*; http://doi.org/10.17882/51662). Only 14 704 of the 33 060 total OTUs retrieved in our dataset could be annotated with functional traits. Phototrophic protists were selected as OTUs with inherent capabilities to photo-autotrophy (with constitutive plastid synthesis, 6756 OTUs), heterotrophic protists were selected as OTUs without inherent phototrophic abilities (without constitutive plastids, 7918 OTUs). In accordance with the various mixotrophic strategies that exist, mixotrophic protists were found among both eukaryotic phytoplankton and heterotrophic protists.

We studied the ratio of the two trophic strategies across our samples (Figure 24), the non-annotated OTUs were discarded although they could represent an important part of reads (44% of the total read number in our dataset). The trophic ratio was also compared to the taxonomic composition of protists in the same samples (Figure 25). Within size-fractions, the micro-plankton showed the lowest abundance of heterotrophs (ranging from 1.6 % to 15 % of read by sample, with a maximum of 39% in March) compared with the two smallest-size fraction (ranging in between 1.2%-25% of read by sample, with a March maximum of 58% and 40% respectively in nano and pico-plankton) (Figure 24).

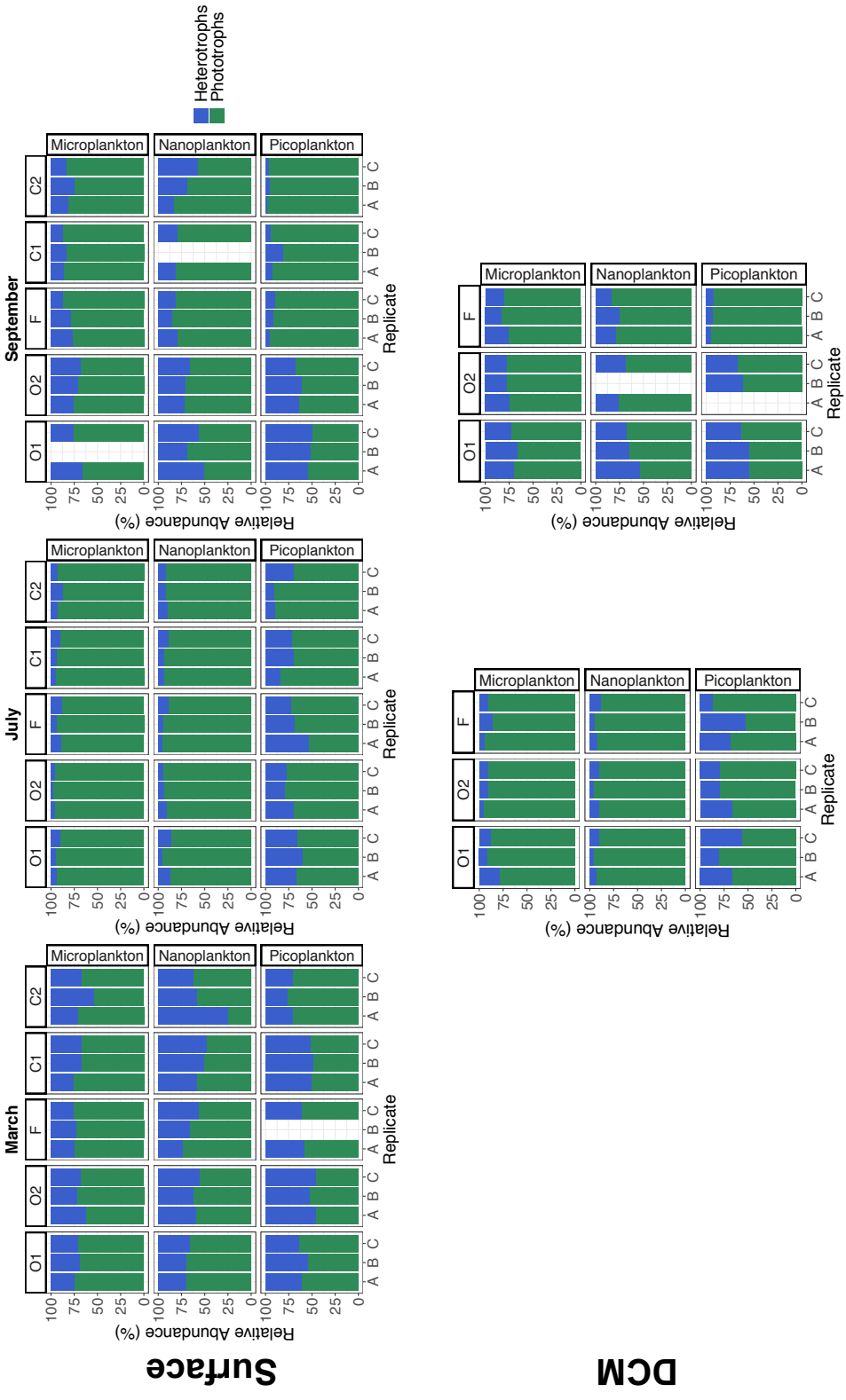

Figure 24 : The trophic ratio of marine protists across our sampling survey of the Iroise Sea. Samples are organized by replicates, size-fractions, sampling stations (from the open-ocean to the coast, left to right), depth and season. 14 704 OTUs were sorted into 'phototrophs' if they had constitutive synthesis of functional plastids and into "heterotrophs if they did not. The relative abundance of this ratio was calculated using the total number of reads that these OTUs represented.

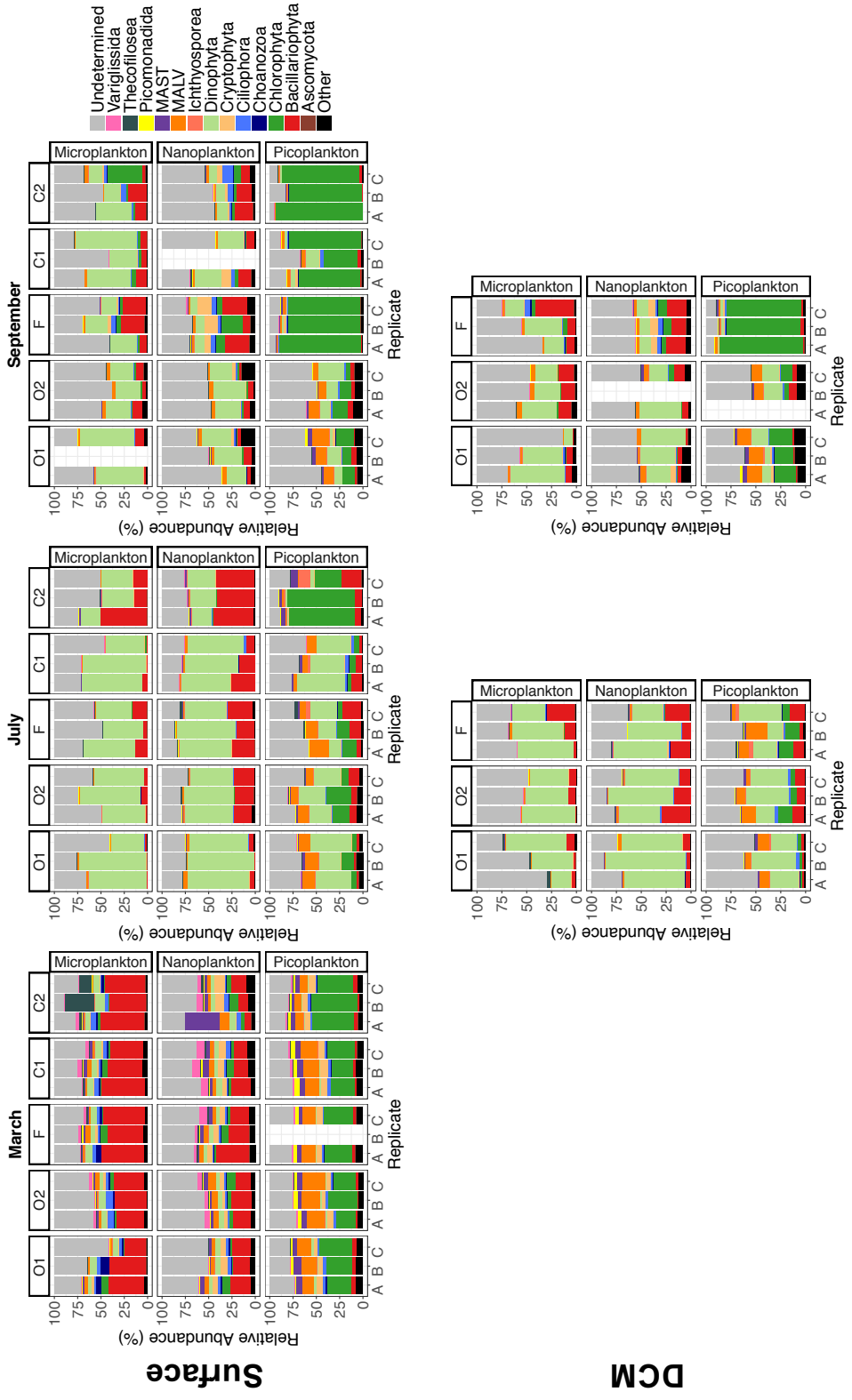

Figure 25: Distribution of the distinct protistan taxa estimated by metabarcoding in the Iroise Sea in March, July and September 2015. Samples are organized by replicates, size-fractions, sampling stations (from the open-ocean to the coast, left to right), depth and season. Relative abundance was calculated based on the number of reads of OTUs corresponding to the shown taxa, 'Other' represented the read number of taxonomic ranks with a relative abundance < 10%, 'Undetermined' represented the read number of OTUs with a low taxonomic level. Same as Figure 19.

Across samples duplicated along depth, there were no significant difference in the trophic ratio (Figure 24). Along seasons, heterotrophs were the most abundant in March and across all size-fractions (from 11 to 57% of read by sample) (Figure 24). In the micro-plankton, the heterotrophs were represented by a marked abundance of Thecofilosea and MAST at the most coastal station C2 (peaks of 30 % compared to values < 5% elsewhere), that corresponded to maxima of heterotrophic proportion (Figure 24 and 25). In the micro and nano-plankton there existed also a wide phylogenetic diversity of heterotrophic taxa in low abundance notably Choanozoa, Variglissida, Picomonadida and Ciliophora (Figure 25). In the picoplankton, the heterotrophic strategy was represented by the Picomonadida, MAST but mostly by the parasitic MALV (ranging in between 20 and 30%; Figure 25).

In July, the abundance of heterotrophs decreased in the micro and nano-plankton (in betwee 1 to 7% of read by sample), but decreased markedly less in the pico-plankton (from 8 to 30%), the minimum values in the picoplankton were observed at the most coastal station C2 (Figure 24). Within the higher size-fraction, there existed a high proportion of Dinophyta (dinoflagellates) in the offshore waters across the higher size-fractions (Figure 25). Although dinoflagellates can also represent heterotrophic strategies, phototrophs dominated these size-fraction in July (Figure 24), implying that most dinoflagellates were phototrophic. Among pico-plankton, MALV still represented the most important heterotrophic group. Pico-plankton was relatively homogenous except for the most coastal station (C2), where the higher proportion of phototrophs (Figure 24) was dominated by Chlorophyta (Figure 25). Parasitic Ichtyosporeans were also observed in pico-plankton at the coastal stations (C1, C2) and the frontal station F but in weak abundance (1 to 5%).

In September, the range of heterotrophs in the micro (1 to 11% of read by sample) and nano-plankton (4 to 20%) slightly increased (Figure 24) and corresponded to a higher portion of ciliates (Figure 25). In picoplankton, the proportion of heterotrophs decreased in the three stations the closest to the coast (F, C1 and C2, with values < 10%) (Figure 24) due to a strong domination of Chlorophyta (Figure 25). A higher proportion of pico-heterotrophs was maintained in the most offshore stations (with values in between 10 and 30% of read by sample) dominated by MALV and in lesser extent by MAST and Picomonadida (Figure 25).

The environmental drivers that could explain the fluctuations in the trophic ratio were studied by the means of the spearman rank correlation (Figure 3). In addition to the nitrate + nitrite concentration presented previously, other nutrients are used in this section (Phosphate PO_4^{3-}, Ammonium NH_4^+ and Silicate $Si(OH)_4$), they were measured with a Seal Analytical AA3 HR automatic analyzer, following the procedures described by Aminot & Kérouel (2007). Across all size-fractions, the correlations showed mostly a higher proportion of heterotrophs in the environmental conditions of March (lower temperature and higher nutrient concentrations) (Figure 3a). In a second time, the correlations were thus investigated only across July and September (Figure 3b). In the micro-plankton, correlations showed a greater proportion of heterotrophs when nutrients were higher (Figure 3b), probably indicating that heterotrophs where more present when the uptake of phytoplankton was limited. In the nano-plankton, heterotrophs correlated with lower salinity although this seems surprising as they were more abundant in the most offshore, and supposedly saline, stations in September (Figure 1). In the pico-plankton, higher proportion of heterotrophs were found far from the coast (Figure 3b), as we observed in September that they were found mostly in the most offshore stations (Figure 1, O1 and O2). The offshore area probably corresponded to environment with a lower silicate concentration as pico-heterotrophs were also correlated negatively to silicate (Figure 3b).

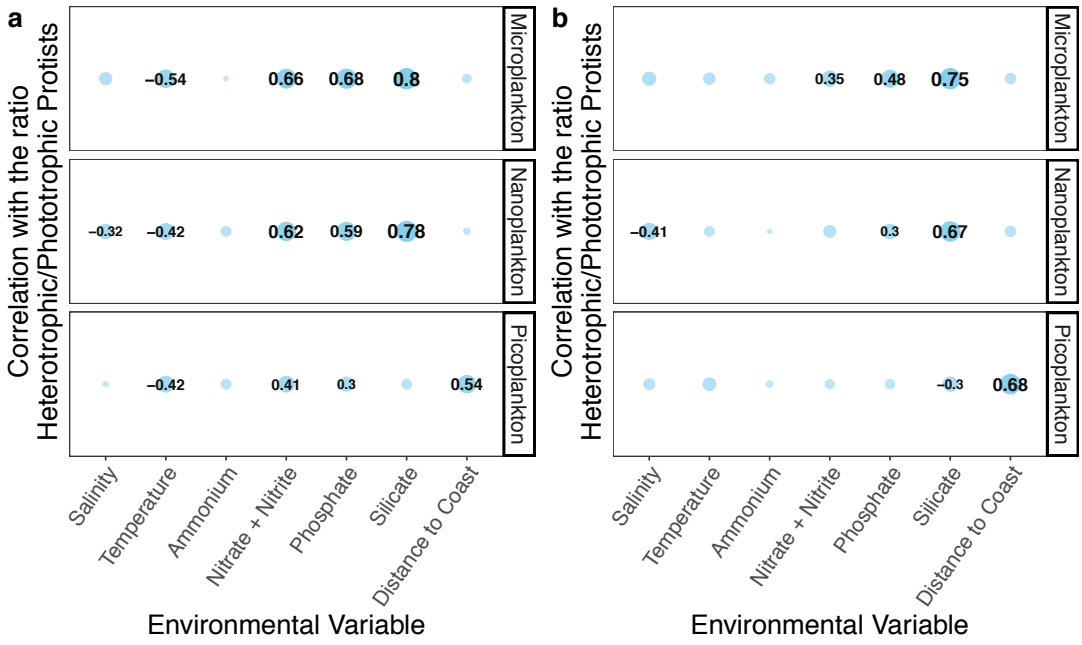

Figure 26: Correlation between the trophic ratio (relative abundance of heterotroph/phototroph protists) in micro-, nano and pico-plankton of marine protists and environmental variables measured in the Iroise Sea throughout 2015. The Spearman rank correlation is used. The correlations were studied based on a) all sampling campaigns (March, July and September) and b) only the productive periods (July and September).

b) Heterotrophic protists diversity

To summarize heterotrophic protists diversity across our dataset, OTU richness was calculated for each season (3), station (5), depth (2 when the DCM was sampled) and planktonic size-fraction (3), corresponding to 183 distinct samples. To account for this great number of measures, the variability of heterotrophic protists richness was first studied using boxplot of values across seasons, stations and size-fractions (Figure 27a). Secondly, OTUs from distinct replicates and depth were united to represent the total richness across seasons, stations and size-fractions (45 distinct measures, Figure 27b). To focus on the spatial structuration of heterotrophic protists diversity, OTUs were flagged as 'ubiquitous' if they were shared by at least two stations of the same season, and as 'Specifics to Station X' if they were retrieved only at station X of the same season. Differences in OTU richness across stations were tested with the Kruskall-Wallis test.

Across size-fractions (Figure 27a), micro-plankton samples presented less heterotrophic protist OTUs richness by sample, values ranged from 678 to 62 OTUs

by sample. Nano-plankton, 884 to 21 OTUs, and pico-plankton, 811 to 47 OTUs by sample, presented a higher richness. Along seasons, and at the same time that the average chlorophyll *a* biomass increased (see previous section, Figure S15), heterotrophic protist richness declined in all size fractions (Figure 27a). Indeed, the maxima of heterotrophic richness markedly decreased from March (maximum of 678, 884 and 811 OTUs by sample respectively for micro, nano and pico-plankton) to July and September (maximum of 296, 381 and 580 OTUs by sample respectively for micro, nano, and pico-plankton).

Heterotrophic protist richness increased when we calculated the total heterotrophic richness across stations, seasons and size-fractions (maximum of 1561 OTUs; Figure 27b). Like for phytoplankton (Figure 20), this indicated that replicates were useful into discovering new heterotrophic protist OTUs at single location and thus in the sampling effort. Throughout the summer increase in phytoplankton biomass, total heterotrophic protist richness declined (from values above 800, 900 and 600 to values under these thresholds respectively for micro, nano and picoplankton; Figure 27b), supsosing a decresase in smapling effort due to filter clogging. In comparison with phytoplankton (see previous section), no station was spared by this decrease. When testing for statistical differences in richness across stations during the productive periods, there existed only a significant difference in between the station C1 and other stations (Kruskall-Wallis test, p-value = 0.019), due to the even lower values found at this station. Still in September, the frontal station observed the maxima in heterotrophic protist richness within the highest size-fractions (590 and 880 OTUs in the micro and the nano-plankton) implying that heterotrophs could also be influenced by dispersal at the front. This peak also corresponded to a slightly higher proportion of OTUs specific to the frontal station in September, however this pattern was not significant statistically (Kruskall-Wallis test, p-value = 0.4651) and contrarily to phytoplankton there existed few patterns of marked 'specific' OTU proportion (Figure 27b).

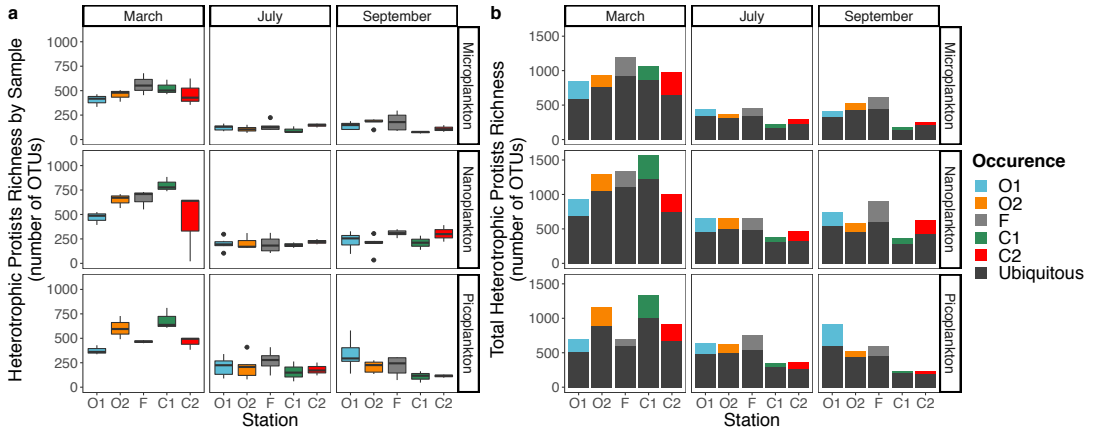

Figure 27: Heterotrophic protist OTUs richness in the Iroise Sea in March, July and September 2015. a) Heterotrophic protist OTUs richness was calculated in each of our 184 samples and the variation of richness is presented as boxplots across station, season and size-fraction. b) Total heterotrophic protist richness when cumulating the number of OTUs retrieved in each station, season and size-fraction. OTUs were colored according to their occurrence in two stations of a same season ('ubiquitous', black) or in one unique station in the same season ('specific', colored).

c) Abundant heterotrophic protists and their traits

Like in the first section on phytoplankton, we computed a co-inertia analysis on the 'abundant community' of heterotrophs (OTUs > 0.01 %of the total read number associated to heterotrophic protists). The analysis was computed with 1/ three presence-absence table, for each sampling campaign, composed of the abundant heterotrophic protist OTUs found across our 5 stations, and 2/ a table composed of 13 traits describing these OTUs. Stations were spread onto a two-dimension space according to the OTUs that they have in common, the traits that explains the most distance in between station are fitted to the two dimensions, and here, we also represented the position of OTUs found only in a single station (Figure 28).

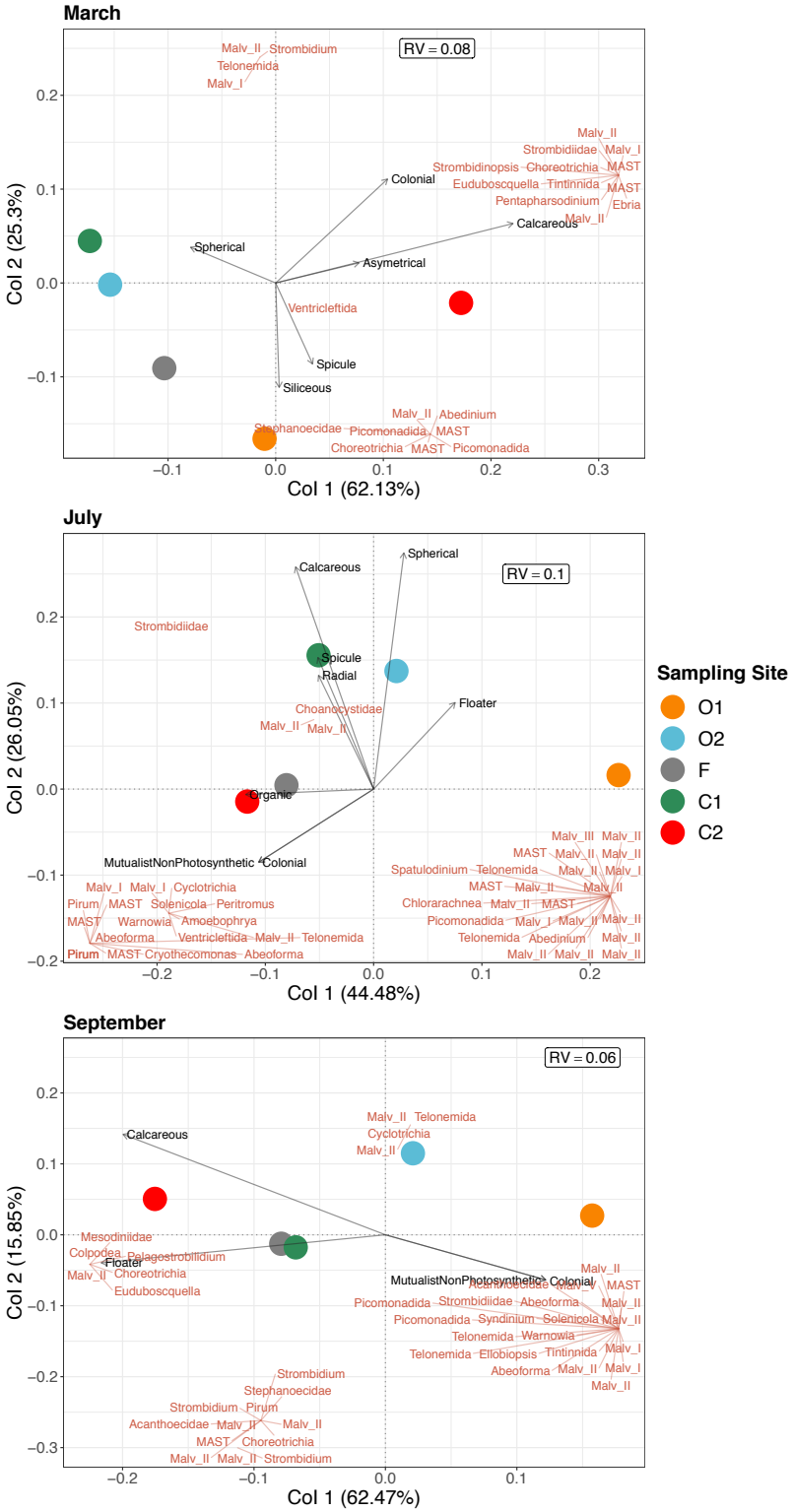

Figure 28: Co-inertia analyses of the abundant community of heterotrophic protists in the Iroise Sea in March, July and September 2015 and the dominant trait expressed. A table composed of the presence-absence of OTUs from the 'Abundant' heterotrophic community (> 0.1% of the total read number in each season) in our 5 stations, was analyzed with principal component analysis (PCA). The table of 13 traits that described the organisms found in the 'abundant' community, was transformed by fuzzy correspondence analysis (FCA). Based on the PCA and the FCA, co-inertia fits each station, OTUs and modality of traits into a common constrained analysis. The RV coefficient is a measure of co-inertia (ranging in between 0 and 1).

RV coefficients details the extent of co-inertia of two datasets (Legendre and Legendre, 2012), here, the extent to which the traits explained the patterns of presence-absence of heterotrophic protist OTUs across stations. All RV coefficients were low (0.1 are under) indicating that traits explained poorly the diversity patterns. We still studied the most representative traits and their repartition across stations. The two dimensions of the co-inertia were sufficient to represent 70% or more of this co-inertia in each season, thus, other axes were not investigated (Figure 28).

In March, the strongest differences in between stations were represented by the most coastal station (C2, distinct on axis 1) and the most offshore station (O1, distinct on axis 2) (Figure 28). The most coastal station fitted well with calcareous, colonial and asymmetrical organisms, which were respectively represented by the dinoflagellate *Pentapharsodinium sp*. (calcareous), the colonial rhizarian *Mataza hastifera* (observed in station C1 and C2 and thus not showed on the graph), and the many asymmetrical ciliates and dinoflagellates represented at the right of the graph. The most offshore station (O1) fitted well with a *Stephanoecidae* OTU, known to bear siliceous spicules on its cell cover. The other station showed an overall higher proportion of spherical organism although none was specific to these stations (Figure 28).

In July, there existed three groups of stations. The frontal and the most coastal stations (at the left of the first axis F and C2) were close indicating similarities in community composition (Figure 28). However, the traits that fitted well with these stations indicated uniquely the presence of *Solenicola setigera* at the frontal station, a colonial organism endosymbiont of diatoms (Figure 28). The most offshore station was markedly distinct from other stations (right of the axis 1), the station presented a lot of OTUs retrieved abundant only at this station (see taxa at bottom right) although these OTUs did not fit with any particular trait. The last group composed of the station C1 and O2, fitted well with the traits of OTUs found across all stations (Floater: *Noctiluca scintillans*, Calcareous and spicule bearing: *Pentapharsodinium sp*., and many dinoflagellates, ciliates and MALV with spherical or radial symmetry), inconsistently, the spicule bearing and siliceous *Choanocystidae* OTUs was found only at station C1 (Figure 28).

In September, all stations were spread along the axis 1 supposing the presence of a single gradient distinguishing the most offshore stations (O1 and O2, at the right of axis 1) from the other stations (F, C1, C2, at the left of axis 1) (Figure

28). The traits that fitted well with this gradient indicated the presence of the colonial endosymbiont of diatoms *Solenicola setigera* at the most offshore station O1 (Figure 5), while the floater *Noctiluca scintillans* was found only at station C1 and C2, and the calcareous *Pentapharsodinium sp*. was found at C1, F and O1.

Overall this analysis highlighted that our traits explained rather poorly the distribution of abundant heterotrophic protist OTUs across our station, this was notably envisioned in the low RV coefficients. The co-inertia was mostly driven by the traits of a single OTUs present in a single area. However, this indicated that a high number of abundant OTUs were shared between stations, although there existed also a higher number of specific OTUs in July and September indicating a higher community structuration by station in these periods (Figure 28).

4) Discussion

a) Trophic ratio of marine protists

The trophic ratio of marine protists has a strong importance into the metabolism of aquatic ecosystems, notably into the balance of CO_2 and O_2 and its effect on the carbon export on marine ecosystems (Duarte, 1998). Here, because environmental sequencing only gives relative abundance it was impossible to quantify its influence on the Iroise Sea. The heterotrophic strategy was more dominant in the smaller size-classes as evidenced previously (see Chapter I), and in contradiction with recent models that hypothesize the dominance of phototrophy in smaller size-classes (Andersen *et al*., 2014; Ward and Follows, 2016). Correlations in between the trophic ratio and environmental variables indicated that higher proportions of heterotrophs were found mostly when the uptake of phytoplankton was limited (Figure 3). This typically illustrates the alternation in between primary production and microbial loop processes (Legendre and Rassoulzadegan, 1995). Pico-sized heterotrophic protists were less structured by these processes but presented higher proportions towards the open ocean. However, this phenomenon was strongly influenced by the domination of phototrophic chlorophytes in the coastal area during

productive periods, the hypothesis of a greater abundance of heterotrophic protists in the open-ocean remains to be tested with quantitative tools.

b) Heterotrophic protisan community

The ecology of protistan heterotrophs is poorly studied in the environment. Members of Thecofilosea were markedly observed at C2 in March (Figure 25). This heterotrophic phylum is ubiquitous; being observed along coasts, oceans and even fossil records (Massana *et al*., 2004; del Campo *et al*., 2013; Ohtsuka *et al*., 2015). Thecofiloseans are part of the cercozoans that were associated with parasitism and microbial loop in the East English Channel (Christaki *et al*., 2014; Genitsaris *et al*., 2015), however, little is known of their ecology. Similarly, MASTs were dominating a nano-plankton replica at C2 in March (Figure 25). These organisms are widespread but can represent a high proportion of reads in the coasts (Massana *et al*., 2004, 2006; Hu *et al*., 2016). Their dynamics might however be better explained by the presence of bacterial preys (Massana *et al*., 2009) than by other protists. Reversely, ciliates (Ciliophora) were retrieved more abundantly in September, mostly at the coastal and frontal stations (F, C1 and C2, Figure 25). A study combining sequencing and microscopy of micro-zooplankton, showed a decrease in the abundance of Ciliates from the coast to the ocean, that was explained by a decrease in phytoplankton biomass (Santoferrara *et al*., 2016). Here, the frontal area (station F) was still productive in September (see previous section, Figure S15), so this environment probably maintained high ciliates abundances (Figure 25). Inconsistently, in our survey there existed no correlation in between the relative abundance of ciliates and the phytoplankton biomass (estimated by chlorophyll *a;* see Figure 29a). Other heterotrophic protists could include the dinoflagellates although we evidenced that they were were mostly part of the phototrophic protists (Figure 24 and 25). However numerous phototrophic dinoflagellates are able to carry out mixotrophy and could still take part in the grazing activity (Jeong *et al*., 2010), most notably during their high abundance in July and September (Figure 25).

The parasitic MALVs were abundantly observed across all samples, except when Chlorophytes over-dominated pico-plankton (> 75%). This might be explained by the remanence of numerous dinospores, that can transform into resting stages (Guillou *et al.*, 2008; Gleason *et al.*, 2014; Scholz *et al.*, 2016), but also by an over-

estimation of their abundance because of a high DNA copy number by cell (Massana, 2011). Ichtyosporeans, another group of parasites, were also observed in the coast and the front in July (Figure 25). These organisms are mostly pathogens of fish, bird or mammal (Mendoza *et al.*, 2002), and have been retrieved in other coastal sequencing surveys (del Campo *et al.*, 2015). The distribution of the global parasites diversity might be influenced by 1) presence of specific preys or 2) increasing in total prey biomass, as some can be generalists (Chambouvet *et al.*, 2008; Lafferty *et al.*, 2008). There appeared no evidence of an increase of parasites abundance in parallel to phytoplankton biomass (Figure 29b), however there existed a high number of MALV (clade I, II and III) and ichtyosporean OTUs (e.g. *Abeoforma spp.* and *Pirum spp.*) found specifically at a single station in our co-inertia analysis (Figure 28). This could imply that parasites were abundant when preferential preys occurred at single station, in accordance with a high prey specialization across some MALV clades (Coats and Park, 2002; Guillou *et al.*, 2008).

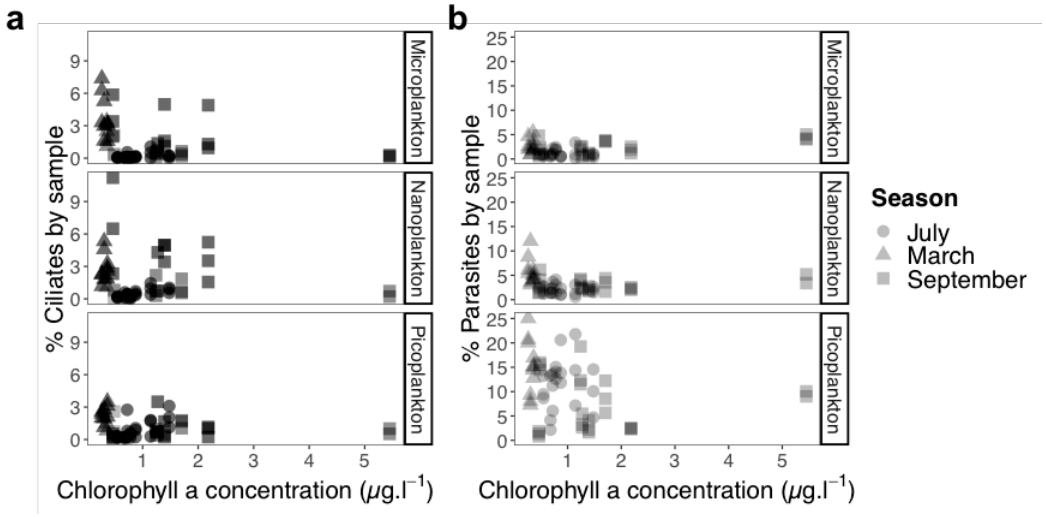

Figure 29: Relationship in between a) ciliates relative abundance (estimated by metabarcoding) and phytoplankton biomass (estimated by chlorophylla) and b) parasites relative abundance and phytoplankton biomass, throughout our survey of the Iroise Sea in 2015.

Being part of micro-zooplankton, heterotrophic protists could highlight microbial-loop processes involved in remineralization and regenerated production (Azam *et al*., 1983), that have been observed at the Ushant tidal front (Le Corre *et al*., 1993). Heterotrophic protists might also be structured by the size-spectrum of dominant phytoplankton organisms (Hansen *et al*., 1994; García-Comas *et al*., 2016). It is possible that both Thecofiloseans and MASTs, but also the other small predators

observed in March (e.g. Choanozoa, Variglissida, Picomonadida), represented an active microbial loop in March. Interestingly, the heterotrophic strategy in September showed a higher proportion of the larger ciliates and potentially dinoflagellates. Supposing that the available food size increases during a typical productive period (Kiørboe, 1993), the succession between nano-flagellates, with a low optimal prey size, and ciliates associated with dinoflagellates, with both larger optimal prey size (Hansen *et al*., 1994), could represent a change in the nature of the microbial loop influenced by phytoplankton.

However, these hypotheses could not be verified by our trait approach. Cell size which usually correlates with the spectrum of size available for predators (Hansen *et al*., 1994), was not expressed throughout our dataset (Figure 28). This was probably due to the constant presence of small-sized MALV parasites within the pico-plankton which tended to normalize the size of heterotrophic protists across stations and seasons.

c) Heterotrophic protistan diversity

Contrary to phytoplankton, heterotrophic protist diversity was weakly structured across our samples (Figure 27). Similarly, we observed the influence of a lower sampling effort in the productive periods due to filter clogging, which markedly decreased the number of OTUs in July and September (Figure 27). Dispersal of water masses towards the front could also have influenced the slightly higher values of heterotrophic protist OTUs richness found in the higher size-fractions at the front in September (Figure 27). Dispersal can indeed markedly structure the communities of heterotrophic protists (Dolan *et al*., 2007), as much as to create neutral patterns.

Our traits poorly explained the patterns of heterotrophic protist diversity. The traits that highlighted the most relevant patterns represented strategies found in single location. In addition, our traits were not enough adapted to heterotrophic protists to understand the advantages of the ecological strategy in each area. Our traits represented strategies to avoid predation (cell cover, spicules or coloniality), but the pressure of predation on heterotrophic protists could not be estimated. Still in September, the mixed area (the front and the coastal area) correlated with organisms with a 'floating' type of motility which could indeed represent a good strategy to remain at surface without investments on motility (Margalef, 1978; Franks, 1992).

On the open-ocean, a heterotroph endosymbiont of diatoms, *Solenicola setigera* (Gómez *et al*., 2011), was found dominant. When studying phytoplankton another symbiosis was found in the same area in September (see previous section), perhaps symbioses could represent an ecological strategy found in higher abundance within the open-ocean, this hypothesis needs to be further investigated.

5) Conclusions

Studying a frontal system that markedly structured the diversity of eukaryotic phytoplankton (see previous section), we evidenced a low structuration of heterotrophic protist diversity. Patterns in the trophic ratio of marine protists indicated a higher influence of heterotrophic protists when the uptake of phytoplankton was limited, in accordance with a stronger influence of the microbial loop in these environments. We hypothesized that changes in the phytoplankton community could better influence the diversity of heterotrophic protists. However, our trait approach could not evidence major changes in the functional patterns of heterotrophic protists diversity. The addition of traits related to the phagotrophy of heterotrophic protists will probably help to further explain patterns of their diversity in the environment. In addition, it is still poorly understood what constitutes resource for heterotrophic protists, it can be hypothesized that an increase in prey biomass would favor their occurrence, however heterotrophic protists seems rather specialized (in the size or the taxonomy of their prey) and an increase could as well favor only a part of the heterotrophic community. The key to understand patterns of heterotrophic protists is probably a better sampling and comprehension of what constitutes its resource. The diversity and abundance of heterotrophic protist would also probably better answer to truly quantitative measures of the pelagic community, considering the natural abundance of various prey type (i.e. prokaryotes, size-structured protists) and predation pressure (e.g. viruses, metazoan zooplankton) is necessary to understand the dynamic of heterotrophic protists in their environment.

6) Perspective

Like in other communities of organisms (Loreau, 2001; Scherber *et al*., 2010), the selectivity of protistan predators for their resource has led to question the relationship in between the diversity of preys and predators (Irigoien *et al*., 2004; Saleem *et al*., 2013; Yang *et al*., 2018). Some theories suppose that there exists an interplay in between predation and the effect of intra-guild competition on prey diversity. Saleem *et al*. (2012) notably showed that the predation of heterotrophic protists on bacteria allowed to reduce bacterial competitive exclusion, which increased bacterial diversity by allowing non-dominant species to grow. In return, Yang *et al*. (2018) recently showed that the increase in bacteria diversity also promoted the diversity of protistan predators, supposedly by increasing niche opportunities for various predators. These results contrast with the low correlation in between micro-zooplankton diversity and phytoplankton diversity (Irigoien *et al*., 2004; García-Comas *et al*., 2016), which have been attributed to the low specialization of heterotrophic plankton to phytoplankton (Irigoien *et al*., 2004), or to the facilitation effects present in diverse phytoplankton assemblages, which would decrease the efficiency of predators (Hillebrand and Cardinale, 2004). However, the potential preys of marine protistan heterotrophs range from the bacteria to the largest species of phytoplankton (Sherr and Sherr, 2002), consequently various type of preys needs to be considered in such investigations. In addition, other authors also stressed the necessity to look at the size diversity of both preys and predators (García-Comas *et al*., 2016).

During our survey of the Iroise Sea, environmental sequencing was adapted to the marine protistan community but also to the prokaryotic community. For marine prokaryotes, details about the genetic and bioinformatics process are available (Clarisse Lemonier, IUEM, PhD). In this work, only the smallest size-fraction (3-0.2µm) was studied and considered as the free fraction for prokaryotes. In comparison, the largest size fractions were supposed to represent prokaryotes 'attached' to larger particles and/or organisms. We recognize that studying only this small size-fraction can largely underestimate prokaryotic diversity estimations, as bacterial, and bacterivores, can be found abundant in larger aggregates (Kiørboe *et*

al., 2003). Combined, both the eukaryotic and the prokaryotic datasets could help us tackle questions about the effect of heterotrophic protists on other communities.

In a first attempt to do so, and inspired by a recent study on the subject (Yang *et al*., 2018), we investigated the correlation between the diversity of heterotrophic protist, eukaryotic-phytoplankton and prokaryotic community. To study diversity relationship, we followed a protocol similar to Yang *et al*. (2018) and used a generalized linear mixed–effects model (GLMM) with a Gaussian distribution. This analysis was computed with the MASS package (Venables and Ripley, 2002). To acknowledge the distinct number of OTUs by season due to sampling bias, the distinct seasons were considered as a random effect in the GLMM and we used the Shannon index (Piélou, 1966), a diversity metric less influenced by the total richness in a sample. We tested the correlation in between the diversity of the heterotrophic communities from the micro-, nano- and pico-plankton with the diversity of a) the phytoplankton community of the same size-class, b) the phytoplankton community of inferior size-classes, c) the heterotrophic community of inferior size-classes and d) the prokaryotic community. Correlations were estimated on the basis of our 63 environmental samples retrieved from the Iroise Sea in 2015. In these preliminary results, parasites were discarded from the diversity of heterotrophic protists as they could complicate correlations across size-classes (with preys present in all size-fractions). When the p-value of the GLMM was under 0.05, we considered that there existed a good relationship in between the diversity of the two communities and fitted a linear model to the data, the correlation was then tested with the Pearson correlation coefficient (Venables and Ripley, 2002). Preliminary results are presented in Figure 30.

It is necessary to stress that a good correlation in between the diversity of distinct communities do not represent a preferential diet but the effect of predation on the potential prey diversity. Furthermore, optic confirmation of this predation should be evidenced to sort-out the fortuitous correlations. Nevertheless, we observed a good fit in between the diversity of micro-heterotrophs and micro-phytoplankton ($R^2 = 0.71$), nano-heterotrophs ($R^2 = 0.61$) and pico-heterotrophs ($R^2 = 0.55$). The diversity of nano-heterotrophs fitted well with the diversity of pico-heterotrophs ($R^2 = 0.62$) and correlated negatively with pico-phytoplankton ($R^2 = -0.43$). While the diversity of pico-heterotrophs only correlated negatively with the diversity of pico-phytoplankton ($R^2 = -0.25$).

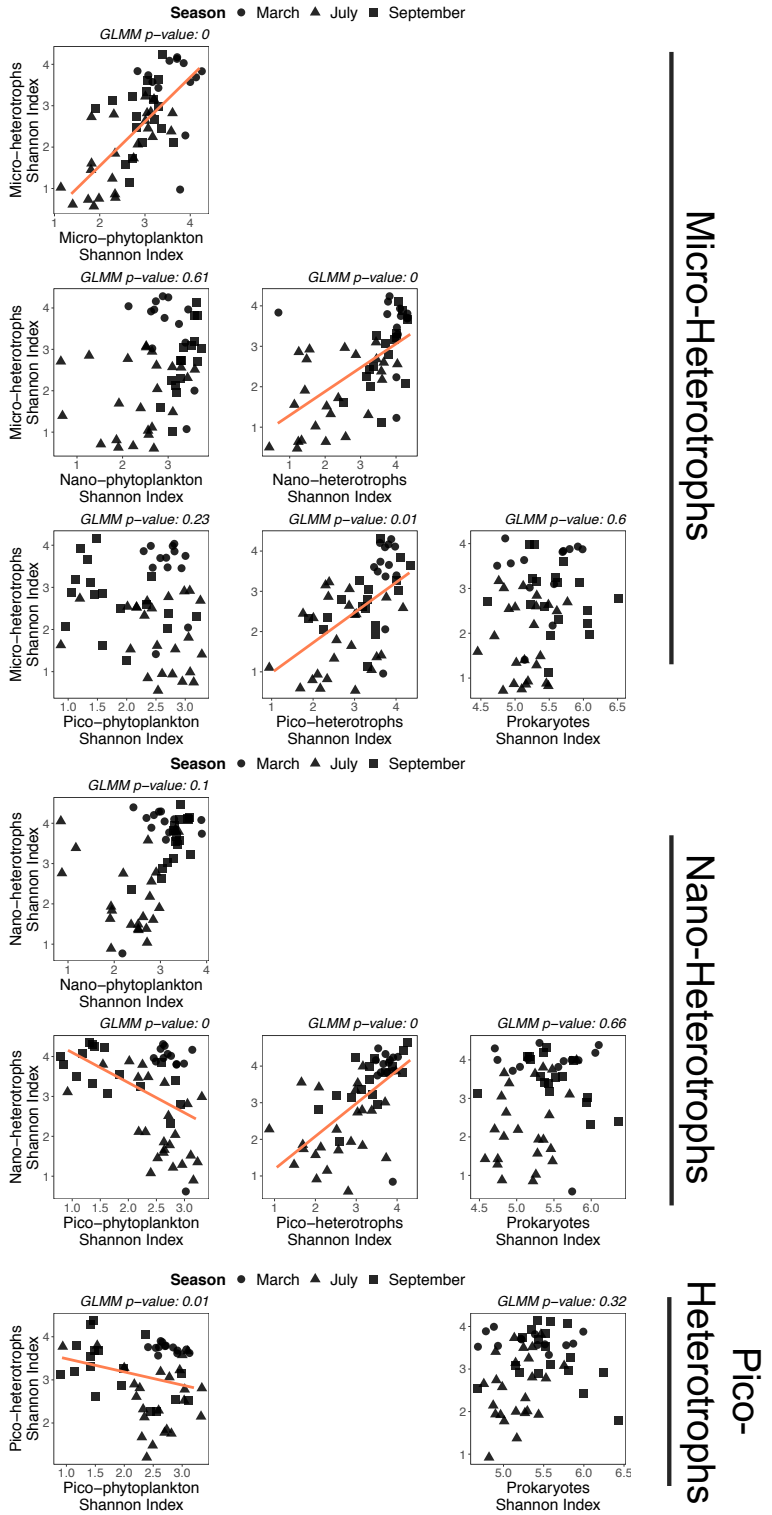

Figure 30: Relationship between the diversity of micro, nano and pico-heterotrophic protists and the diversity of their potential preys (smaller heterotrophic protists, same size and smaller phototrophic protists and Prokaryotes), based on 63 environmental samples retrieved in the Iroise Sea. Diversity was estimated with the Shannon Index. The significance of the relationship was teste with a GLMM and a Gaussian distribution (as in Yang et al., 2018), when p-values < 0.05 a linear model was plotted and the correlation was tested with the Pearson coefficient (referenced in the text).

There exists multiple bias that could explain a good correlation in between the diversity of two communities in our dataset. First, a higher sampling effort across a single season could favor the correlation in between samples by increasing species richness on both communities (in our survey this bias is present in March). Secondly, in size-fractionated samples contamination are frequently observed and could lead to a good correlation in between size-fractionated communities. These biases could be avoided by considering multiple diversity metrics (Yang *et al*., 2018) and metrics less influenced by the sampling effort (e.g. considering OTUs richness only above > 1% of the total abundance by sample). Despite these artefacts, our preliminary results could indicate:

- That as supposed by Fenchel (1982a, 1982b), heterotrophic protists can predate on smaller heterotrophs, and this affects positively the diversity of smaller heterotrophic protist preys by decreasing competitive exclusion (Saleem *et al*., 2012). This would indicate that there exist intricate diversity patterns within the microbial loop (Azam *et al*., 1983).
- That the link in between the diversity of heterotrophic protists and prokaryotes can be missed when studying environmental patterns (contrary to in-silico; Saleem *et al*., 2012) or when focusing on size-classes that do not contain only bacterivorous organisms (only bacterivores selected in Yang *et al*., 2018).
- That the supposed effect of heterotrophic protists on their phototrophic counterparts could be distinguished across size classes. Indeed, the good fit in between micro-heterotrophs and micro-phytoplankton supposes the typical theory in which diversity of preys would promote a diversity of predators (Scherber *et al*., 2010). At the contrary, the negative correlation in between nano- and pico- heterotrophs and phytoplankton would corroborate the theory which supposes that a low specialization of protistan heterotrophs (Irigoien *et al*., 2004) and facilitation process in phytoplankton rich communities (Hillebrand and Cardinale, 2004) would decrease predator diversity.

Other prospect of this study would be to study the correlation with the functional diversity of heterotrophic protists. Although our trait approach was not effective into sorting distinct strategies of heterotrophic protists, the size diversity of the heterotrophic protist could correlate with an increase in the variability of prey (García-Comas *et al*., 2016).

CHAPTER III: THE FUNCTIONAL ROLE OF PARASITISM IN A COASTAL ECOSYSTEM

Résumé (en français)

L'abondance des protistes parasites dans le milieu marin a largement été mis en avant par les méthodes d'échantillonnage génétiques. Si nous savons encore peu de choses sur les espèces parasites, leur rôle fonctionnel dans l'écosystème pélagique est mieux compris et apparaît comme crucial dans la régulation des abondances de leurs proies, la facilitation de transferts trophiques et la création d'interactions maintenant la stabilité des écosystèmes. Afin de mieux comprendre le fonctionnement de ce compartiment dans l'écosystème côtier nous nous sommes intéressés à la communauté de protistes parasites associés à des blooms d'une espèce de dinoflagellé toxique *Alexandrium minutum* (Halim, 1960). Les blooms d'*Alexandrium minutum* constituent une nuisance pour les côtes bretonnes depuis les années 90. Ces blooms semblent être favorisés par l'eutrophisation due aux activités humaines et sont fréquemment infectés par des parasites des genres *Amoebophrya* et *Parvilucifera*. Récemment les blooms d'*Alexandrium minutum* se sont répandus dans la rade de Brest, notamment à l'embouchure de la rivière de Daoulas où *A. minutum* a atteint un maximum de 40 millions de cellules/L en 2012. Nous avons échantillonné des blooms d'A. minutum à l'embouchure de la rivière Daoulas en 2013, 2014 et 2015, et nous avons étudié la communauté de protistes associés à ces blooms par une approche génétique. Les protistes parasites d'*A. minutum* ont été identifiés par deux manières : 1) en comparant notre marqueur génétique à des séquences génétiques de références associées à des parasites reconnus d'*A. minutum*, et 2) en étudiant l'association statistique entre des protistes identifiés comme parasites avec *A. minutum*. Notre analyse démontre la présence de parasites des genres *Amoebophrya* et *Parvilucifera*, ces organismes semblent maintenir des interactions avec *A. minutum* qui sont stables et répétées au cours des trois blooms. Ces résultats supposent que le rôle fonctionnel des protistes parasites est joué par des espèces fortement spécialisées à leur hôte.

Context

Parasitic protists are recognized as architect of the marine pelagic food-web. They multiply species interactions in an ecosystem, contribute to terminate phytoplankton blooms and to biogeochemical cycles by creating new pathways of organic matter. Questioning the nature and dynamics of the parasitic function in marine ecosystems is thus crucial to understand and predict global patterns of protistan diversity. Advances in the sampling of marine protistan parasites arose with the development in genetic and sequencing methods that stressed their natural abundance in the environment. Using the observations of environmental DNA markers and new methods for network inference also helped to identify host/parasite complex in pelagic ecosystems. Here, we apply this methodology to identify the protistan parasites associated to *Alexandrium minutum,* a harmful dinoflagellate that bloomed in the Bay of Brest in 2013, 2014 and 2015. First by combining genetic markers and sequence homology with genetic references, we identified known-parasites of *A. minutum* in our survey. As a second step, we used network inference to study the parasites well associated to *A. minutum* and the interaction repeated over blooms. Our results demonstrate that the parasitism of *A. minutum* was played by few taxa recurrent over years and blooms, supposing a strong specialization in their host.

Author Contributions

The samples used in this chapter have been retrieved by members of Ifremer lab and staff from the Daoulex project. I took part in the genetic procedures for the 80 samples of the 2015 survey, the other samples (2013 and 2014) were processed by Sophie Schmitt (Ifremer de Brest). Environmental variables have been measured members of Ifremer. All samples were sequenced by the Genotoul platform and bioinformatics were carried out by Stéphane Audic (SBR). Gabriel Metegnier, Mickael Le Gac, Annie Chapelle, Samuelson Nzeneri (Ifremer de Brest) and Laure Guillou (SBR) all participated in discussions that have structured this paper. I have carried out all the analysis presented in this chapter. I have written this manuscript under the supervision of Raffaele Siano and Marc Sourisseau. This manuscript is still under preparation, it will potentially be submitted to the Journal of Microbial Ecology.

Recurrent parasite/host interactions during blooms of the toxic dinoflagellate *Alexandrium minutum* in the Bay of Brest

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Abstract

Protistan parasites seem to play many roles in coastal ecosystems, one of them is the regulation of other harmful protists when they bloom. *Alexandrium minutum* is a harmful dinoflagellate that causes an environmental issue to the coast of Brittany (West-Atlantic, France) since the 90's. The protistan parasites that regulate the blooms of *A. minutum* were thus extensively studied, and members of the parasitic genera *Amoebophrya* and *Parvilucifera* were detected. However, recent investigations highlighted the existence of two cryptic species of *A. minutum* in the coasts of Brittany, questioning the extent of specialization of its parasites. *A. minutum* recently spread in the neighbor bay of Brest (Brittany, France), genetic investigations proved that three blooms in 2013, 2014 and 2015 were dominated by a single cryptic species of *A. minutum*. We investigated the protistan parasites associated to these blooms with a metabarcoding approach and statistical analysis. A focused was made on A) the known parasites of *A. minutum* and B) the parasites with a recurrent interaction with *A. minutum* across blooms. We illustrate that the interactions were carried out by few specialized parasites.

1) Introduction

Parasitic protists of planktonic organisms have been mostly neglected by marine ecologist, this neglect arose from the difficulties into sampling these organisms and to understand their complex life cycle and ecology (Skovgaard, 2014). Advances in the sampling of marine protistan parasites came with the development in genetic and sequencing methods that stressed their natural abundance in the environment (López-García *et al*., 2001; Guillou *et al*., 2008). From then on, a particular attention has been given to parasites of Harmful Algal Blooms because some parasites had already been described (Coats, 1999; Erard-Le Denn *et al*., 2000; Coats and Park, 2002; Park *et al*., 2004), and because these parasites seemed to be very specialized to their preys which allowed them to efficiently terminate HAB bloom (markedly reduce the abundance of the HAB, thus ending its bloom) (Chambouvet *et al*., 2008).

Alexandrium minutum (Halim, 1960) is a HAB dinoflagellate known for its production of saxitoxins that causes Paralytic Shellfish Poisoning (PSP; Anderson *et al*., 2012). *A. minutum* has been signaled along the French coasts around 1985 (Lassus and Bardouil, 1988; Sournia *et al*., 1990; Belin, 1993) and, soon after its detection, *A. minutum* started to grow in considerable proportions within the rivers of north Brittany (up to 10^7 cells.L^{-1} in the rivers of Morlaix and Penzé, Chapelle *et al*., 2007) thus creating toxic events for neighboring shellfish communities. The factors favoring the proliferation of *A. minutum* were extensively studied, early summer conditions with high water temperature, high phosphate, in addition to a higher retention time due to low hydro-dynamism were all incriminated (Andrieux-Loyer *et al*., 2008; Chapelle *et al*., 2010; Guallar *et al*., 2017; Sourisseau *et al*., 2017). In the Penzé and Rance estuaries (Brittany, France), biotic regulations were also studied and distinct parasitoids of the genera *Parvilucifera* and *Amoebophrya* were shown to infect the blooms of *A. minutum* (Erard-Le Denn *et al*., 2000; Chambouvet *et al*., 2008; Lepelletier *et al*., 2014). Genetic investigations of the *A. minutum*/parasites complex further showed that there existed rapid and co-evolved genetic differentiations in between the host and its parasites (Dia *et al*., 2014; Blanquart *et al*., 2016), supposing a local adaptation and specialization of the parasites. However,

a recent study highlighted that there existed two distinct cryptic species of *A. minutum* within these estuaries (Le Gac *et al*., 2016), thus questioning the sources of genetic differentiations and the specialization of *A. minutum*'s parasites.

More recently, the blooms of *A. minutum* have spread in the Bay of Brest (Brittany, France), notably at the mouth of the Daoulas river where concentrations exceeded 40 $x10^6$ cells.L^{-1} in summer 2012 and were maintained in considerable proportions throughout the summers of 2013, 2014 and 2015 (Chapelle *et al*., 2015; Klouch *et al*., 2016). The following summers of 2016, 2017 and 2018, showed weaker blooms of *A. minutum,* reduced both in proportion (but still few maxima of 10^6 cells.L^{-1}) and in time (one to few days) (Nzeneri *pers. comm*.). A recent work, highlighted that only one of the cryptic species of *A. minutum* dominated the blooms of 2013, 2014 and 2015 within the bay of Brest (Metegnier *et al*., submitted).

In this still preliminary study, we analyzed the parasite community associated to the blooms of *A. minutum* in 2013, 2014, 2015 at the mouth of the Daoulas river. Environmental DNA was analyzed through a metabarcoding approach and parasite OTUs were detected with a trait table previously developed (see Chapter I, http://doi.org/10.17882/51662). Considering a single cryptic species of *A. minutum* during the three years sampled, our aim was to study if the parasite community associated to the blooms was consistent (composed of the same parasitic species) or variable over time. This analysis will help in elucidating whether the function of parasitism in a costal ecosystem is represented by specialist parasites or by a changing community of parasitic species varying over time.

2) Material and Methods

a) Sampling strategy

At the mouth of the Daoulas river of the Bay of Brest (Brittany, France) (Figure 31), cell counts of *A. minutum* are estimated on a weekly basis by the French monitoring REPHY (http://envlit.ifremer.fr) and in the frame of specific research project (Daoulex, AlezBreiz). During this study, water sampling was increased in frequency, when *A. minutum* cell-count was close to 10 000 Cell.L^{-1} (Figure S20), this threshold

also represents the concentration above which *A. minutum* is considered potentially toxic (as defined by the REPHY). This sampling resulted in the monitoring of three blooms of *Alexandrium minutum*, in 2013 (from 08 July to 08 August), 2014 (from 30 May to 18 August) and 2015 (from 15 June to 11 August) with a three- to five-day interval. In this mixed tidal estuary, samples were taken at subsurface (0-1 m), and in a two-hours period around the high tide of each sampling day, to sample a similar water mass. Overall, blooms started in early June and ended in early to mid-August, however in 2014 the bloom started earlier and ended later, therefore during this year more environmental samples were collected (2013: 11, 2014: 22, 2015: 16). Our sampling yielded a total of 49 distinct environmental samples, collected in duplicate (98 samples). A seawater differential filtration approach was used to separate the communities of micro- (> 20 μm), nano- (20-3 μm) and pico- (3-0.2 μm) plankton. Carbonate membrane filters of 47 mm in diameter (Main Manufacturing, Michigan, USA) were used for each pore sizes. Particles of the two first size fractions were separated by consecutive filtration with a peristaltic water pump and swinnex filter supports, due to filter clogging the volume filtered ranged in between 1.5 and 5L. The residual filtrate was used for separate filtration onto the 0.2 μm filters. Only samples of micro- and nano- plankton were collected in duplicates, resulting into 242 water filters, in total for the three years. After filtration, filters were flash-frozen in liquid nitrogen and stored at -80 °C until DNA extraction.

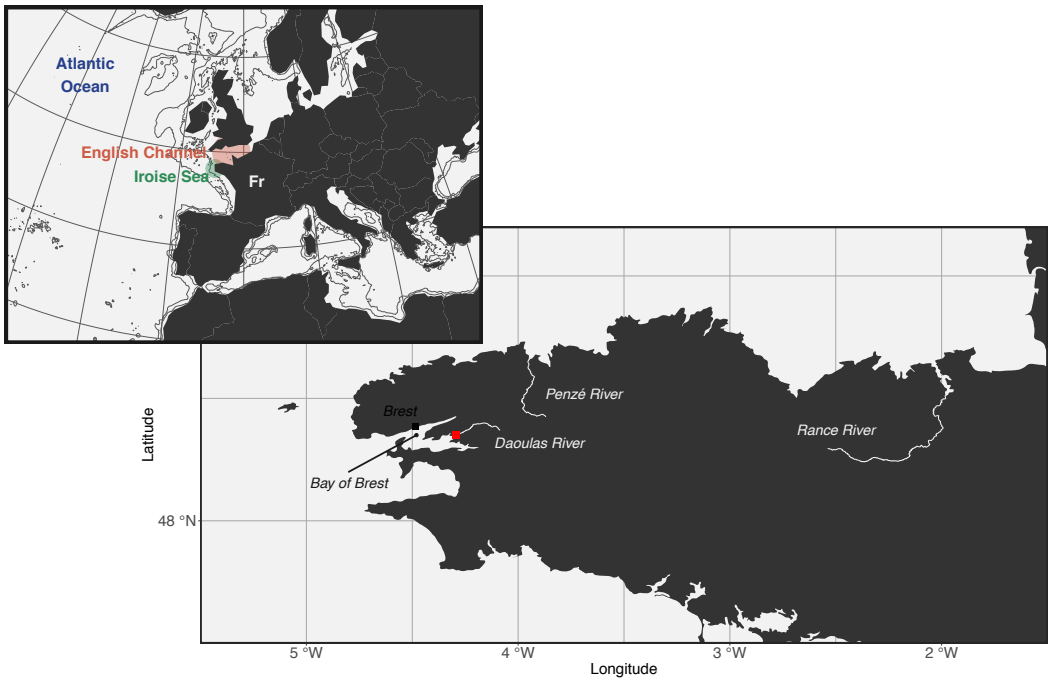

Figure 31: Geographical context and sampling point position within the Bay of Brest.

Temperature and salinity were measured with a manual CTD probe. Additional water samples were taken to estimate nutrient concentrations (Nitrate NO_3^-, Nitrite NO_2^-, Phosphate PO_4^{3-}, Ammonium NH_4^+ and Silicate $Si(OH)_4$) measured with a Seal Analytical AA3 HR automatic analyser, following the procedures described by Aminot & Kérouel (2007).

b) Genetic procedures

A metabarcoding approach was adopted to characterize the genetic diversity of the protistan community associated with the blooms of Alexandrium minutum. The hyper-variable V4 domain of the 18S rDNA region was chosen as a barcode for its conservative character within the eukaryotic microbial community and its relatively high length (230-520bp; Nickrent & Sargent 1991) which allows a good genetic distinction of marine protists (Stoeck et al., 2010; Behnke et al., 2011; Dunthorn et al., 2012). Genomic DNA, issued from the cells collected on water filters, was isolated following the protocol of DNA extraction kit Nucleospin Plant II (Macherey-Nagel, Hoerdt, France). In parallel, some blank extractions (Millipore filtered water) were carried out to check and validate the extraction procedure. DNA quality (proteins/DNA absorbance: A260/A280) and concentration of purified

products were respectively measured using a BioTek FLX 80 spectrofluorophotometer and a Quant-iT PicoGreen dsDNA quantification kit (Invitrogen, Cralsbad, CA, USA) following the manufacturer's instructions. Final DNA concentration of all extracts was normalized to 5-10 ng/μL. PCR was then ran with V4 markers assembled with the GeT-PlaGe adapters of the sequencing platform Genotoul (http://get.genotoul.fr/ ; Forward : V4f_PlaGe: 5'CTT-TCC-CTA-CAC-GAC-GCT-CTT-CCG-ATC-TCC-AGC-A(C/G)C-(C/T)GC-GGT-AAT-TCC'3, Reverse: V4f_PlaGe 5'GGA-GTT-CAG-ACG-TGT-GCT-CTT-CCG-ATC-TAC-TTT-CGT-TCT-TGA-T(C/T)(A/G)-A'3). The process of PCR amplification was carried out three times for each DNA extract (representing a unique filter). The amplification protocol consisted of a denaturation step at 98°C for 30s, followed by two set of cycles 1) 12 x [98°C (10s), 53°C (30s), 74°C (30s)] and 2) 18 x [98°C (10s), 48°C (30s), 74°C (30s)]. The cycles were followed by a final elongation at 72°C for 10 min. Amplification results were verified by gel electrophoresis, triplicate reactions were pooled and purified using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Hoerdt, France). Purified products were diluted to obtain equimolar concentrations before library construction at Genotoul for Illumina MISeq (2x250) sequencing. A single library was assembled; sequencing results are available at:
doi.org/10.12770/16bc16ef-588a-47e2-803e-03b4acb85dca.

c) Bioinformatics analyses

Bioinformatics were carried out on a larger sequencing dataset comprising (7 libraries, see Chapter I) to increase the number of sequences which allows a refined OTU construction and error detection. The cleaning steps and the rest of bioinformatics are the same as in (Ramond *et al.*, submitted, see Chapter I). After cleaning steps, sequences were annotated taxonomically with PR^2 (Guillou *et al.*, 2013) and clustered into OTUs with swarm2 (Mahé *et al.*, 2014). Each OTUs was then given the taxonomic reference and the nucleotide sequence of its most abundant metabarcode.

The dataset used in this study (protistan communities from the Daoulas river in 2013, 2014, 2015) contains 38 227 OTUs, annotated to 1167 distinct taxonomic references and cumulating into 7.5 10^6 reads. Sampling quality was evaluated by

rarefaction curves (reads vs. OTUs numbers) calculated with the *rarecurve*() function of R package "vegan" (Oksanen *et al*., 2016; Figure S21). The difference in community composition across replicated samples, estimated by the OTUs relative abundance, was tested with a Permutational Multivariate Analysis of Variance (PERMANOVA; *adonis()* function of R package "vegan").

d) Detection of A. minutum

We retrieved 2724 OTUs annotated to *Alexandrium minutum*. We used the sequence representative of each OTU and tested their identity with genetic references of *Alexandrium minutum* (NCBI's taxa identification number [39455], regrouping 12 accession number JF521634.1, JF521633.1, JF521632.1, JF521631.1, AY883006.1, AY831408.1, AJ535388.1, DQ168664.1, AJ535380.1, JF906998.1, JF521635.1, U27499.1). The percentage of identity between our sequences and the reference sequences selected was calculated using the 'megablast' algorithm (Altschul *et al*., 1990), the values ranged in between 78 and 100%. Only the OTUs with a percentage of identity of 100% to the reference sequence were considered in this study, the remaining OTUs were discarded. The relative abundance of these OTUs was calculated to represent the proportion of *A. minutum* across our samples.

e) Parasites of A. minutum

The selection of the OTUs that presented a parasitic lifestyle was carried out with a trait-based approach previously developed (see Ramond et al., *submitted*; http://doi.org/10.17882/51662). Briefly, using their taxonomic references and an extended bibliography, our OTUs were annotated with 13 biological traits (SizeMin, SizeMax, Cell Cover, Cell Shape, Presence of Spicule, Cell Symmetry, Cell Polarity, Coloniality, Motility, Plastid Origin, Ingestion method, Symbiosis type and Resting Stage during the life cycle). Because of the low taxonomic resolution of some OTUs (i.e. assigned only at the family level, class or domain) and/or the lack of scientific information, these traits could only be annotated to a subset of 948 out of the 1167 distinct taxonomic references (corresponding to 20 382 of the 38 227 total OTUs)

retrieved in our dataset. Parasites OTUs were identified with the trait "Symbiosis type" and the modality "parasitic".

To study the diversity and ecology of the potential parasites of *A. minutum* we followed two distinct approaches. In the first approach, we selected parasites of *A. minutum* described in the literature Based on the website AQUASYMBIO (http://aquasymbio.fr/en), an online database of known parasites and endosymbioses in aquatic ecosystems, 3 taxa of known parasites of *A. minutum* were targeted: *Parvilucifera rostrata* (Karpov and Guillou, 2014), the species complex uniting *Parvilucifera infectans* (Norén & Moestrup, 1999) and *Parvilucifera sinerae* (Figueroa, Garcés, Massana & Camp, 2008) (a complex recently put together by Jeon *et al*., 2018) and the species complex *Amoebophrya ceratii* (MALV II clade 1; Cachon, 1964). The sequences of theses parasites have been searched in the NCBI's database. Within our database, we selected the OTUs with a taxonomic reference identified at least as the genera *Parvilucifera* (Norén, Moestrup & Rehnstam-Holm, 1999) or *Amoebophrya* (Koeppen, 1894), and then, the sequences of these OTUs were compared with the genetic references of the parasites identified with AQUASYMBIO, again using the 'megablast' algorithm (Altschul *et al*., 1990) and NCBI's database. Only the OTUs whose sequences matched at 100% with the genetic reference of species identified with AQUASYMBIO were considered as known-parasites associated to *A. minutum* and selected by our first approach.

As for the second approach, we applied a protocol based on statistical associations between parasite OTUs (comprising unknown parasites of *A. minutum*) and the OTUs of *A. minutum*, in order to identify new potential associations and to analyze the whole parasite community in our ecosystem. First, a dataset per each size fractions (micro, nano and pico-plankton) was constituted (3 dataset composed of 49 samples). Across these datasets, all OTUs in less than 5 samples (10% of each dataset) were discarded. To estimate the pairwise-association between two OTUs we used the coefficient of 'proportionality' (Quinn *et al*., 2017). As advocated recently, 'proportionality' represent a better alternative than correlation to study pairwise associations within compositional datasets (data forced to semi quantitative abundance, i.e. most of sequencing datasets) (Quinn *et al*., 2017). We thus used a R package (R Core Team Development, 2015; Quinn *et al*., 2017) that computes 1) a 'centered log-ratio transformation' of OTUs read abundance (a transformation that considers the compositional aspect of a dataset) and 2) cross-OTUs proportionality

coefficients. The coefficient of proportionality ρ_p ranges in between -1 and 1, respectively indicating negative or positive pairwise association. Three matrices of pairwise-associations, based on the proportionality coefficient ρ_p, were constituted on the basis of the dataset representing each size-fraction. Then, the three matrices of associations were merged together into one single matrix. For the pairwise-associations present in all the size fraction matrices, we selected the maximal ρ_p value across the three matrices. Pairwise association could not be calculated for a pair of OTUs found strictly in distinct size-fractions, they were given the association value of 0. Out of the selected association, we then considered only associations with an absolute value of $\rho_p > 0.5$ as representative of potential interactions occurring in our dataset. Since some parasites of *A. minutum* are known to infect other dinoflagellates (Park *et al.*, 2004; Chambouvet *et al.*, 2008; Figueroa *et al.*, 2008), associations with other dinoflagellates OTUs were thus also investigated. The parasites that presented a good association with the *A. minutum* OTUs (proportionality > [0.5]) constituted a community of well-associated parasites identified by our second approach.

f) Ecological analysis

To test the robustness and repeatability of the interactions between the well-associated community and *A. minutum*, we rigorously repeated our statistical protocol for each distinct year. A Venn diagram was established by counting the number of initial interactions retrieved and/or shared across 2013, 2014 and 2015.

Using the OTUs from the well-known parasites we investigated the dynamic of the potential host/parasite complex over the blooms monitored. The influence of environmental factors on the host/parasite complex was studied using the Spearman rank correlation (better adapted to our compositional dataset; Legendre and Legendre, 2012).

For both the well-known and the well-associated parasitic communities, the variation in the composition over years was tested with a PERMANOVA analysis, using the relative abundance of all these OTUs in our samples.

3) Results

a) Protist community diversity across the A. minutum blooms

The samples of eDNA (environmental DNA) collected during three blooms of *A. minutum* sampled in 2013, 2014 and 2015 (Figure S20), allowed to identify the protistan diversity and community composition that occurred during the blooming periods. The saturation curves failed to reach the asymptote when studied throughout a single bloom or size-fraction (Figure S21). PERMANOVA indicated that there existed no difference between samples and their replicates for the micro- and nano-plancton (R^2: 0.001 with 9999 permutations). Therefore, for the further analyses we focused on the environmental replicate (single water bottle) from which all the three size fractions were collected.

To show the protistan community of the Daoulas river, the relative abundances of the major taxonomic clades were studied (Figure 32a). OTUs from which the taxonomy could not be well determined represented a low portion of reads across our dataset (10% of read by sample in average), with a maximum of 40% within the pico-plankton in 2015, (Figure 32a). Within the micro-plankton, Dinophyta markedly dominated all samples (in average 69 % of reads by sample), followed by Diatoms (Bacillariophyta, 17% on average by sample) and Ciliates (Ciliophora, 5% on average) (Figure 32a). In nano-plankton (Figure 32a), Dinophyta (19% on average) and Ciliophora (4% on average) decreased while Diatoms showed a higher proportion (on average 23%). Smaller taxa also showed a higher abundance, notably Chlorophyta (13% on average), Cryptophyta (10% on average), but also numerous small heterotrophs (MAST: 3%, Picomonadida: 2%, Thecofilosea: 2% on average). The taxa found in the nano-plankton were in equivalent proportion within the pico-plankton (Figure 32a), Chlorophyta were however in higher relative abundance (28% on average) which decreased the abundance of Diatoms (12% of read by sample). This size-fraction also showed the highest proportion of the Marine Alveolate group (MALV, 3% by sample in average).

b) Identification and dynamic of Alexandrium minutum

The 2724 OTUs corresponding to the genus *Alexandrium* spp., accounted for 34 % (2.6 x10^6 of the 7.5 x10^6) of the total reads retrieved in our dataset (Figure S22). Out of these 2724 OTUs, 169 OTUs presented a V4 marker sequence that corresponded to 100% of blast identity to NCIBI's genetic references of *Alexandrium minutum* [taxid:39455]. These OTUs still contributed to 2.45 10^6 reads in our dataset, representing 33% of the total read number across our dataset and 94% of the initial read number associated to the genus *Alexandrium* (Figure S22), notably one single OTU accounted for 2.44 10^6 reads across our dataset.

The blooms of *A. minutum* had distinct phenology during the three years (Figure 32c). In 2013, *A. minutum* reached a concentration above 10 000 cell.L^{-1} from July-15 to August 01 with a maxima of 250 000 cell.L^{-1} observed on July-18 and July 22 and second maxima of 200 000 cell.L^{-1} on August-21. This pattern corresponded well to higher proportions of *A. minutum* OTUs within the micro-plankton with values around 40% of reads by sample (Figure 32b and 32c). The maxima also corresponded to peak proportions of *A. minutum* within the two smallest size-fractions (both around 30% read by sample). During the bloom of 2014, *A. minutum* presented two major peaks in cell counts on June-06 (10^6 cells.L^{-1}) and June-20 (5.5 x10^5 cells.L^{-1}), both surrounded by a fast increase and decrease in cell abundance. Still in 2014, a peak of 100 000 cell.L^{-1} on July-21 was identified, while cell abundances was still around 10 000 cells.L^{-1} from July-11 to August-14 (Figure 32c). During all these events the OTUs of *A. minutum* represented around 40% of reads by sample within the micro-plankton, and only the peak-abundance in cell counts coincided with a higher proportion of *A. minutum* in the pico and nano size-class (around 40% compared with 5% of read by sample elsewhere, both in nano- and pico-plankton) (Figure 32b and 32c). In 2015, the concentration of *A. minutum* was in lower numbers than in the previous years and only reached the maxima of 50 000 cells.L^{-1} on June-26 and 30 000 cells.L^{-1}on July-23, with surrounding dates of concentrations above 10 000 cell.L^{-1}, but the rest of samples had concentration under 10 000 cell.L^{-1}. This lower proportion was not traduced in the read proportions of *A. minutum* OTUs in the micro-plankton, which accounted for 40% of reads by sample, but a notable decrease from July-23 to the end of monitoring on August-08 (close to 20% of read by sample) was observed (Figure 32b and 32c).

A. minutum OTUs relative abundances in the micro-plankton obtained with our metabarcoding dataset, well coincided with the cell concentrations obtained by

microscopy analyses. Within nano- and pico-plankton, the proportion of *A. minutum* was lower but reached values above 20% during brief peaks (Figure 32b). The peaks within the smallest size-fraction coincided with events when the cell count of *A. minutum* at the Daoulas river was high (ca. above 50 000 cells.L^{-1}) (Figure 32c).

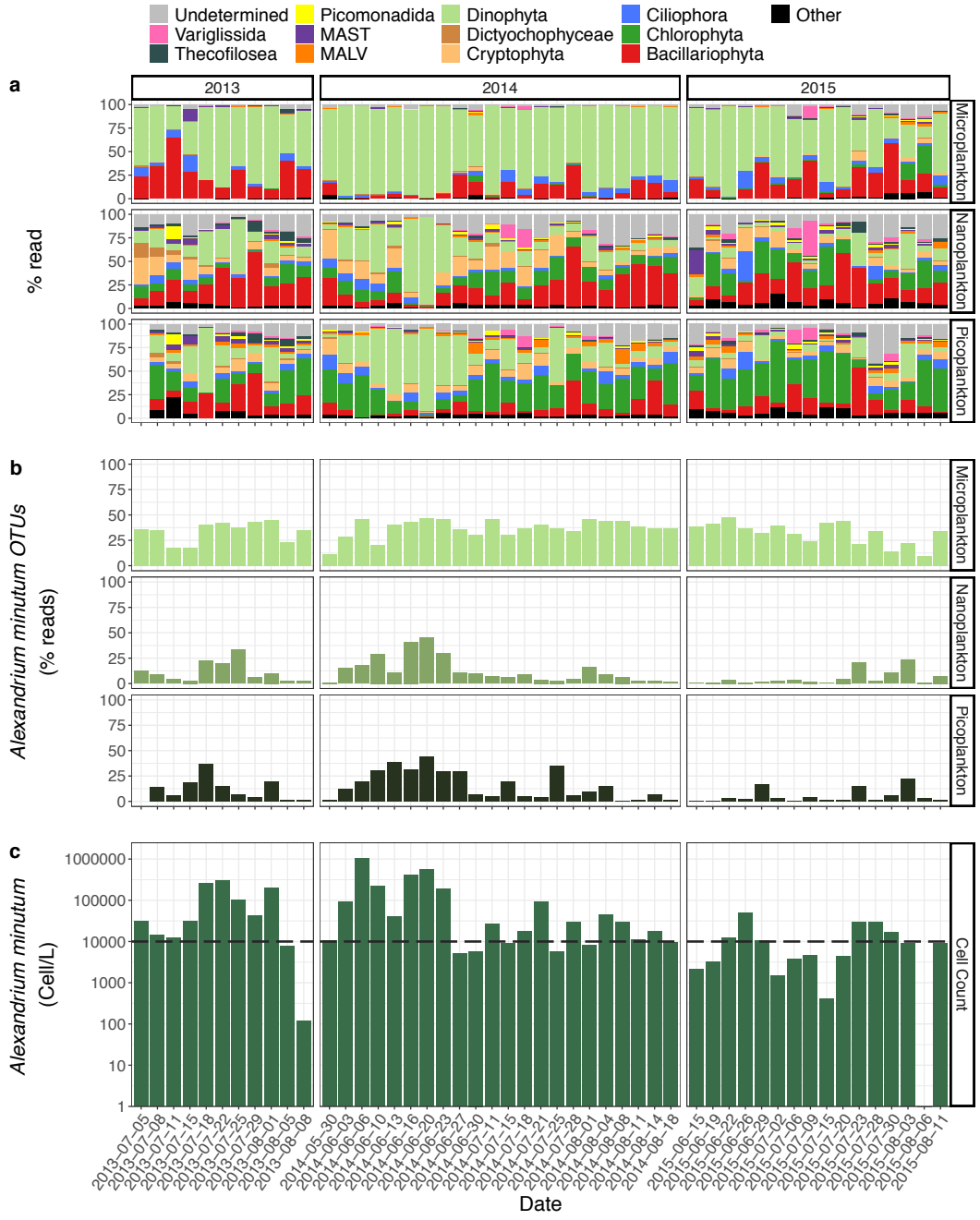

Figure 32: Results from the metabarcoding of the protistan community at the mouth of the Daoulas river and comparison with cell count (cell/L). a) Distribution of the distinct protistan taxa estimated by metabarcoding across our dataset. One replicate comprising all size-fractions is shown for each of the 49 environmental samples and three blooms of 2013, 2014, 2015. Relative abundance was calculated based on the number of reads of OTUs corresponding to the shown taxa, 'Other' represented the read number of taxonomic ranks with a relative abundance < 10%, 'Undetermined' represented the read number of OTUs with a low taxonomic level. b) Relative abundance of A. minutum OTUs from which the sequence matched at 100% with the genetic references of A. minutum in NCBI. c) Cell count of A. minutum carried out in parallel to our metabarcoding survey, the scale is logarithmic.

The proportion of *A. minutum* OTUs within the micro-plankton, correlated only with high phosphate concentration (Figure 34a). Within the smallest size-fractions, *A. minutum* correlated with a decrease in salinity, in ammonium and silicate, however it correlated positively with NOx (nitrate +nitrite) concentration (Figure 34a).

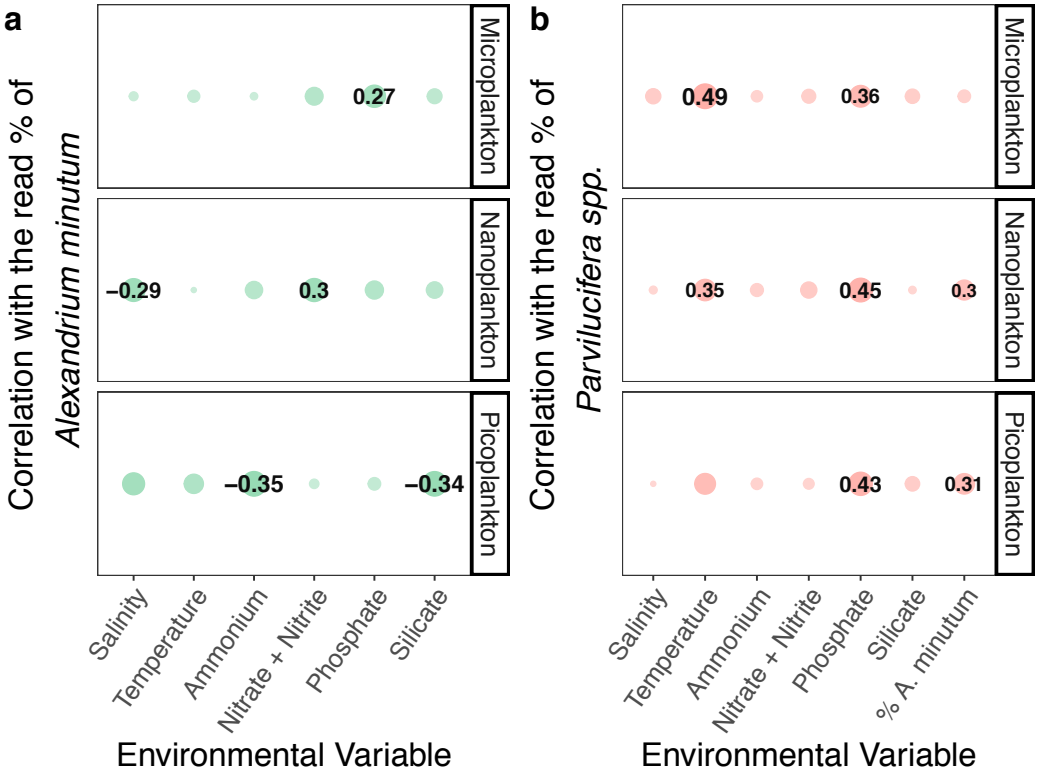

Figure 33: Correlation between environmental variables and the read relative abundance of a) A. minutum and b) Parvilucifera in micro-, nano and pico-plankton, measured at the mouth of the Daoulas river in 2013, 2014 and 2015. The Spearman rank correlation is used.

c) Identification and dynamics of known parasitic interactions

Within our metabarocding dataset, 1627 OTUs were associated to the functional group of parasites. Numerous parasite OTUs were known to infect larger metazoan organisms and were thus discarded from further analysis. However only, 84 OTUs corresponded to the genera *Amoebophrya* (62) and *Parvilucifera* (22) known for parasitic interactions with A. minutum. No OTUs corresponded at 100% with a genetic reference of *Amoebophrya* known to infect *A. minutum*, however 4 OTUs matched at 100% of identity with *Parvilucifera infectans-sinerae*. The 4

Parvilucifera infectans/sinerae OTUs were regularly present across *A. minutum* blooms, the PERMANOVA indicated that the composition in these 4 OTUs did not vary significantly across the years (R^2: 0.1; with 9999 permutations).

Although very low, the cumulated relative abundance of the 4 OTUs corresponding to *Parvilucifera infectans/sinerae* correlated well with high temperature and high concentrations of phosphate across size-fractions (Figure 34b). Within the smallest size fraction, *Parvilucifera* well correlated with a higher proportion of *A. minutum* (Figure 34b).

To investigate the dynamic of the supposed host/parasite complex within our dataset we used the number of read (Figure 34b) because the relative abundances of the *Parvilucifera* OTUs were too low and no dynamics were perceptible (the proportion of these OTUs was mostly under 0.01%). The number of reads of all *Parvilucifera infectans/sinerae* OTUs was cumulated to represent a single species-species interaction. Only one OTU out of the 4 accounted for 4584 reads (0.6% of the total read number). The other OTUs accounted for less than 15 reads across all the dataset. These peaks of *Parvilucifera* reads were retrieved within the pico-plankton (3-0.2 μm), although less-frequently, reads were also found in the higher size-fractions (Figure 34b).

The peaks occurred after a primary phase of high abundance by *A. minutum*; estimated in terms of read proportion, cells/L and read abundance (Figure 34a). Two peaks of *Parvilucifera* above 200 reads were observed in 2013 (on July-25 and August-5), the first within pico-plankton and the second within the micro-plankton (Figure 34b). These two peaks coincided with two dates that followed peaks in the blooms of *A. minutum*, 293 000 cells.L^{-1} on July-22 and 203 000 cell.$^{L-1}$ on August-01 (Figure 34a). During 2014, two peaks of *Parvilucifera* reads were observed on June-23 (600 reads) and August-01 (250) (Figure 34b). The first peak in *Parvilucifera* occurred, again, at date that followed a maxima in *A. minutum* abundance, 552 000 cell.L^{-1} at June-20, while the second peak occurred in a period where *A. minutum* showed constant and moderate abundance around 10 000 cell.L^{-1}, 8201 cell.L^{-1} and at the date of the peak (August-01) (Figure 34a). No peak of *Parvilucifera* was observed in 2015 where the number of reads was constantly under 10 except at a maximum of 52 reads at August-03, at the end of our monitoring (Figure 34b). The overall low abundance of the parasite in 2015 corresponded to a year with markedly lower *A. minutum* abundances (Figure 34a).

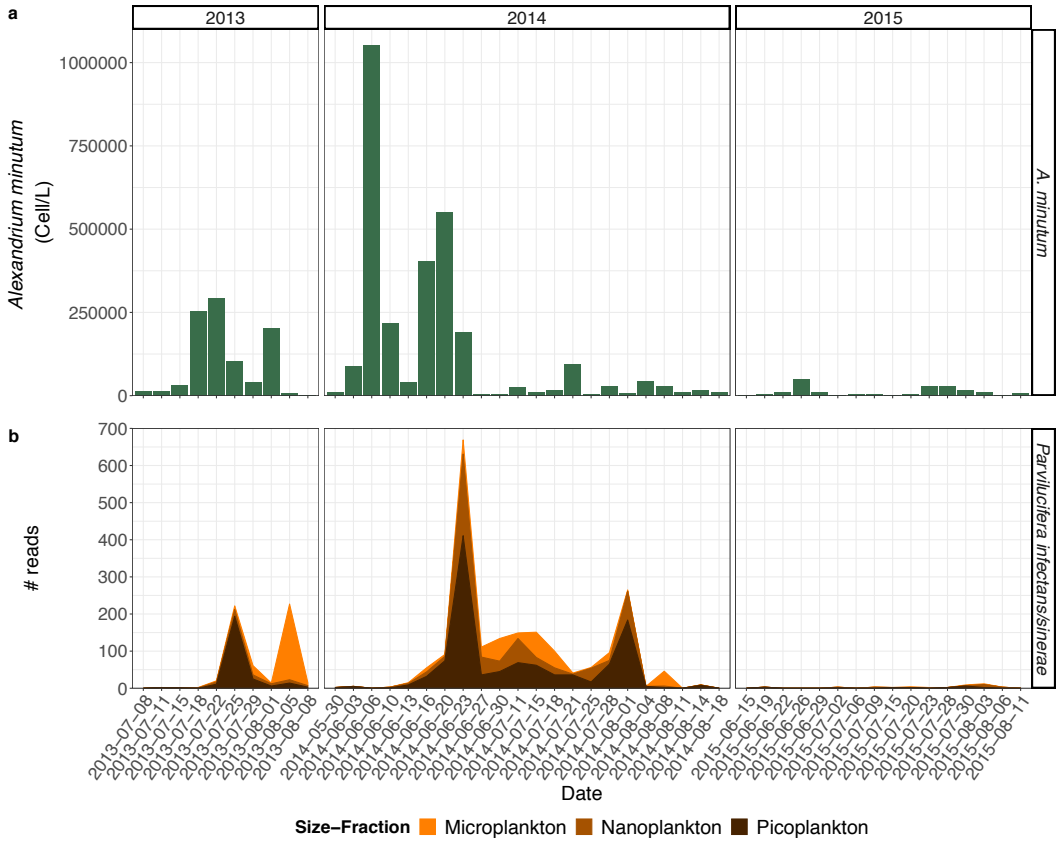

Figure 34: Dynamic of the Alexandrium/Parvilucifera complex throughout our monitoring at the mouth of the Daoulas river in 2013, 2014 and 2015. a) Cell count of A. minutum and b) read number of Parvilucifera across size fractions (colors of the area).

d) Other potential host-parasite interactions

We identified 12 OTUs well associated with *A. minutum* which could represent unknown potential interactions (Figure 35). The 12 parasitic OTUs identified all belonged to the Marine Alveolate Group, or Syndiniales, i.e. *Amoebophrya spp*. and the less precisely annotated OTUs of Malv I, II and III. All these OTUs correlated at least with one *A. minutum* OTUs but also with other dinoflagellate genera (Figure 35), like the phototrophic *Prorocentrum*, *Gonyaulax* or *Gymnodiunium* (although some are also mixotrophic), and the heterotrophic *Protoperidinium*, *Warnovia,* or *Pentapharsodinium*. Across the 12 parasite OTUs, the OTUs annotated to *Amoebophrya* were the most abundant (in general > 5 with a max of 19 reads by sample; Figure 36), other OTUs were observed in few number of reads by samples but they repetitively appeared across years (Figure 36). PERMANOVA analysis indicated that the composition of the 12 OTUs parasites well associated to *A. minutum* did not variate significantly across years (R^2: 0.04 with 9999 permutations).

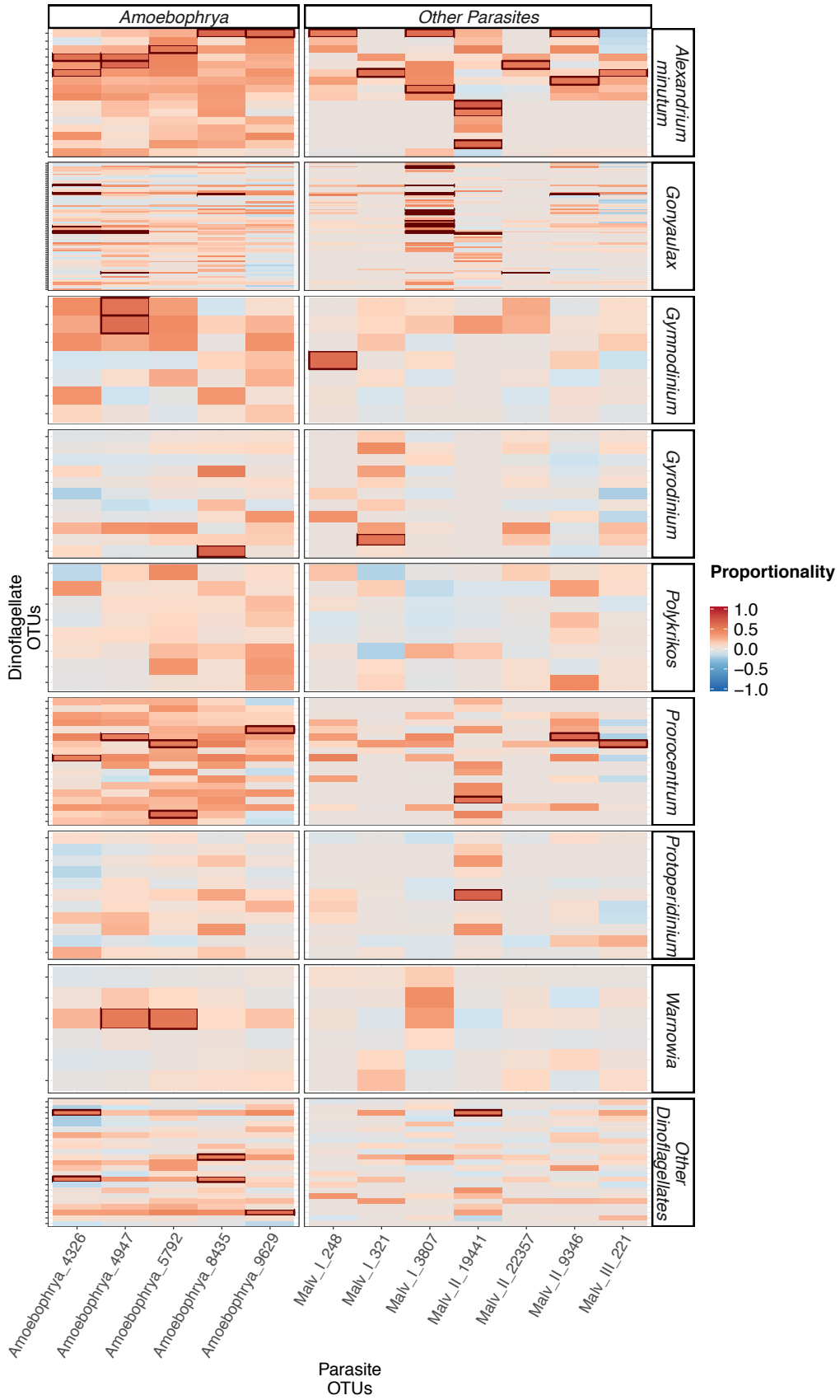

Figure 35: Heatmap representing the proportionality coefficient of association between the parasite OTUs well associated to the OTUs of A. minutum (Axis X) and the OTUs from A. minutum and other dinoflagellate genera (Axis Y). Good associations (absolute proportionality > 0.5) have been framed in dark red.

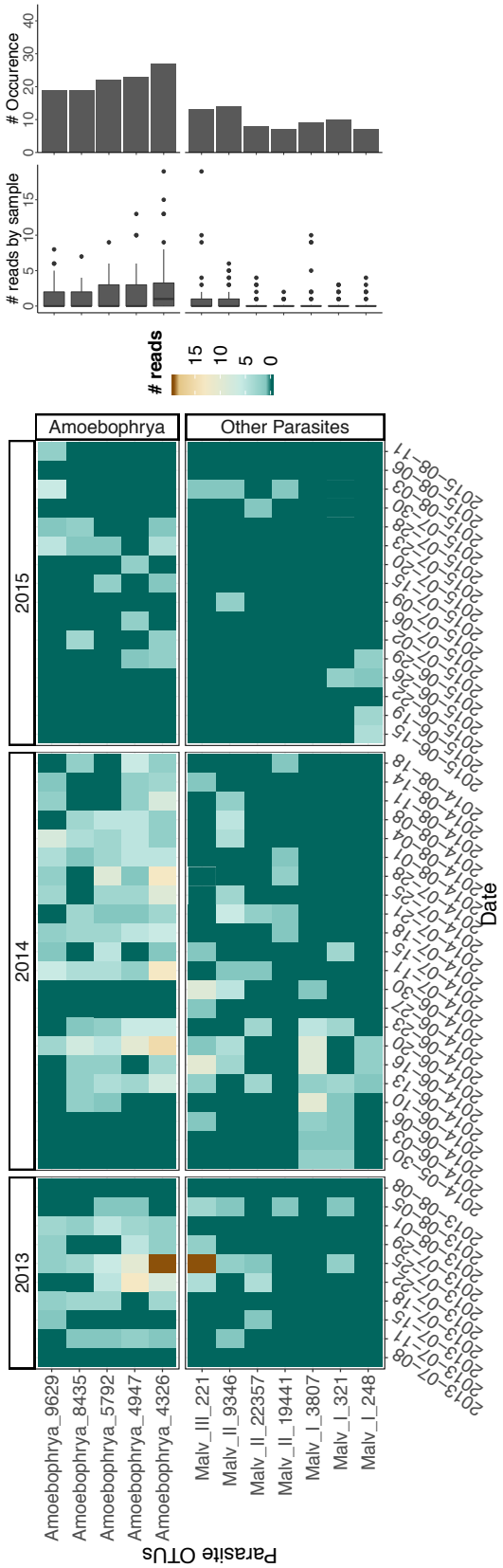

Figure 36: Heatmap of the total read abundance of each parasite OTUs well associated to A. minutum throughout our survey at the mouth of the Daoulas river (read vertically). At the right are also represented the distribution of values in read number by sample (boxplot) and the number of occurrence (number of sample in which the OTU is found).

The 12 parasite OTUs well-associated to *A. minutum* (10 distinct *A. minutum* OTUs involved in these interactions) accounted for 18 potential interactions with OTUs of *A. minutum* and 42 with OTUs of other dinoflagellates (potential interactions corresponds to a pairwise association with $\rho_p > 0.5$, red frames in Figure 35). However, these pairwise associations were not stable across time (Figure 37). Amongst the 18 interactions with *A. minutum* only 4 were strictly recurrent across each bloom (Figure 37). Those 4 OTUs included 3 OTUs of *Amoebophrya* and one OTU of Malv II clade 4, that currently contains only members of *Amoebophrya* (Guillou *et al.*, 2008). Interactions with other parasites and *A. minutum* were less stable and more specific to one bloom or two (Figure 37). Among the 42 pairwise associations with other dinoflagellates only two were stable along time (Figure 37), they involved two parasite OTUs of *Amoebophrya*, with an OTU of *Prorocentrum* and an OTU of *Gonyaulax spinifera*.

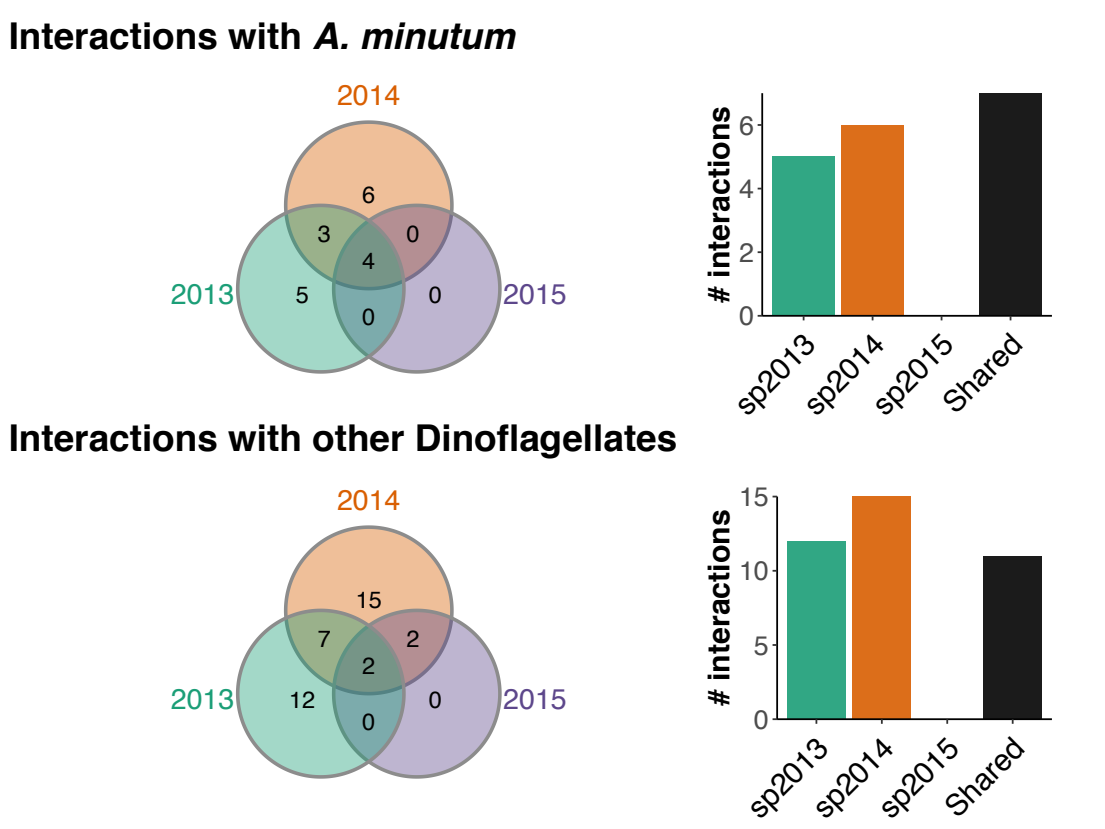

Figure 37: Recurrence of the interactions in between the well associated parasite OTUs and A. minutum as well as with other dinoflagellates throughout the three blooms surveyed at the mouth of the Daoulas river in 2013, 2014 and 2015. The occurrence of the pairwise associations (18 with with A. minutum, 43 with other dinoflagellates) in each bloom has been represented in a Venn diagram (at the left), the total number of interactions specific to each bloom or shared between blooms is shown at the right.

4) Discussion

Three consecutive blooms of *Alexandrium minutum* were observed in 2013, 2014 and 2015 at the mouth of the Daoulas river within the bay of Brest. Previous metatranscriptomic analyses carried out on environmental samples highlighted that these blooms were dominated by a single population of *A. minutum* (Metegnier *et al*., submitted). The aim of this study was to verify if the parasitic community accompanying these blooms was recurrent or variable over the time. Recurrence in the interaction implies that the parasitic association is very specific, reversely variations in time supposes that the parasitism function in a coastal ecosystem can be assured by different species. With a metabarcoding approach we searched for parasite protists that could potentially interact and regulate the bloom of the HAB species. During the three years of bloom monitoring, we regularly evidenced the presence of parasite OTUs from the species complex *Parvilucifera infectans/sinerae* that is known to infect *A. minutum*. The host/parasite data analysis showed good correlation supporting a potential interaction. However, only few records of drops in host abundances were observed in the presence of the parasites, complicating the analysis of a potential contribution of the parasite in the bloom termination of *A. minutum*.

With a statistical approach based on pairwise associations between OTUs across our dataset, we searched for other potential interactions between parasites, *A. minutum* and additional dinoflagellates. Supposing that our statistical associations were a potential for interactions, we evidenced 18 new potential parasites of *A. minutum*. However most of these interactions were not recurrent across the monitored years, indicating a more opportunistic nature in these interactions. Here we discuss the contribution of these results to the *A. minutum*/parasites complex and to the understanding of parasitism and its role within the marine ecosystems.

a) Metabarcoding approach for the study of the dynamic of Alexandrium minutum

Metabarcoding is increasingly used as a tool for the description of environmental microbial community interactions. Thanks to a primary taxonomic annotation of

genetic markers by PR^2 (Guillou *et al*., 2013), we evidenced classical taxa from coastal ecosystems in the Daoulas river (Massana *et al*., 2015; Hu *et al*., 2016), comprising notably Dinoflagellates, that contains *A. minutum*, and MALV, that contains known-parasites of Dinoflagellates, but also other phytoplankton taxa as Diatoms, Chlorophytes or Cryptophytes, and classical heterotrophic protists among Ciliates and the recently described Picomonadida, Variglissida, or MAST.

In a second time, a Blastn check (Altschul *et al*., 1990) with the NCBI database detected 169 OTUs annotated to *Alexandrium minutum*. This high number of OTUs was probably caused both by intraspecific genetic variability of the genetic region (V4 of the 18s) within the genus *Alexandrium* (Anderson *et al*., 2012) and the elevated read proportion facilitating sequencing errors. However due to parallel works on genetic and transcriptomic we have good reasons to consider that these OTUs are descriptors of a single species (Metegnier *et al*., submitted). The causality of this micro-diversity (Needham *et al*., 2017) remains therefore unknown. Although rarely reported (Zimmermann *et al*., 2014; Malviya *et al*., 2015; Abad *et al*., 2017; Groendahl *et al*., 2017), we also observed a good fit in between read proportion and cell counts of *Alexandrium minutum* (Figure 32), this allowed us to use the proportion of OTUs associated to *A. minutum* as a proxy for its dynamic across blooms.

The three blooms of *Alexandrium minutum* at the mouth of the Daoulas river in 2013, 2014 and 2015, had distinct phenology (Figure 32 and S20). As expected, due to its size range between 17-29 μm (Balech, 1989), *A. minutum* was found mostly in the micro-plankton size fraction. Classically, the phenology *A. minutum* in this size-class was influenced by elevated phosphate concentrations (Figure 33a). Indeed, in estuaries, pulsed phosphate inputs issued from the sediments often favors *A. minutum* (Andrieux-Loyer *et al*., 2008), which is competitive in the uptake of phosphate under high concentrations and uses a storage capability that later becomes a selective advantage under phosphate-depleted conditions (Labry *et al*., 2008; Chapelle *et al*., 2010). The presence of *A. minutum* within the smallest size-fractions coincided with high cell abundance of *A. minutum* in the field, the contamination could be due to clogging with a high number of *A. minutum* cells and cell breakage from the higher size-fraction.

b) Known parasites of A. minutum and their dynamic

Thanks to our functional approach we identified 1627 parasite OTUs at the mouth of the Daoulas river. This diversity was probably underestimated due to the high number of parasites that remains uncultured, overlooked and from which we do not have genetic information (Strassert *et al*., 2018). Among these parasites, several were known for infecting larger metazoan; e.g. *Polyplicariida spp*., polychaete-infecting gregarines (Cavalier-Smith, 2014), *Abeoforma spp*. and *Pirum spp*. that infects mussels and peanut-worms (Marshall and Berbee, 2011), or the suctorian ciliates *Ephelota spp*. that are epizoic organisms found on krill, hydrozoan or other benthic metazoans (Stankovic *et al*., 2002; Chen *et al*., 2008; Tazioli and Di Camillo, 2013). Considering their host preference, these parasites were discarded from our analyses, since marine dinoflagellate parasite were our principal target.

We used a strict approach, based on the taxonomic annotation of PR^2 and further sequence homology with NCBI's reference sequence, to select known parasite of *A. minutum*. This approach allowed us to retrieve 4 OTUs from the species complex *Parvilucifera infectans/sinerae*. These OTUs blasted at 100% of identity with both NCBI's references for *P. infectans* and *P. sinerae*. Although previously distinguished (Garcés and Hoppenrath, 2010; Lepelletier *et al*., 2014), these two species were recently brought together by highlighting the few divergence in morphology, hosts preferences, and most notably nucleotides within their small subunit (SSU) rDNA (Jeon *et al*., 2018). Although some sequences were also annotated at 100% with some *Amoebophrya* spp found in NCBI's database, the lack of information about a parasitic interaction of these taxa and *A. minutum* hindered them from being taken into consideration.

The peaks in read abundance of Parvilucifera (Figure 34b), coincided relatively well with high abundances of *A. minutum* (Figure 34b). In addition, few events of marked decrease in *A. minutum* followed by an increase in *Parvilucifera* (Figure 34) suggested a potential host/parasitic dynamic in the bay of Brest. The *Alexandrium/Parvilucifera* complex is supposed to be widespread and has been described among the coasts of Sweden (Norén *et al*., 1999), Mediterranean Spain (Delgado, 1999; Figueroa *et al*., 2008) or in the harbors of Korea (Jeon *et al*., 2018). In France, the parasitism of *Parvilucifera* was described and retrieved from *A. minutum* for the first time in the estuaries of North Britany (Erard-Le Denn *et al*.,

2000), notably in the Penzé and the Rance rivers (Lepelletier *et al*., 2014). Here we report for the first time the presence of *Parvilucifera infectans*/*sinearae* associated to *A. minutum* blooms within the bay of Brest. The association between *A. minutum* and *Parvilucifera* was positive and recurrent within our dataset (Figure 33 ad 34). Our data indicate that 1/ the parasite's infection was found only above a threshold in the abundance of the host (see e.g. Holt *et al.*, 2003), and 2/ that the parasite did not cause enough mortality to the host to create a negative relationship in the timescale of our study (3-5 day frequency) (see e.g. Blanquart *et al.*, 2016; Berdjeb *et al.*, 2018). Positive associations have also been interpreted as a practical proof of rapid co-evolution processes in between the parasites infectivity and host resistance (Rabajante *et al.*, 2015; Berdjeb *et al.*, 2018). However, such interpretations necessitate measures of infectivity and a proxy for adaptive changes in both the host and the parasite (e.g. genotyping, Blanquart *et al.*, 2016), therefore they co-evolution processes could not be analyzed with our dataset.

The parasite was found mostly within the smallest size fractions (Figure 34), this supposed that the read abundance was dominated by free-living stages (i.e. zoospores ranging in between 1.2 and 2.7 μm), (Figueroa *et al*., 2008; Garcés and Hoppenrath, 2010). Some events however showed the presence of the parasite within micro-plankton, suggested that at these points *Parvilucifera* was present within a micro-planktonic cell host, whether at the zoosporic or at the sporangium life-stage, i.e. an intra-host stage ranging between 13.4 and 44.9 μm (Figueroa *et al*., 2008). In the case of *Parvilucifera*, the intra-host life cycle can last one to six days (Norén *et al.*, 1999; Figueroa *et al.*, 2008), while the liberated zoospores last only few minutes in the absence of hosts in the environment (Delgado, 1999). In consequence, with our sampling strategy we could only encounter punctual events of infection (when the parasite was found in micro-plankton) and zoospore releases (when the parasite was found in pico-plankton). This peculiarity of the host-parasite interaction and the consequent bias of our dataset could probably justify the unperfected correlation between *Parvilucifera* and *A. minutum* in our study (Figure 33). A daily sampling could have been preferable to better characterize this interaction. The parasite *Parvilucifera* also correlated well with phosphate and temperature (Figure 33). Figueroa *et al.* (2008) notably stressed the importance of phosphorus limitations in the infectivity of some parasites and hypothesized that similar limitations could influence the life-cycle of *Parvilucifera*. As for temperature, an increase could

highlight a decrease hydrodynamism favoring water warming, this would also decrease the water dilution rate and this process rather than a simple increase in temperature have been hypothesized to favor parasitic infection (Llaveria *et al.*, 2010; Siano *et al.*, 2011). It is also probable that the parasite could be fortuitously correlating with the environmental preferences of its host, as *A. minutum* also correlated with phosphate (Figure 33).

The OTUs of the parasite were all found across the three years (PERMANOVA), in consequence *Parvilucifera* might have played the role of the parasitism across the blooms of *A. minutum* monitored. However, the very low abundance of *Parvilucifera* in 2015 (Figure 33) indicated that to grow the parasite probably needs its hosts to be very abundant, as typical for parasitic interactions (Holt *et al*., 2003). The nature and intricacies of this interaction remains to be confirmed by further environmental observations and microscopic investigations.

c) Other potential parasitic interactions

Following a newly developed protocol for estimating microbial associations based on 'proportionality' (Quinn *et al*., 2017), we identified 12 parasites potentially interacting with *A. minutum* over our three-year survey of the Daoulas river (Figure 35). This approach did not identify the *Parvilucifera* OTUs from which we supposed an interaction with *A. minutum*, this result highlights that biological interpretations from our statistical associations should be interpreted with caution. The potential parasite OTUs belonged to protist groups from which parasitic species of *A. minutum* can be classically retrieved, notably the group MALV II which includes the species complex *Amoebophrya ceratii* (Coats and Park, 2002; Park *et al*., 2004), but also the MALV group III and I which are known for infecting other Dinoflagellates, protists and metazoan (Strassert *et al*., 2018). The composition of this parasitic community did not change significantly over the years (Figure 36; PERMANOVA), however the interactions of these parasites with both *A. minutum* and dinoflagellates were largely unstable along time (Figure 37). Indeed, across blooms, these interactions appeared only occasionally because they were found only in a single year (Figure 37). Among the repeated interactions with *A. minutum*, we found only members of the MALV II group, the larger clade that contains all known *Amoebophrya spp*., some of which are

known parasites of *A. minutum* with low prevalence and annual recurrence even at low density of host (Chambouvet *et al.*, 2008). Given the high genetic variability found within this clade (Guillou *et al.*, 2008), these OTUs could potentially represent either a single species or a strain of *Amoebophrya* infecting the population of *A. minutum* in the Bay of Brest. The real occurrence and the strain-specificity of this relationship with *A. minutum* could only be explained by host-specificity experiments of *Amoebophrya spp.* in culture (Coats and Park, 2002). In addition, the inter-parasitic competition between *Amoebophrya* and *Parvilucifera* could lower host-infectivity (as observed in the Rance river in Blanquart *et al.*, 2016), that could explain the success and long duration of blooms of *A. minutum* in the bay of Brest.

Finally, opportunists (non-obligate, non-specialist parasites with seldom repeated interactions) and specialists (parasites with a species-specific repeated interaction, like *Parvilucifera* and/ or *Amoebophrya*) both co-occurred at the same time during our sampling. The co-occurrence in low abundance of opportunist parasites could highlight distinct mechanisms and strategy within the functioning of parasitism. The co-occurrence of this two strategies in the environment is increasingly brought forward (Brown *et al.*, 2014). In our ecosystem, we also observed that the bloom of *A. minutum* maintained both successful specialist parasite and less successful parasites, in lower abundance. This phenomenon is crucial as these parasites can represent a bank of interactions that could later be involved in the regulation of other dinoflagellate blooms or populations and thus take part in the larger role of parasites within coastal ecosystems (Logares *et al.*, 2015; Jousset *et al.*, 2017).

5) Conclusion

The sampling of three blooms of *A. minutum* within the bay of Brest with a metabarcoding approach allowed us to identify commonly known parasites of *A. minutum*. We hypothesized that members of *Parvilucifera infectans/sinerae* and *Amoeborphrya* infected *A. minutum* across the three blooms and that this interaction was stable across the time. We stress however the need for further microscopic investigations and cultural experiment with local strains to verify theses interactions. The parasitic function in our studied coastal ecosystem and for our monitored species seemed therefore regulated by specific parasite interactions, these interactions probably cannot be interchanged with other species playing the same role. These parasites were not associated to a bloom termination, which might partially explain the success and the duration of *A. minutum* blooms across 2013, 2014 and 2015. Finally, with help from a statistical approach we supposed that the blooms of *A. minutum* helped to maintain host-specific but also opportunistic parasites, a phenomenon crucial to the functioning of parasitism among coastal ecosystems that should be given more attention.

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6) Supplementary Material

Rephy monitoring and samples taken

At the mouth of the Daoulas river of the Bay of Brest (Brittany, France) (Figure 31), cell counts of *A. minutum* are estimated on a weekly basis by the French monitoring REPHY (http://envlit.ifremer.fr). We carried out further sampling when the abundance of *A. minutum* reached 10 000 cell.L^{-1} (Figure S20). Our sampling missed some samples at the very beginning of the blooms (> 10 000 cell.L^{-1}) due to the delay in between the observations and our implementation of the monitoring. Our sampling ended after the maintaining of A. minutum < 5 000 cell.L^{-1} (Figure S20), however sometimes the blooms continued at low abundance after our survey (see in 2014, Figure S20).

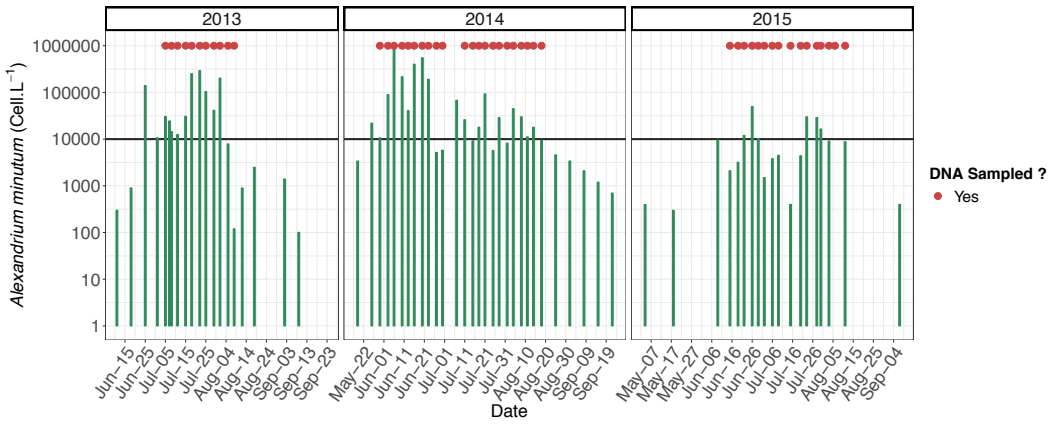

Figure S 20 : Cell count of A. minutum estimated by the Rephy http://envlit.ifremer.fr at the mouth of the Daoulas River (the scale of Axis Y is log transformed) and the dates of our sampling (red dots above).

Biodiversity saturation

The next step was to investigate the protistan community found in the samples at the mouth of the Daoulas river. This was carried by metabarcoding with a sequencing of environmental DNA, and to test if more samples would have brought more distinct OTUs we computed rarefaction curves (Figure S21). As the rarefaction curves did not reach an asymptotic plateau it was considered that the biodiversity of marine protists was not saturated and more samples would have brought more OTUs (Figure S21).

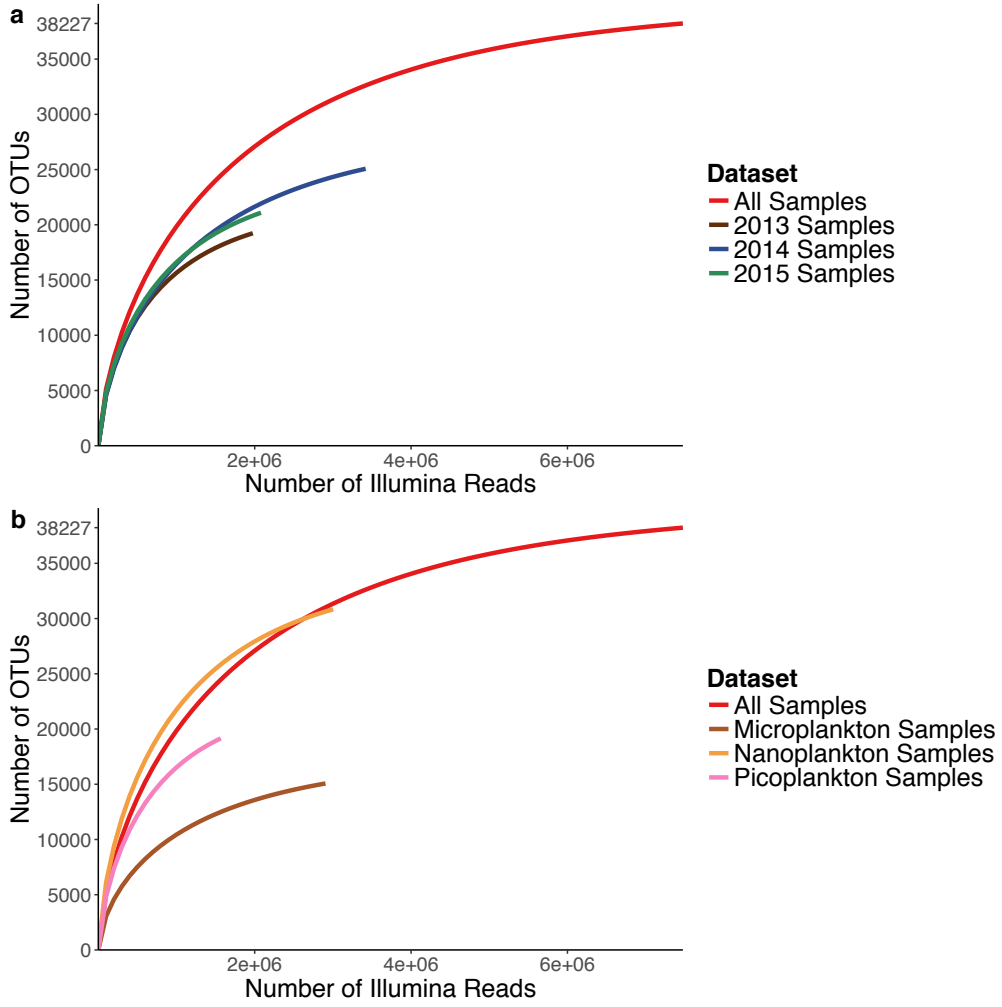

Figure S 21: Rarefaction curves built for the samples of our monitoring at the mouth of the Daoulas River. Curves were constructed by cumulating the samples of all size fraction and for each size fraction independently. The sequencing depth is represented by the number of reads in relation to the species richness as the number OTUs. The function [rarecurve() function of "vegan" (Osaksen et al., 2016)] samples an increasing number of reads with a rate of 100 000 reads/sample and without replacement. Rarefaction curves we constructing all samples presented in our paper, a) by sampling years (blooms of A. minutum in 2013, 2014 and 2015) and b) by size fractions.

OTUs estimation of A. minutum

Due to its high abundance and large genetic variability, *A. minutum* was represented by 2724 OTUs at least annotated to the genus *Alexandrium*. To be certain that we used OTUs corresponding to *A. minutum* we selected 169 OTUs from which the sequence matched at 100% with NCBI's genetic references. However, this had low impact on the total number of reads associated to *A. minutum* in our survey (Figure S22). The 169 OTUs still contributed to 2.45 10^6 reads in our dataset, representing 33% of the total read number across our dataset and 94% of the initial read number associated to the genus *Alexandrium* (Figure S22), notably one single OTU accounted for 2.44 10^6 reads across our dataset.

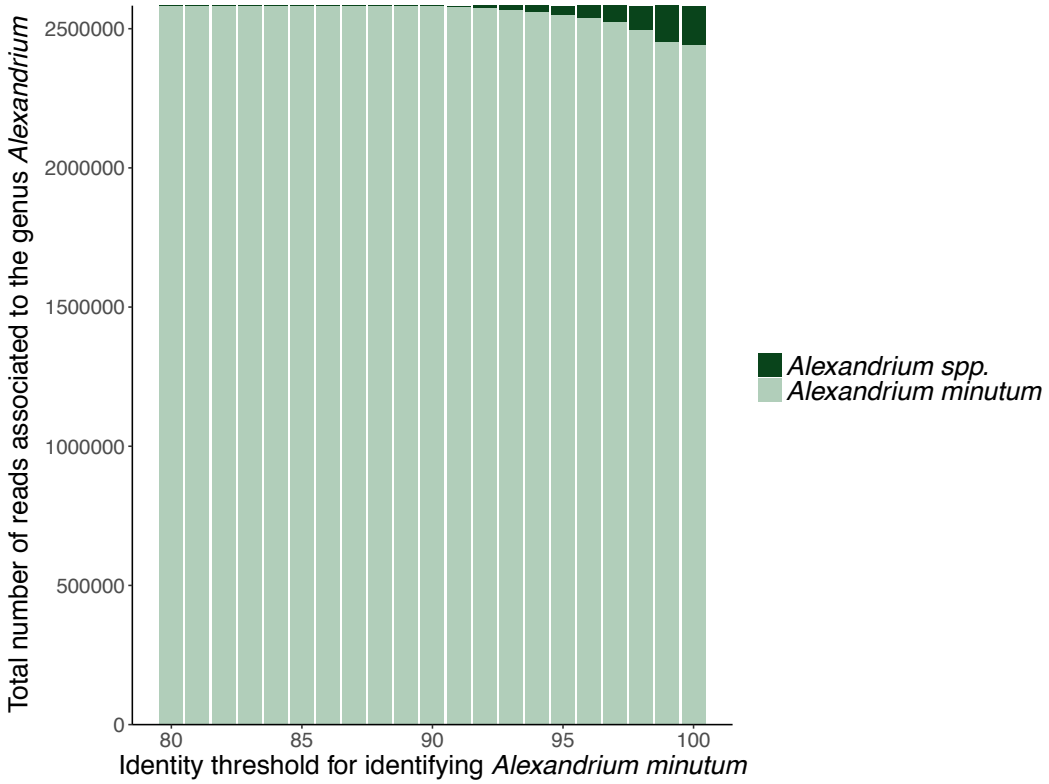

Figure S 22: The total read number of OTUs associated to A. minutum at the mouth of Daoulas and according to distinct identity thresholds. The 2724 OTUs associated at least to the genus Alexandrium were blasted to NCBI's genetic references of A. minutum, we studied the effect of increasingly stringent threshold to define A. minutum, from 80 to 100% of identity to a genetic reference. The read abundance of OTUs that did not match the criterion was cumulated into the category Alexandrium spp.

CONCLUSION AND PERSPECTIVES

The new organisms discovered by DNA-based taxonomy throughout the last 20 years are still poorly described. Indeed, this task is complicated by their small size and the difficulties to grow them in-silico. In this work, I have postulated that a simple trait-based approach on the characters of marine protists, inspired by works on larger organisms (e.g. fish, benthic fauna, plants), could be used to better understand their ecology and their functional diversity within the coastal ecosystem. Using this work, I have then focused on three main problematics. Here, I will recall each problematic and the results emanating from this work. I will finally discuss the limits and the possible short-terms perspective of this approach

1/ Is the functional diversity of marine protists expressing distinct patterns of taxonomical diversity in the environment? Or, do marine protists express a functional redundancy in the environment?

If DNA-based taxonomy of protists has been used to describe patterns of protistan diversity across many ecosystems (de Vargas *et al*., 2015; Massana *et al*., 2015; Mahé *et al*., 2017), only too few times the functions of these organisms have been studied in their environment (de Vargas *et al*., 2015; Genitsaris *et al*., 2016). Furthermore, with the advent of –omic methods (Caron *et al*., 2016), microbiologists are rushing to infer functional diversity with environmental DNA, RNA, or proteins (Muller *et al*., 2018). However, it has been hypothesized that the ecological roles of marine protists remain rooted in trophic behaviors, morphology or ecological preferences (Worden *et al*., 2015), that are harder to decipher from molecules than do metabolic pathways (Keeling and del Campo, 2017). With few morphological and trophic traits adapted to the OTUs of a metabarcoding approach, I have estimated the natural abundance of protistan functional groups corresponding to ecological strategies (distinguishable by trait trade-offs) but most importantly to known functional roles of marine protists (i.e. various strategies for phototrophic and heterotrophic protists). I could then propose that protistan taxonomic and functional diversity were tightly coupled in coastal ecosystems.

Indeed, it existed a good correlation between variations in protist diversity structure and the functional properties displayed by the protists in the coastal environment. In contrast, it has been hypothesized that the taxonomy of prokaryotic communities was decoupled with its functional diversity (Louca, Jacques, *et al*.,

2016; Louca, Wegener Parfrey, *et al*., 2016), and I discussed the distinct methodology and evolutionary processes at stake. Recently, Galand *et al*. (2018) reopened the debate for marine prokaryotic diversity and showed contrasting results that were more in accordance with our observations on marine protists. Galand *et al*. (2018) also stressed that the microbial functional redundancy should be tested at various scales, e.g. temporal vs. spatial or global vs. local, as the coupling between taxonomy and functional diversity can vary across these scales (Galand *et al*., 2018). Our dataset included this variability of scale, comprising meso/local and spatial/temporal scales. In addition, I stressed the need to study functional diversity across size-classes, as I showed that, pico-plankton was dominated by similar functional groups but showed a wider functional diversity by sample. In order to further speculate about to the issue of scale it would be interesting to develop a trait approach to a larger dataset of marine protistan diversity, e.g. the Tara Ocean Dataset (de Vargas *et al*., 2015). However, it can be hypothesized that the taxonomic diversity of organisms found in a global survey such as the Tara Ocean dataset, would far exceeds the one we retrieved in few coastal ecosystems, which would increase significantly the work of trait annotation. It remains also unknown if the functional groups that we retrieved in coastal ecosystems would be the similar in an oceanic survey. As an example, coloniality was a notable trait for phytoplankton in our approach, the trade-off of this trait is a better defense against predators and better floatability at the price of a decrease in the cell surface used for nutrient assimilation (Reynolds, 2006; Litchman and Klausmeier, 2008). This ecological strategy would seem less advantageous in the oligotrophic open-ocean. Another result from our second chapter was the evidence of symbioses favored on the open-ocean side, this strategy could represent also an interesting trait for marine plankton in the oligotrophic ocean in comparison with coastal ecosystems (Decelle *et al*., 2012).

Our traits were mostly composed of morphological and trophic traits, these traits were sufficient to infer functional roles. However, it could be asked whether new functional traits and especially traits of other type would change the coupling we observed. For phytoplankton, physiological traits seemed more related to phytoplankton phylogeny (Bruggeman, 2011) than were morphological groups (Kruk *et al*., 2011). The extent to which the addition of new traits would change our

patterns and the coupling in between protistan taxonomic and functional diversity remains an open question.

The high proportion of non-annotated OTUs in our dataset also hindered the generalization of our results. These proportions were partially surprising, indeed DNA-based taxonomy already stressed the natural abundance of uncultured organisms and this large part of organisms has yet to be described. In consequence, discovering and describing more and more species will help in the field of marine protistan functional diversity. In respect to functional ecology, every further description of behavior, swimming, storage capabilities, prey preference, size of cytosome or mouth opercula (for phagotrophic protists) is required and will surely help to understand the pattern of protists in their environment. To counteract the bias of non-annotated organisms and/or traits, Galand *et al*. (2018) proposed to study simply the coupling in between all, non-annotated and annotated, OTUs and genes expressed (environmental transcriptomic profiles) in a same sample. This method seems risky as the non-annotated genes expressed could be involved in "maintenance" process (housekeeping genes) unrelated to the functional role of the organisms. In addition, due to their conservative nature (Lv *et al*., 2015), housekeeping genes could favor a tighter link in between taxonomy and functional diversity. Application of transcriptomic methods to the functional diversity of marine protists seems further complicated has only few genes can be annotated to this day and only few genomes of protists have been sequenced (Keeling *et al*., 2014; Caron, 2016). Still, the first results from eukaryotic transcriptomics looks promising (Alexander *et al*., 2015), we can only stress that these methods should consider more and more the implication of gene expression in the functional roles of marine protists.

As a consequence, in addition to their description, the markers and the genomes of newly discovered organisms should be sequenced to later estimate their abundance and the abundance of their functions in environmental samples. Sequenced markers but also all sampled information about new and previous taxa could then form an integrative database of curated taxonomy and possibly trait annotation. Such an approach is currently discussed among protistologists (Berney *et al*., 2017) and could represent a giant step forward for marine microbiology.

Briefly, taxonomic and functional diversity seemed to covariate in the marine ecosystems. Flaws in our methods (i.e. scales, non-annotated OTUs and traits) prevented us from generalizing our results. These biases could likely be overcome by considering other temporal and spatial scales but also by upgrading the taxonomy and functional diversity of marine protists. In comparison with larger terrestrial plants (Tilman *et al*., 1997), benthic (Bremner, 2008) or fish communities (Mouillot *et al*., 2014), the functional study of marine protists is more challenging and remains yet incomplete, nevertheless this is also what makes marine protists so interesting to study.

2/ How does the environment affects marine protistan diversity at the submesoscale? and, does the environment selects distinct organisms according to the ecological strategies they have adapted?

The sub-mesoscale physics of the ocean are increasingly recognized as drivers of planktonic production (Lévy *et al*., 2015; Mahadevan, 2016). If it is known that physical processes can enrich the sunlit surface layer, a phenomenon that triggers phytoplankton blooms worldwide, the distinct patterns of marine protistan diversity coincident with these processes are poorly understood. Because the ocean physics are better understood as mathematical equations, the coupling between phytoplankton and the ocean submesoscale physics has been studied most notably by modelling (Clayton *et al*., 2013; Lévy *et al*., 2015). In this chapter, I argued that coupling DNA-based taxonomy and our trait approach could represent a good method of field observation to test the hypotheses brought forward by modelling. I notably tested the hypothesis that tidal fronts could represent diversity hotspots for eukaryotic phytoplankton (Cadier *et al*., 2017). By selecting OTUs with constitutive abilities to phototrophy I studied patterns of eukaryotic phytoplankton. In the Iroise Sea, nutrient inputs, decrease in competitive exclusion, dispersal, and intermediate disturbances all allowed the maintaining of a higher diversity of phytoplankton, in taxonomic and functional terms.

These factors were expected as they shape global marine phytoplankton diversity (Barton *et al*., 2010; Huisman, 2010; Chust *et al*., 2013), however in this chapter I stressed their seasonal effect over a continental shelf. If our approach was successful, other markers more adapted to phytoplankton could have been adapted to our study, like plastidial 16S rDNA for microeukaryotes (see PhytoREF, Decelle *et*

al., 2015), or with other markers also targeting phototrophic prokaryotes, thus comprising the whole extent of marine phytoplankton, like plastidial 23S rDNA marker (Yoon *et al*., 2016). Another default of our approach was the semi-quantitative estimation of abundance by DNA-based taxonomy. If environmental sequencing gives reliable results of the relative proportions of organisms within a same size-class (Giner *et al*., 2016), the real abundance and/or biomass are necessary to infer the influence of a taxon on a biogeochemical flux (Leblanc *et al*., 2018), on primary production (Agawin *et al*., 2000), or simply to estimate taxonomic turnovers, successful strategies and competition processes (Props *et al*., 2017). Quantitative PCR or Fluorescence In-Situ Hybridization could represent alternatives, however both have been criticized respectively for a lack of consistency when comparing results from distinct studies and the low possible size of the sampling power (ca hundreds of cells; Props *et al*., 2017). Perhaps the most interesting approach would be to combine metabarcoding and flow-cytometry. In this coupling, flow cytometry would give the abundances of distinct size-fractions and/or plastidic and non-plastidic organisms, while in exchange metabarcoding would give the diversity and relative abundance of taxa within these size fractions. Recently, authors even carried out the sequencing of water pre-counted by flow cytometry (Li *et al*., 2017), however, in this last study only the pico-eukaryotes were investigated. Indeed, a flow-cytometry device that would measure the abundance throughout the whole size-spectrum of plankton is required. This approach remains a good perspective for coupling quantitative estimations and sequencing surveys.

The interpretation of environmental effects on phytoplankton was allowed by a strong body of literature investigating the abiotic dynamic of the Iroise Sea (Le Fèvre and Grall, 1970; Morin *et al*., 1985), but phytoplankton surveys would also probably benefit from field studies with a wider sampling of the environment and with historical/paleological records of fluctuations in the environment. Higher frequency of marine microbial communities would likewise help to understand the dynamic of planktonic communities (Needham *et al*., 2018), but at the sub-mesoscale this task is further complicated by the need to increase the spatial sampling as well. Such investigations could however be carried out in modeled hydrological configurations. In a master internship that I have co-monitored with Marc Sourisseau, Cécilia Teillet has tried to introduce our trait approach to a numerical

model of phytoplankton. On the basis of the trade-offs I observed in phytoplankton (see our three functional groups SWAT, FLAT and CAT) and a literature survey Cécilia modeled distinct phytoplanktonic strategies and their dynamic within a virtual hydrological ecosystem. The main factors limiting the comparison in between my results and the results of her model was the semi-quantitative nature of my sequencing survey. In a second step, we also tried to study the dominant size of phytoplankton organisms along time. Most of our phytoplankton OTUs were annotated with size, however as we sequenced the 3 size-fractions separately (to increase diversity), the relative abundances as well as the number of reads associated to OTUs from distinct size-fractions was not directly comparable and could not informed us on the dominant size. Notwithstanding, our results on phytoplankton diversity patterns in the Iroise Sea were in general agreement with an applied model based on phytoplankton functional types (Cadier *et al.,* 2017). More interactions in between modelers and microbiologists would benefit the comprehension of protistan diversity and its dynamic in the marine environment.

For heterotrophic protists patterns were less clear. The factors of the front mostly influenced phytoplankton by resource availability (nutrients) and dispersal. Dispersal can also influence patterns of heterotrophic protists (Dolan *et al*., 2007). However, for heterotrophic protists 'resource availability' cannot be traced back as easily as nutrient concentrations. As an example, a high quantity of a certain prey could be utilized only by a subset of heterotrophic protists and have thus a lesser impact on the whole community. In addition, certain protists predators have taxonomic or size preferences (Hansen *et al*., 1994; Massana *et al*., 2009; García-Comas *et al*., 2016) and this selectivity is even truer for protistan parasites (Guillou *et al*., 2008). The fact that heterotrophic protists depends on biological interactions to survive, added to their higher resource specialization thus renders the dynamic of heterotrophic protists far less predictable than in phototrophic protists. This realization remains even more relevant for organisms from which prey preferences are not even known. In our study, heterotrophic protists seemed poorly explained by patterns of phytoplankton, we supposed that heterotrophic protists could have been better correlated to other preferential preys, notably marine prokaryotes (Yang *et al*., 2018). Logically, short-term improvements to our method would be to consider other pelagic compartments (e.g. prokaryotes or zooplankton) and quantitative

observations (e.g. by flow cytometry) to investigate the top-down regulation as well as the microbial loop processes in which marine protists take part.

To explain patterns of heterotrophic and phototrophic protists other specific traits could have been studied, e.g. functional response and grazing rates of predators (Fenchel, 1982; Massana *et al*., 2009). A good prospect for the ecology of marine heterotrophic protists would be to target few prey types and to study the preferences of the newly described heterotrophs for these preys. More precise estimations on the growth rate and uptake affinity of phytoplankton are also needed to understand intra-guild interactions and competition (Hillebrand and Cardinale, 2004; Edwards *et al*., 2012; Marañon *et al*., 2013). Despite the existence of mixotrophy, the addition of trophic-specific traits will be necessary to better understand the patterns of marine protists and their role in the environment (Weisse *et al*., 2016).

In short conclusion, our approach was successful to address how the environment drives the diversity of eukaryotic phytoplankton. Patterns of heterotrophic protists were less influenced by the environment and we hypothesized that including other biological compartments could help to better understand their dynamic. Ultimately, improving our sampling of the marine environment, the sampling of marine pelagic communities (qualitatively and quantitatively), and annotating trophic-specific traits to either phototrophs or heterotrophs, will help to further disentangle the effects of the environment on marine protists.

3/ How many taxa do play the role of parasitism within blooms of the same phytoplankton species? Is the parasite community composed of the same species during three distinct blooms of a dinoflagellate species, or is the interacting parasitic community fluctuating across blooms?

In the bay of Brest three recurrent blooms of *Alexandrium minutum* were sampled and we searched for the occurrence of known parasites of this harmful species. In still preliminary results, we detected OTUs corresponding to the parasitic species complex *Parvilucifera infectans/sinerae* known to infect *A. minutum* in other seas (Garcés and Hoppenrath, 2010; Jeon *et al*., 2018), and in neighbor estuaries of French Britanny (Lepelletier *et al*., 2014; Blanquart *et al*., 2016). Other results showed the regular presence of members of *Amoebophrya spp*. from which statistical evidences supposed a possible interaction with *A. minutum*. Statistics also helped to

identify other parasitic OTUs well associated to *A. minutum* but from which the interaction was more opportunistic (found only during a single bloom,). We thus hypothesized that the parasitic function across these blooms was played by the same taxa, and that it exists only few functional redundancy in this ecological role. We also suggested the role of *A. minutum* blooms in maintaining other opportunistic, generalist parasites forming a reservoir of species that could potentially interact with other members of the coastal communities.

To allow a fast-exploratory statistical approach we used 'proportionality' as a coefficient of pairwise associations (Quinn *et al.*, 2017), which had the advantage of running relatively fast (5 to 10 seconds). Other association coefficients were studied previously, SparCC or SPIEC-EASY (Kurtz *et al.*, 2015). Local similarity analysis (Ruan *et al.*, 2006) is also increasingly used to infer networks of associations on the basis of temporal metabarcoding datasets (Christaki *et al.*, 2017; Berdjeb *et al.*, 2018), and could have been tested in our study. A major limiting factor for the use of network analysis on our dataset came from our sampling strategy, size-fractionated sampling can indeed represent a major bias by a) decoupling the sampling of host and preys and b) sequencing distinctly hosts and potential preys which could blur the natural abundances of the host-parasite complex. Although size-fractionated sampling is necessary to understand the distinct ecological strategies and ecology of organisms along the size-spectrum (e.g. in our first chapter), an increasing number of studies uses less size-fractions (Berdjeb *et al.*, 2018), or pool together the filters before DNA extraction and PCR (Christaki *et al.*, 2017).

Another aspect relevant in a more theoretical way, is the extent of which *A. minutum* and its parasite(s) can migrate. If *A. minutum* has been retrieved in sediments of the bay of Brest dated to 1870, it has been proposed that its relative success since 2012 could be explained by the migration of a new and more adapted population (Klouch *et al.*, 2016). *A. minutum* have notably been studied in neighbor estuaries of Brittany (Dia *et al.*, 2014), in which two infective species of *Parvilucifera* were also described (Lepelletier *et al.*, 2014). Migrations of host/parasite complex are crucial in the dynamic of parasite-infectivity and host-resistance (Morgan *et al.*, 2005; Greischar and Koskella, 2007; Zhang and Buckling, 2016), thus studying the connectivity of *A. minutum* and its parasite(s) in between the bay of Brest and neighbor ecosystems is necessary to the understanding of the

host/parasite complex. In addition, genotyping the hosts and the parasite populations at high frequency could help us disentangle the co-evolution processes in between *A. minutum* and its parasites (Blanquart *et al*., 2016).

Finally, evidences for opportunistic parasitism is increasingly recognized outside of the planktonic community (Brown *et al*., 2014). Opportunistic interaction might be favored by the high availability of resource (Kinnula *et al*., 2017), like in our application with a high quantity of *A. minutum* as preys. Attacks of non-optimal preys have also been observed among specific parasites, although the infections proved non-productive (Coats and Park, 2002; Chambouvet *et al*., 2008). Investigating the natural prevalence of parasites among planktonic protists remains a difficult task, coupling both metabarcoding surveys and fixed water samples (e.g. with formaldehyde) has proven useful to verify putative interactions (Chambouvet *et al*., 2008; Villar *et al*., 2015).

In summary, with our metabarcoding approach we identified known interactions in between *A. minutum* and parasites, these interactions were constant over time and played by the same taxa. This implied a strong specialization in the parasitic regulation of the blooms of a single species. As these results are still preliminary, the short-term prospects of this study are advances in the network analysis. We also cannot stress enough that interactions should be verified under the microscope and be seen in the context of co-evolution.

Perspectives for the field of functional diversity and marine protists

DNA-based taxonomy shook the phylogeny of the organisms previously described morphologically, questioned the species concept, discovered new organisms in the ocean and overall changed the modern paradigm of marine microbial ecology (Caron *et al*., 2012; de Vargas *et al*., 2015; Worden *et al*., 2015; Fišer *et al*., 2018). In our study, we tried to reconcile DNA-based taxonomy with functional ecology through a trait approach. We evidenced simple functional groups (i.e. different kind of phototrophic groups, heterotrophic, mixotrophic, decomposers, parasites) that associated typical roles to the OTUs of our metabarcoding dataset. However, other approach could be used to study the functional traits of marine protists.

Obviously, methods considering the natural abundances of organisms, e.g. by cell counts under the microscope can represent a quantitative alternative to DNA-

based taxonomy. Relevant processes have been described in the functional diversity of phytoplankton with cell counts (Kruk *et al.,* 2011; Edwards *et al.,* 2013). Due to their quantitative nature, the results from this method can also more easily integrate models (Barton *et al.,* 2013). Models also investigates more and more the trait trade-offs highlighted by physiological surveys (Edwards *et al.,* 2012) and their impact on success under distinct environmental conditions (Våge *et al.,* 2013; Ward and Follows, 2016). The advantages of metabarcoding surveys are more related to the description of the natural abundance of small protists and general diversity patterns. However, ecological patterns have been brought forward too. The high abundance of heterotrophic protists within the smallest size-fractions is often highlighted by sequencing surveys, these results questions the models that predicts the domination of phototrophs in the smallest size-fractions (Andersen *et al.,* 2014; Ward and Follows, 2016). The interaction between microbiologists developing genomic methods and modelers has proven powerful (Coles *et al.,* 2017). We can only advocate for more integrations of ecological problematic in genetic-based surveys and more joint efforts with modelers.

As mentioned in the previous section, cultivating organisms remains the best ways to observe their behaviors and ecological preferences. This remains a difficult task, the principal issues being a) the difficulties into isolating the newly discovered organisms, and b) the complexity of the life-cycles and growth requirements of these organisms. To carry out these processes researchers can now count on microfluidic and other microfabrication methods that allows to confine single cells into controlled micro-environments (Weibel *et al.*, 2007; Rusconi *et al.*, 2014). This methodology already allowed to describe traits of marine microbes like complex swimming behaviors among marine protists (Kantsler *et al.*, 2013) or chemotaxis in bacteria (Lambert *et al.*, 2017). These methods could clearly help to further describe the small organisms that are constantly found in sequencing surveys but from which we know so little. By doing so we will obviously enhance our knowledge the functional diversity of marine protists.

The (meta-)genomics and (meta-)transcriptomics of marine protists are still hindered by the large size and the complexity of their genomes (Caron *et al.*, 2009, 2012; Hou and Lin, 2009). However, these methods have already been successful into sorting distinct ecological strategies of phytoplankton (Alexander *et al.*, 2015)

and new advances look particularly promising for the field of functional ecology. Burns *et al*., (2018) recently described a new method to predict trophic modes based on gene-homology, briefly these authors compared the genomes of a multitude of eukaryotic phagotrophs with the genome of a poorly known archaea and were able to test for the presence of phagotrophy within the archaea. This process would require further morphological evidences, at the time the method still refers to a potential for phagotrophy. The prediction of trophic abilities or other traits across marine protists could probably help us to end many debate; e.g. which marine protists are mixotrophs? Sequencing more genomes of marine protists remains thus a good perspective for marine protists functional ecology.

It is also necessary to stress that genes and gene expression represent only a potential for metabolic processes, as post-transcriptional and translational processes are multiple. One way to better apprehend this subject have been demonstrated for prokaryotes (Muller *et al*., 2014, 2018). This second method propose a multi-omic analysis binding together results from DNA-taxonomy, meta-transcriptomic and meta-proteomic. In a second time, based on all results and a correlation network the method helps to estimate 1/ the taxa involved in 2/ gene expression, itself involved in 3/ protein or enzyme synthesis (Muller *et al*., 2018). Again, the application of this method to the functional ecology of marine protists supposes first that enzymes or proteins involved in functions should be detected (Keeling and del Campo, 2017), as first step calibrating this approach to phagotrophic (Burns *et al*., 2018) and phototrophic traits (Alexander *et al*., 2015) could represent an interesting first step. Then, comparing the taxa involved in both photosynthesis and heterotrophy could help us disentangle the effects of heterotrophic, mixotrophic and phototrophic protists on global biogeochemical flux.

Finally, it is my personal belief that all these methods should be combined for studying common problematics. Collaborations between researchers with distinct backgrounds and methods (e.g. modeling, statistics, field surveys, genetics, evolution) are probably the key to expand our understanding of contemporaneous ecology.

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ANNEXES

Information about our sequencing run.

The first table represents the number of distinct sequences (Distinct Seq), the total number of sequences (# Seq) and the number samples (# Samples) in each sequencing library and in all our dataset after our first quality checking. The second table represents the same metrics but after a second quality filtering. The distinct sequences present in less than 4 reads and 3 samples were discarded (as in de Vargas *et al*., 2015). The third table represents the effects of these filtering steps on the initial sequencing libraries (% loss of distinct sequences and total number of sequences). After these steps, sequences were annotated taxonomically with PR^2, and clustered in OTUs with swarm 2

Infos BDD

After USEARCH Filter

RUN	Distinct Seq	# Seq	# Samples
RUN1 : DA (2013, 2014, 2015)	851127	9622061	244
RUN2 : MB (09/14; 03/15), SE, Other datasets not present in this study	961947	6924899	235
RUN3 : RA	490633	4711842	138
RUN4 : MB (07/15; 09/15)	761635	4928449	231
RUN5 : PI (2013), PH (2013), PE, DY	1064259	9562085	240
RUN6 : PI (2015), PH (2015), Other datasets not present in this study	1156017	8564698	129
ALL	4040348	44314034	1217

After Singleton Filter

RUN	Distinct Seq	# Seq	# Samples
RUN1 : DA (2013, 2014, 2015)	318752	8851381	244
RUN2 : MB (09/14; 03/15), SE, Other datasets not present in this study	362395	6200630	235
RUN3 : RA	208368	4393188	138
RUN4 : MB (07/15; 09/15)	290420	4392854	231
RUN5 : PI (2013), PH (2013), PE, DY	472810	8668956	240
RUN6 : PI (2015), PH (2015), Other datasets not present in this study	350520	7541278	129
ALL	943961	40048287	1217

Loss

RUN	Distinct Seq Loss %	# Seq Loss %
RUN1 : DA (2013, 2014, 2015)	62.5	8.0
RUN2 : MB (09/14; 03/15), SE, Other datasets not present in this study	62.3	10.5
RUN3 : RA	57.5	6.8
RUN4 : MB (07/15; 09/15)	61.9	10.9
RUN5 : PI (2013), PH (2013), PE, DY	55.6	9.3
RUN6 : PI (2015), PH (2015), Other datasets not present in this study	69.7	11.9
ALL	76.6	9.6

Publications:

- Liénart, C., Savoye, N., David, V., **Ramond, P**., Rodriguez Tress, P., Hanquiez, V., et al. (2018) Dynamics of particulate organic matter composition in coastal systems: Forcing of spatio-temporal variability at multi-systems scale. Prog. Oceanogr. 162: 271–289.
- **Pierre Ramond**, Marc Sourisseau, Nathalie Simon, Sarah Romac, Sophie Schmitt, Fabienne Rigaut-Jalabert, Nicolas Henry, Colomban de Vargas, Raffaele Siano (*submitted* 2018) Coupling between taxonomic and functional diversity in protistan coastal communities. *Environ. Microbiol.*
- **Pierre Ramond**, Raffaele Siano, Colomban de Vargas, Laurent Memery, Marc Sourisseau (*under-submission* 2018) Pattern of protistan diversity across a tidal front.
- **Pierre Ramond**, Marc Sourisseau, Sophie Schmitt, Nicolas Henry, Colomban de Vargas, Laure Guillou, Raffaele Siano (*work in preperation*) Parasites of the marine protistan community in blooms of the toxic dinoflagellate *Alexandrium minutum*.
- Mickael Le Gac, Gabriel Metegnier, **Pierre Ramond**, Raffaele Siano… (*work in preparation*) Species specific gene expression from metatranscriptomic datasets.

Conferences :

- Phytoplankton's taxonomic and functional diversity patterns over a coastal tidal front, (SFE, Rennes, 2018). **Pierre Ramond**, Raffaele Siano, Colomban de Vargas, Mathilde Cadier, Marc Sourisseau
- Protist functional diversity across size-fractionated coastal planktonic communities (ICHA, Nantes, 2018). **Pierre Ramond**, Marc Sourisseau, Colomban de Vargas, Raffaele Siano*
- Protist functional stability in pico-nanoplanktonic marine coastal communities. **Ramond Pierre**, Sourisseau Marc, Audic Stephane, Simon Nathalie, Romac Sarah, Schmitt Sophie, Rigaut-Jalabert Fabienne, De Vargas Colomban, Siano Raffaele (2017). ICOP 2017 - 15th International Congress of Protistology. 30th july - 4th august 2017, Prague.

Datasets:

- **Ramond Pierre**, Siano Raffaele, Sourisseau Marc (2018). Functional traits of marine protists . SEANOE . http://doi.org/10.17882/51662
- **Ramond Pierre,** Siano Raffaele, Sourisseau Marc (2016). Metabarcoding of Coastal Ecosystems. IFREMER - SISMER. http://doi.org/10.12770/16bc16ef-588a-47e2-803e-03b4acb85dca

Internship supervisor:

- "Structuration fonctionnelle des protistes dans l'écosystème côtier de la rade de Brest" (Cécilia Teillet, 2017), Stage de M2 de l'Université de Bordeaux [U.F Sciences de la Terre et de l'Environnement Sciences et Technologies], Encadré par Marc Sourisseau et **Pierre Ramond**.

The Functional Diversity of Marine Protists in Coastal Ecosystems

Abstract

Protists are the eukaryotic share of microbial communities, in the ocean they represent the first link between the harsh aquatic environment and its biocenosis. The distinct roles and adaptations of marine protists to their environment constitutes their functional diversity. A number of marine protist have been discovered by DNA-based taxonomy, however due to their recent discovery the functional diversity of these organisms is still unknown. In this project, the functional diversity of marine protist is studied by coupling a genetic survey (V4 marker of 18S rDNA) of 1145 distinct samples from various coastal ecosystems and a trait approach constituted of 13 traits describing the ecological strategies of marine protists. As a first step, in terms of functional redundancy, changes in the community of marine protists were tightly coupled with changes in the functional role it expressed. These results contrasts with observations about prokaryotes and the distinct evolutionary process at stake are commented. The smallest size-fractions also displayed a higher functional diversity probably influenced by less stringent requirement and the higher pelagic resource availability for this compartment. In a second application associated to a tidal front, the influence of the environment on marine protists is studied. The phototrophic protists presented a maximum of taxonomic and functional diversity at the front. The diversity maximum was influenced by dispersal (at an ecotone) but also by physical cycles of nutrient inputs and stratification, which allowed to decrease competitive exclusion and to alternate the dominant ecological strategy. Reversely, the diversity of heterotrophic protists was less structured over this environment. It is postulated that heterotrophic protists could be influenced by similar processes as dispersal and resource availability, however because their nutrition is related to biological interactions, their distribution is less influenced by the environment. In a last section, parasitism of a single dinoflagellate species was showed to be carried out by few specialized protistan parasites. These results underline that the predation role of protistan communities might be dictated by the extent of specialized interactions involving heterotrophic protists and their prey.

Keywords: marine protists, ecology, functional diversity, metabarcoding, coastal ecosystems, environmental microbiology

La Diversité Fonctionnelle des Protistes Marins dans l'Ecosystème Côtier

Résumé

Les protistes sont les organismes eucaryotes du compartiment microbien. Dans l'océan ils représentent le premier lien entre l'environnement aquatique et sa biocénose. Les différents rôles et adaptations de ces organismes dans leur milieu constituent leur diversité fonctionnelle. Cependant, parce qu'un certain nombre d'entre eux a été découvert récemment par des méthodes d'échantillonnage génétique, la diversité fonctionnelle des protistes marins reste peu connue. Dans cette thèse, la diversité fonctionnelle des protistes marins de 1145 échantillons de l'écosystème côtier a été étudiée en couplant la taxonomie génétique (marqueur V4 de l'ADNr 18S) et une approche de 13 traits décrivant les stratégies écologiques des protistes. Dans un premier temps, à l'inverse des procaryotes, un fort lien entre taxonomie et fonctions est mis en évidence, impliquant que des changements de composition de la communauté sont susceptibles de modifier le fonctionnement de l'écosystème. Les protistes des petites tailles semblent également soutenir une plus grande diversité fonctionnelle, probablement influencée par une plus grande disponibilité en ressource pour ces organismes moins exigeants. Dans une seconde application associée à un front de marée, l'influence de l'environnement sur la diversité des protistes est étudiée. Les protistes phototrophes démontrent un maximum de diversité taxonomique et fonctionnelle au niveau du front. Ce maximum est influencé par la dispersion (existence d'un écotone) ainsi que par des cycles physiques d'apport en ressources (abiotiques) et de stratification qui permettent de diminuer localement la compétition exclusive et de faire s'alterner des stratégies écologiques dominantes. Inversement, la diversité des protistes hétérotrophes semble moins structurée par l'environnement. Il est postulé que les protistes hétérotrophes sont également influencés par la dispersion et la disponibilité en ressource, cependant parce que leur nutrition se fait par des interactions biotiques complexes leur distribution est moins expliquée par l'environnement. Dans une dernière partie, nous observons que les protistes hétérotrophes parasites sont particulièrement spécialisés à leurs proies. Ces résultats soulignent que le rôle de prédation des communautés de protistes passe par l'intermédiaire d'interactions spécifiques entre les protistes hétérotrophes et leurs proies.

Mots-clefs : protistes marins, écologie, diversité fonctionnelle, metabarcoding, écosystèmes côtiers, microbiologie environnementale