# From data to knowledge: integrating observational data to trace phytoplankton dynamics in a changing world

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*To my mother,*

*a bright example of courage, determination, love*

# Abstract

Phytoplankton are a fundamental component of marine systems. They are involved in the global regulation and functioning of biogeochemical and trophic processes and are strictly connected to human health and well-being through the provision of essential ecosystem services. Despite their fundamental importance, there is still no broad consensus on the mechanisms underlying their seasonal and interannual variability, while even less is known about the ecology of individual species and their response to climate variability given the scarcity of comprehensive long-term observation sets. Here, by taking advantage of high-frequency oceanographic and biological data collected over more than 25 years in a coastal pelagic Mediterranean site, I applied a set of statistical methods in order to investigate different aspects of community and individual species' ecology, with a particular emphasis on the environmental factors and the mechanisms underlying phytoplankton phenology. Further, I analyzed long-term meteorological variations in the area and their relationships to large-scale climatic oscillation in order to address their impact on the planktonic system during the different seasons of the year. Finally, I integrated the data from 10 worldwide-distributed coastal time-series and investigated the adaptive potential of ubiquitous phytoplankton species to local conditions using a niche-based approach. The results of these analyses highlighted an impressive regularity in the annual occurrence of phytoplankton community and individual taxa despite a highly variable environment. Light was the predominant factor regulating species turnover and replacement and seemed to regulate endogenous biological processes associated with species-specific phenological patterns. Over the time series, a considerable stability was shown by individual species and the whole community, while the effects of climate fluctuation on the abiotic and biotic components revealed a strong dependence on the season. The comparison of phytoplankton niches across diverse biogeographical regions supported the idea of evolutionary adaptation, further emphasizing the importance of long-term ecological observations in the context of climate change. Overall, the results of these studies highlight the considerable resilience and the active role that phytoplankton plays under different environmental constraints, which contrasts the view of these organisms as passively undergoing external changes that occur at different temporal scales in their habitat, and show how, under certain conditions, endogenous biological processes prevail over environmental forcing.

# Acknowledgements

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# 1 Introduction

## 1.1 Phytoplankton

The surface of the earth is covered 71% by water. This vast fluid and apparently indistinct medium is populated by an impressive complexity of organisms that have evolved over millions of years. Among these, there is a group of organisms collectively mentioned as 'plankton', a Greek word meaning 'wandering' or 'drifter' for their inability to oppose to water currents, which includes organisms ranging from a size 0.2 µm (picoplankton) to more than 200 cm (megaplankton) (Fig. 1.1). The autotrophic component of plankton is generally smaller than 0.2 mm and is usually referred to as 'phytoplankton', a composite word that comes from the Greek *phyton* (plant) and *plankton*. Although these organisms populate the enclosed, transitional and open waters of the entire globe since the beginning of life on earth, only in recent times science has made significant progress in understanding their biology and ecology. The first to study plankton (as well as to coin the term plankton) was the German biologist Victor Hensen, who moved the first and fundamental steps forward in developing tools to sample and study these organisms (Mills, 2012), and perceived their importance by defining what he saw under the microscope as 'this blood of sea'. Indeed, marine phytoplankton is the main responsible for the primary production in the seas, generating biomass from solar energy and inorganic nutrients. Although they account for less than 1% of the photosynthetic biomass on Earth, phytoplankton contributes almost half of the world's total primary production (Field et al., 1998) providing food directly and indirectly to every other marine creature, and, fixing $CO_2$ and releasing oxygen as a byproduct of the photosynthesis. In this process that occurs virtually on all surfaces of the oceans, phytoplankton exports the atmospheric $CO_2$ in the form of organic carbon to the deepest layers and sediments in a process called 'biological pump', contributing significantly to regulating and balancing the quality of the air we breathe.
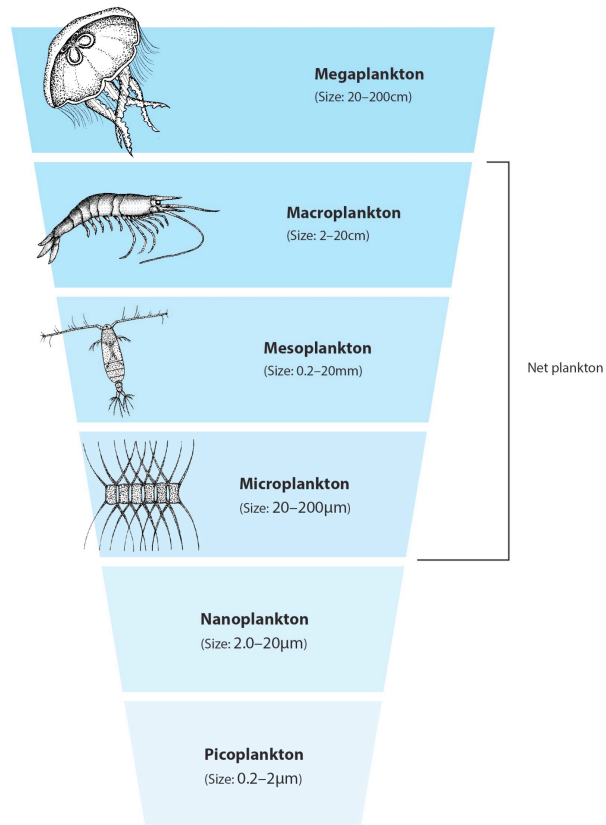
Figure 1.1. Subdivision of planktonic organisms into size classes. Organisms ranging from 20 μm to 20 cm are generally sampled through special plankton nets (taken from https://www.seattleaquarium.org/).

# 1.1.1 Phytoplankton diversity

Although the term phytoplankton is common in scientific literature, it can be elusive and reductive for such a complex group of organisms. The term phytoplankton encompasses a polyphyletic group of organisms that include deeply divergent lineages of both prokaryotes and eukaryotes. In terms of abundance, cyanobacteria are thought to be the most representative group of phytoplankton and are the only existing group of prokaryotic photoautotrophs. The first eukaryotic photosynthetic organisms are thought to have originated through an endosymbiotic process between cyanobacteria and a eukaryotic cell containing already a mitochondrion (De Clerck et al., 2012). Since that time a variety of eukaryotic clades have emerged (Fig. 1.2) which are included in two main lineages. The 'green' lineage is characterized by taxa with chlorophyll-$b$ in their photosynthetic pigment set and includes Chlorophyta, Euglenophyta and Chlorarachniophyta. All terrestrial plants (Embryophyta) have derived from this lineage. The second lineage, the 'red' lineage, includes most of the current eukaryotic phytoplankton groups such as Cryptophyta, Haptophyta (including coccolithophores), Heterokonta (including diatoms), and Dinoflagellata.

A group of Heterokonta, Bacillariophyta (diatoms), is among the most representative group of microalgae in diverse aquatic environments, from the open ocean to coasts and estuaries and internal waters. Their main characteristic is the presence of the frustule, a silica cell wall composed of two overlapping thecae. Given their ubiquity and their dominance in coastal waters, diatoms are among the most studied microalgae, while the wide variability of sizes and structural details associated with the frustule facilitate their taxonomic identification using a microscope. Diatoms lack motility structures such as cilia and flagella, have relatively high sinking velocities compared to other microalgae, and are supposed to transport ~40% of organic carbon to the deep ocean (Jin et al., 2006). They are well adapted in nutrient-rich environments and therefore are generally dominant and representative of nutrient-rich environments such as estuaries and coastal waters. Similarly, to diatoms, a group of haptophytes, the coccolithophores, produce an exoskeleton made of calcareous plates (coccoliths) instead of silica. The characteristic calcareous coccoliths make coccolithophores particularly interesting for the study of the effects of acidification on the oceans (see Meyer & Riebesell, 2015). In particular, a well-known representative of coccolithophores, *Emiliania*

*huxleyi*, is considered a model organism because of its ubiquity and ability to produce massive blooms in temperate and high-latitude coastal systems. Another representative group of pelagic marine systems includes a variety of organisms of the clade Aveolata, common called dinoflagellates for the presence of two perpendicular flagella that lie in two surface grooves, the cingulum and the sulcus. The presence of flagella confers certain motility to these microalgae which can move in the water column following physical and chemical stimuli, and therefore find the optimal conditions for their growth. They generally represent a substantial part of the algal biomass in coastal areas and can produce massive blooms generally following the diatoms one.

Although phytoplankton is primarily autotrophic, many organisms exhibit other trophic strategies. Among the Alveolata clade, the Apicomplexa are obligate parasites of metazoans, while many dinoflagellates feed on small phytoplankton through phagocytosis. Some of them such as some species of the genera *Tripos* and *Prorocentrum* can exhibit both autotrophy and heterotrophy (Johnson, 2015; Smalley et al., 2003), a condition called mixotrophy. Furthermore, some phytoplankton groups have ecologically-relevant symbiotic relationships with more complex multicellular organisms, such as the well-known association between some dinoflagellates belonging to the family Symbiodiniaceae with the Anthozoa polyps responsible for the building of coral reefs (see Finney et al., 2010).

Until a few decades ago, the primary instrument with which humans approached the exploration of phytoplankton diversity was the microscope with which the species were identified and classified based on morphological criteria. However, with the recent advances of molecular technologies, there is increasing evidence that the diversity of phytoplankton is underestimated (De Vargas et al., 2015), while the combined use of microscopy with molecular tools has highlighted the existence of cryptic species within 'morphospecies' (Amato et al., 2007; Sarno et al., 2005-2007; Zingone et al., 2005) laying the foundations for new experimental and conceptual challenges in the study of the ecology of phytoplankton (Degerlund et al., 2012; Smayda, 2011).
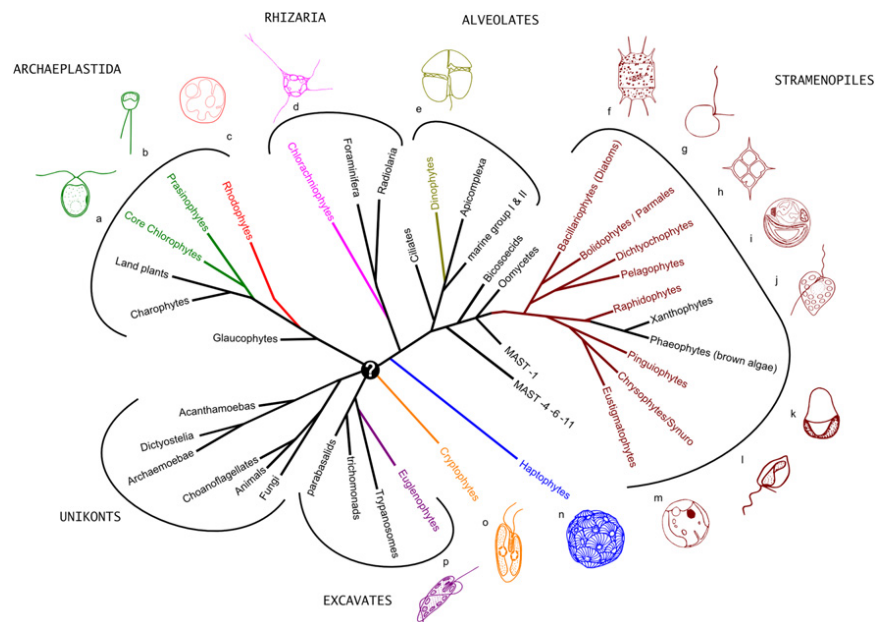
Figure 1.2 Distribution of phytoplanktonic taxa across eukaryote lineages (taken from Not et al., 2012). The incredible diversity of eukaryotic phytoplankton is perceptible considering that metazoans (from sponges to humans) and fungi are all included in the group of unikonts.

# 1.1.2     Consequences of phytoplankton diversity

Phytoplankton taxonomic diversity translates into a huge genetic pool from which a tremendous heterogeneity of life-cycles, adaptations, trophic strategies and ecological functions emerge. Phytoplankton diversity plays an active role in influencing the physics, chemistry and trophic relationships of an ecological system, and expanding the scale, of entire seas and oceans. Different phytoplankton clades and species have different chemical compositions. Cyanobacteria have an N:P ratio of about 22:1, which is higher than in eukaryotic taxa, which is around 13:1 (Deutsch & Weber 2012), while the taxa included in the so-called 'green' lineage have a higher N: P than those of the red line. In addition to have a different stoichiometry, different species have different requirements and affinities for micro and macronutrients. Given the ubiquity and abundance of phytoplankton, these stoichiometric differences assume considerable importance in the functioning of the global biogeographical patterns of nutrients and the chemical composition of marine water and seafloor (Boyd & Trull, 2007; Litchman et al., 2015). Indeed, diatoms are estimated to bury by sinking $6.3 \times 10^{12}$ mol of silicon per year (Tréguer & De La Rocha, 2013), whereas coccolithophores together with planktonic foraminifera are the main responsible for creating and maintaining the vertical gradient of seawater alkalinity, and for the formation of calcium carbonate rock across the globe (Rost & Riebesell, 2004).

The different phytoplankton sizes and shapes related to taxonomic diversity strongly influence the dynamics of biogeochemical patterns. For instance, smaller or flatter cells, tend to have a lower sinking velocity compared to larger and spherical cells, and contribute differently to carbon exports. Also, many metabolic and physiological properties as nutrient uptake, metabolic rates and light absorption all depend on the cell shape and size (Reynolds, 2006; Sommer et al., 2017). Based on the life cycle and adaptation strategies, the sizes of individuals in the same population can be very different. For example, in diatoms, the average cell size decreases as mitosis proceeds (vegetative growth), and they restore the maximum species size by sexual reproduction. Similarly, many species of microalgae (especially the diatoms of the genus *Chaetoceros* and *Thalassiosira*, but also several dinoflagellates) tend to form colonies or chains, some adaptations that confer the colony different physical and trophic properties such as a different buoyancy in the water, as well as greater protection from grazers (see Pančić & Kiørboe, 2018) compared to the individual

cell. The size and the shape of organisms determine their trophic interactions in different environments (Potapov et al., 2019), and in the pelagic one, phytoplankton size and composition have a structural effect on the whole pelagic food web (Karl et al., 2001; Riegman et al., 1993). Although primary consumers have certain trophic plasticity (Sommer et al., 2017), the smaller part of phytoplankton is generally not consumable by most primary consumers, and their energy is mostly directed towards trophic processes that have relatively little energy efficiency (e.g. the microbial loop) compared to the efficiency of energy pathways derived by the consumption of larger-size phytoplankton.

Some microalgae species can affect trophic networks by adopting unique defence mechanisms which, in a cascade effect, activate responses along with the upper trophic levels. If mechanically damaged (as a result of grazing) some species release chemical compounds that act as attractors for secondary consumers and relieve the grazing pressure by a so-called tritrophic interaction (Steinke et al., 2002). A well-known case is a trophic dynamic that follows the release of dimethyl sulfide (DMS) from some microalgae species (including *Emiliania huxleyi*) which would attract seabirds specialized in feeding on primary consumers. In addition to relieving the pressure of grazing, sea birds would benefit phytoplankton by fertilizing seawater through defecation (Savoca & Nevitt, 2014).

Although our direct perception of phytoplankton is limited because of their mostly invisible nature, changes in phytoplankton communities or the presence of particular species can have directly perceivable impacts on both human health and the economy. Some species can reach very high densities in the water column, others can produce toxic compounds, and can cause serious health problems for humans and negatively affect entire coastal marine systems (harmful algal blooms, HAB). Potentially toxic species belong to very distant taxonomic groups (Zingone & Enevoldsen, 2000), among diatoms some species belonging to the genus *Pseudo-nitzschia* can produce a powerful neurotoxin (domoic acid) which, accumulating in invertebrates' tissues, propagates along the food chain causing mass mortality in marine mammals (Lelong et al., 2012) and significant economic damage to human coastal activities (Hoagland & Scatasta, 2006). Among dinoflagellates, *Karenia brevis* and *Gonyaulax spinifera* are often associated with the so-called 'red tides', relatively frequent phenomena which can induce dramatic mass mortality in fish populations (Naqvi et al., 1998). Cyanobacteria are also associated with HAB events, some of them can produce toxins and particular conditions of nutrients and temperature have been seen to promote

their growth (Paerl & Huisman, 2008) besides producing other side effects including hypoxia (Paerl & Otten, 2013). Not all species associated with harmful algal blooms produce toxins, some may damage the gills of fish when reaching a certain density in the water as in the case of the mortality of salmonids in fish farms due to *Chaetoceros concavicornis* and *C. convolutus* (Albright et al. 1993). Nevertheless, while some compounds produced by some species of phytoplankton are harmful, others are currently used for medical applications, and the extreme genetic diversity of phytoplankton has become the subject of important progress in the biotechnological field in recent years (Lauritano et al., 2019).

In summary, the variety of shapes, sizes, life cycles and adaptations of the various species of phytoplankton determine a profound impact on the functioning and structure of marine ecosystems. Such diversity of phytoplankton life-forms presents the interesting condition of being passively affected by the environment and external forcing, and at the same time of being tremendously active in modelling the global chemistry and ecosystems functioning and, by modifying the absorption, surface albedo and surface drag it exerts significant control in global physics (Kahru et al., 1993; Sonntag & Hense; 2011; Webster & Hutchinson, 1994). Phytoplankton diversity translates contextually into a variety of individual and community responses, and this makes the study of the phytoplankton biodiversity and biology particularly relevant in the present time, where there is an urgent need to quantify and interpret the current environmental changes (McQuatters-Gollop et al., 2017).

# 1.2 Fundamentals of phytoplankton ecology

The biodiversity of phytoplankton has always represented a big conceptual headache for phytoplankton specialists and ecologists in general. One of the cardinal principles that ecologists use to decipher species diversity is the competitive exclusion principle, a theory that was already hypothesized by Charles Darwin during his famous journeys (Darwin, 1859) but that is generally attributed to Georgii Gause (Hardin, 1960). According to the competitive exclusion principle, two or more species living in the same geographical environment competing for the same resources cannot coexist. If one of the two had even a small competitive advantage to draw on available resources it would outcompete the other species, and lead it to its local extinction over the long period. The set of resources that a species needs to complete its life cycle and on which it competes by means of evolutionary adaptations is represented by the concept of the ecological niche. Although the competitive exclusion principle was initially conceived on the model of the Elton niche (Elton, 1927), which refers to the trophic-functional position of a species in the environment (e.g., grazers, detritivores, filter feeders), it has also been adapted to other niche models. In particular, G. Evelyn Hutchinson was the first to provide a quantitative argument to the concept of the ecological niche (Hutchinson, 1957), based on which he introduced a question that would stimulate phytoplankton research for years to come. Referring to phytoplankton, he wondered how it is possible for such a large variety of species to coexist in a relatively homogeneous environment.

In addition to light, autotrophic phytoplankton needs the same resources to grow, including inorganic micro and macronutrients (mainly nitrates, phosphates and silicates) for which all varieties of species compete simultaneously. Therefore, according to the competitive exclusion principle, the best-fitted species should overwhelm the others and dominate. Nevertheless, we observe a large variety of species even in summer, when waters in mid-latitude systems generally suffer from a large nutrient deficiency and when the competition among phytoplankton species would be at its maximum. This condition, well known as the 'paradox of the plankton' (Hutchinson, 1961) is one of the most famous classical problems in plankton ecology, and many approaches from diverse disciplines were adopted to contribute to its solution.

Hutchinson himself hypothesized that the aquatic environment, especially marine waters, never reach a stable equilibrium and argued that the time required to 'complete' the competitive exclusion principle in a phytoplankton community was greater than or equal to the time of the actual physical variability in the underwater environment (i.e., weather fluctuations). The aquatic environment would therefore never reach a physical equilibrium (and not even the resources for phytoplankton) preventing the absolute dominance of a single species in a community (Hutchinson, 1961). The resolution of the paradox by a 'nonequilibrium' view of the aquatic environment is one of the streams on which scientists have focused to solve the paradox (Roy & Chattopadhyay, 2007). Several studies have shown that the physical and chemical habitat of phytoplankton is not as homogeneous as previously thought, and this is above all true considering the spatial and temporal scale that phytoplankton cells experience. There is evidence that neither the composition nor the resources for which phytoplankton competes are spatially homogeneous in the aquatic environment, and that both biological and physical processes prevent the competitive exclusion (Bracco et al., 2000; Richerson et al., 1970).

Other efforts aimed to solve the paradox focused more on the biology and trophic relationships of phytoplankton than on the physical habitat. The view that emerges is that the biodiversity that we observe in natural phytoplankton populations is justified by the presence of additional limiting factors beyond light and nutrients. The selectivity and plasticity of grazers in feeding on phytoplankton are now well documented (Löder et al., 2011; Meunier et al., 2013-2016; Riegman et al., 1993), and the variety of microalgae shapes and sizes could reflect defence mechanisms related to specific attack systems (Smetacek, 2001). Other top-down mechanisms can influence the phytoplankton community and avoid the dominance of a single species. The role of viruses and parasites in regulating phytoplankton communities seems to be more important than previously thought (Sommer et al., 2012). According to Thingstad (1997) and Thingstad & Lignell (2000), the coexistence of competing phytoplankton is ensured by the presence of viruses and parasites that would prevalently infect the most abundant algal species in a dynamic generally known as the 'kill the winner' hypothesis.

Besides top-down processes, recent studies investigated the role of different metabolism in microalgae species and suggested that, even in a homogeneous environment, different species make differential use of resources. Thus, even if two species are equal competitors

for one niche dimension (e.g., same affinity for nitrates), they would show different competition behaviours for other niche dimensions (e.g., light absorption) making their coexistence possible without competitive exclusion (Burson et al., 2018; 2019).

Hence, there is evidence that species biology and adaptation strategies together with the effects of environmental variability determine the key for the coexistence of numerous species of microalgae. By collecting empirical data in the lakes of Pyrenees and Catalan coastal marine waters, Ramon Margalef assumed the temporal distribution of phytoplankton populations be the final result of competition dynamics, species adaptation strategies and habitat variability. In particular, Margalef gave much importance to the selective effect that the environment has on phytoplankton composition, and conceived the set of phytoplankton morphological characteristics and adaptive strategies as the expression of the selective process that habitat physical and mechanical properties exert on microalgal communities. He used the expression 'life-forms' to categorize the species adaptive strategies according to what he assumed as the two main abiotic forcings on phytoplankton community, nutrient availability and water mixing (turbulence) (Margalef, 1978). These ideas have been elegantly summarized in a model, the well-known Margalef's mandala (Margalef, 1978; Margalef et al., 1979) which, despite a formulation dating back to the 1970s, keeps being regularly cited and is still a source of inspiration for phytoplankton ecologists.

Margalef's *mandala* describes phytoplankton species in a space defined along the gradient of turbulence and nutrients, which represent the constraints on which the community is structured at a given time. In the *mandala*, the main taxonomic groups are proxies of phytoplankton life-forms (Fig. 1.3). Indeed, fertile conditions (high turbulence and high nutrients concentrations) would favour r-strategist, small and fast-growing species as diatoms, whereas conditions of low turbulence (stratification) and nutrient limitation would promote k-strategist species as larger, slow-growing and mobile forms such as dinoflagellates. In mid-latitude systems, fertile and limiting environmental conditions typically recur cyclically, therefore there is an implicit temporality in the Margalef model which describes the succession of species over the year (Margalef, 1997). Although the rigorous application of the conceptual framework provided by Margalef underestimates much of the taxonomic information, it still provides an excellent guide to the study of

phytoplankton ecology and is generally consistent with what we observe in natural phytoplankton populations (Kiørboe, 1993).
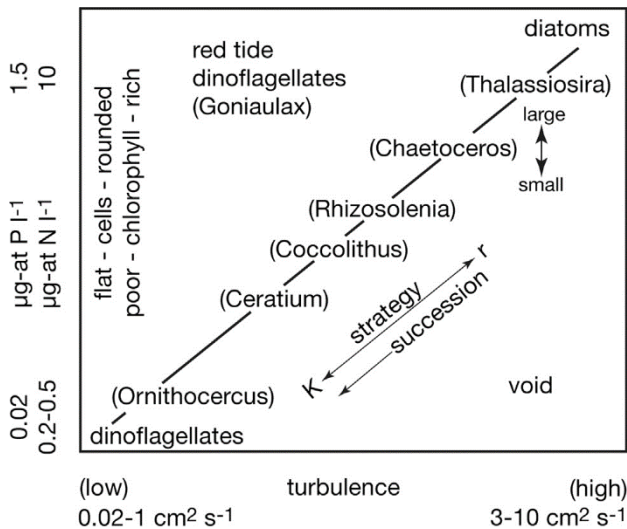


Figure 1.4 Margalef *mandala* (1978) representing the succession and the main strategies of phytoplankton genera in a bidimensional environmental space of turbulence and nutrients. Picture taken from Kemp & Villareal (2018).
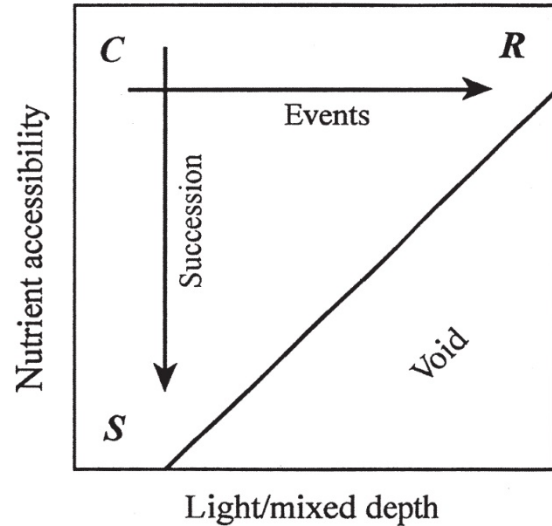
Figure 1.3 Smayda and Reynolds *intaglio* (2001) representing the three main survival strategies in the environmental space defined by resources (nutrients) and energy (light). Autogenic and allogenic succession are associated with succession and events' arrow respectively. Picture taken from Smayda & Reynolds (2001).

However, more recent evidence suggests that the knowledge of the taxonomy and properties of individual species is essential for understanding the ecology of phytoplankton and set important limitations to the Margalef model (Glibert, 2016; Kemp & Villareal, 2018). For instance, increasingly data support the idea that diatoms can regulate their buoyancy (Woods & Villareal, 2009; Gemmell et al., 2016), and this underweight the importance that Margalef attributed to turbulence in structuring phytoplankton communities. Furthermore, in several regions diatoms are an active part of the community even during summer (Cloern & Dufford, 2005; Ribera d'Alcalà et al., 2004; Zingone et al., 1990), a period generally characterized by a strong scarcity of nutrients and therefore potentially limiting diatoms growth. These arguments redefine the position of diatoms in Margalef's *mandala* and suggest that diatoms, rather than occupying the position of r-strategists, form a continuum

along the r/K axis through extensive physiological and adaptive plasticity (Kemp & Villareal, 2018). It is worth saying that this could also apply to other groups less studied and observed than diatoms.

Important advances in the knowledge and description of the phytoplankton ecology have been made based on studies conducted in the freshwater environment by C. S. Reynolds. Reynolds' view of phytoplankton ecology is based on the observation that in certain environmental conditions, a certain combination of species is more likely to occur than other combinations. These combinations, named 'species assemblages', include those species that exhibit high growth rates under those specific environmental conditions or, citing Southwood (1977), when habitat conditions match the 'habitat template' of each species. Reynolds formalized this hypothesis by representing species-specific growth rates using 6 parameters that he assumed as crucial in freshwater species distribution: mean underwater irradiance, mixed layer depth, water temperature, zooplankton filtration rate, carbon dioxide concentration and biologically available phosphorus (Reynolds, 1998). By intersecting the maximum growth-rate measurements for each axis, Reynolds mapped the habitat template of each species under a configuration called 'hexacle' (Fig. 1.4), based on which he categorized species. These categories or species assemblages, grouping species with similar physiological requirements and morphological adaptations, have successfully been used as a tool to summarize environmental variability in freshwater environments (Kruk et al., 2010 – 2011 - 2017).
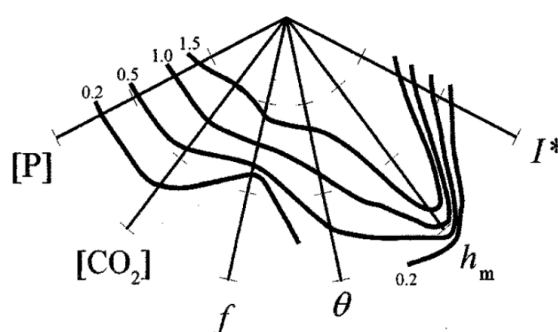


Figure 1.5 Habitat template of Chlorella according to growth rates measured in laboratory conditions associated with different parameters. Picture taken from Reynolds (1998).

Although the classification proposed by Reynolds was initially conceived for freshwater environments, Smayda and Reynolds (2001) adapted it to the marine context by mapping species assemblages into a so-called 'intaglio', obtained by combining an axis representative of the accessibility of the resources (nutrients) and one representative of the energy availability (light) (Fig. 1.5). These two axes are assumed to be the major dimensions of phytoplankton ecological niches, based on which three major survival strategies were distinguished: Colonist taxa (C-strategists) are typical of coastal areas where both nutrients and light are generally abundant and include many forms of flagellates but also some diatoms genera. Ruderal taxa (R-strategists) are light-stress-tolerant organisms and have peculiar morphological adaptations to maximize efficiency in resource uptake. Nutrient stress-tolerant taxa (S-strategists) have a high nutrient affinity and are adapted to nutrient-depleted conditions typical of the open ocean. Coccolithophores and cyanobacteria belong to this group. Like the *mandala*, temporality is implicit in this model. Environmental constraints act as a filter on specific traits and adaptations of species and regulate the persistence of a particular phytoplankton assemblage in a given time, and since the sets of environmental parameters are seasonally recurrent, specific phytoplankton assemblages are recurrent too.

In analyzing the temporal succession of the species, Reynolds distinguished two different processes related to the temporal replacement of the species. Phytoplankton communities experience changes in the physical-chemical environment that can be driven by both their autogenic/ biological activities (i.e., nutrient uptake) and allogenic forcing as changes in light and temperature regimes. The prevalence of autogenic or allogenic succession depends on the investigation area and the period. Indeed, the relaxation of physical forcing in mid-latitude summers promotes a succession driven by the gradual uptake of nutrients and, in absence of external forcing, the prolonged succession would culminate in a dynamic steady state where one species dominates overwhelmingly. However, what happens in nature is that disturbances imposed by external factors (allogenic) have the effect of arresting and/or changing the biological (autogenous) succession. Such allogenic forcing (mentioned as 'events' in Reynolds model (Fig. 1.5) are generally associated with hydrographic disruptions of the water column as a strong mixing or flushing (Sommer et al., 1993).

The approach proposed by Reynolds to cluster species with common environmental sensitivities and tolerances into functional groups is currently emerging as a useful tool to explain phytoplankton temporal variability and predict phytoplankton response to habitat changes (Litchman & Klausmeier 2008; Litchman et al., 2012). Functional traits inspired by Reynolds were successfully tested by Edwards et al. (2013) and Edwards (2016) in the English Channel, and more recently by Wentzky et al. (2020) in the Rappbode Reservoir (Germany). Nevertheless, despite the undoubted large contribution and tools provided by Reynolds' view of phytoplankton ecology, some limitations need to be addressed. Indeed, inferring natural species responses from artificial conditions must be done cautiously, as optimal conditions garnered in laboratory experiments do not necessarily coincide with those that may be effective *in situ* (Cloern & Dufford, 2005). Moreover, results obtained on a strain are hardly representative of natural populations, while phenotypic and genomic traits of strains grown in laboratory conditions can change over time (see Lakeman et al., 2009) making their application to the real world merely speculative. Finally, growing evidence of cryptic species that may have a different ecology while retaining identical morphological traits poses serious limitations to the application to approaches based on morpho-functional traits (Smayda, 2011; Zingone et al., 2003).

The intrinsic greater variability of the marine environment compared to the freshwater one could also play a role in the partial unsuitability of Reynolds' schemes in the ecology of marine phytoplankton. In contrast to Reynolds schemes, long-term phytoplankton data collected in a coastal area showed the regular co-occurrence of forms belonging to very different functional groups, from colony-forming and silica-requiring diatoms to solitary and scarcely silicified diatoms, autotrophic and mixotrophic species, and co-occurrence of motile and non-motile forms and, on the same concept, congeneric species characterized by very similar morphologies occurred in very different environmental contexts (Zingone et al., 2003).

In summary, we are still looking for a 'complete' paradigm of marine phytoplankton ecology despite many signs of progress made in the last 50 years. Given the microscopic dimension of primary producers in the aquatic environment compared to those of terrestrial habitats, the advancements of ecological theories on phytoplankton have strongly been influenced by technical limitations. Freshwater primary producers are microscopic too, but sampling phytoplankton in the marine environment requires very expensive equipment and technologies, which have seen a wide development only in the last decades making the study of marine phytoplankton a relatively younger discipline compared to the freshwater environment. Furthermore, as already argued before, phytoplankton is a group including a wide variety of different microscopic organisms, and their tremendous diversity makes the research for a sufficiently generalizable paradigm a highly demanding challenge, potentially manageable through the integration of data and information from different disciplines, from molecular biology to oceanography.

# 1.3 Phytoplankton as an ecological indicator

In 2001 Dale & Beyeler proposed a conceptual framework aimed to identify and describe the ideal properties of an ecological indicator, and their work is currently one of the most cited papers in the context of ecological indicators. In their view, an ideal ecological indicator should capture the complexity of a given ecosystem in a relatively simple way, and return easily communicable, quantitative information to facilitate management actions. It should also be sensitive to stresses on the system, it should have a predictable response over time, and certainly, it should be easily measurable (Dale & Beyeler, 2001).

Phytoplankton are ectothermic organisms having rapid growth rates, with an average doubling time of 0.5 per day (Laws, 2013) and given their relatively small size they react quite quickly to environmental changes. Indeed, their physiological properties and their metabolic rates are highly sensitive to temperature and light variations, as well as to nutrient concentrations (Reynolds, 2006). Furthermore, given their impossibility to move autonomously in the water column, phytoplankton are strongly influenced by physical forcing as surface currents, convective motions and stratification processes, which makes them an ideal candidate for describing and tracing both small and large-scale environmental changes (Harris, 1980). Phytoplankton biological and ecological processes take place relatively quickly compared to organisms characterized by longer life cycles, whereby the rapidity with which species respond to external forcing is a property that scales up to the community level. For instance, while the ecological succession of tree species in a forest is observable over a time interval of decades, the succession of microalgae species takes place in much shorter times and is generally observable and describable over a solar year (Sommer et al., 1986 - 2012). These properties contribute prominently defining the suitability of phytoplankton as an ideal environmental indicator. In fact, by knowing the general patterns of succession in relation to the environment, it is theoretically possible to set a reference useful to be compared with possible anomalous records, and constituting an invaluable advantage in the effort to understand the prominent factors involved in a certain change and act on them with the right management measures. Moreover, phytoplankton aggregated measures are relatively easy to sample and quantify.

Nowadays there is a large variety of instruments and methodologies useful to sample and quantify phytoplankton, from the classic nets and bottles coupled with microscopy generally used for the taxonomic identification, to methods based on fluorescence and chromatography for the quantification of aggregate properties such as chlorophyll and pigments, up to more recent semi-autonomous techniques such as flow cytometry, remote sensing, Imaging FlowCytobot (IFCB) and Environmental Sample Processor (ESP) and, remarkably, along with the development of each of these methodological areas there is a parallel effort aimed at the implementation of ecological indicators. Surely, each indicator obtained from different methodologies and techniques has its defined investigation scale and therefore its limitations, whereas the integrated use of them can provide an exhaustive framework to assess the environmental status (see Beaugrand, 2005), and ideally, to determine the spatio-temporal scale and the impact of a certain disturbance event on the many levels of biological organization.

Aggregate measurements of phytoplankton such as chlorophyll and microalgal density are among the parameters most commonly sampled in all aquatic systems and are often used as indicators of the trophic status and environmental quality of the system. For instance, relatively high values of chlorophyll can indicate a dystrophic condition of the system. Especially in coastal or semi-enclosed areas such as estuaries or lagoons, particular conditions such as enhanced nutrient fluxes increased temperature and man-made physical modifications of the habitat and removal of predators can favour an uncontrolled growth of phytoplankton biomass (Cloern, 2001), a phenomenon known as eutrophication (Nixon, 1995), with serious consequences on the whole system (Cloern, 2001; Rabalais et al., 2009). A case study represents the Baltic Sea which, given its geographical shape, the increase in population and industrial activities along its coasts, and the concurrent climate change is particularly exposed to eutrophication problems (Rönnberg & Bonsdorff, 2004), and the microalgal biomass is historically used as an indicator of the environmental status of the area.

Other indicators are based on the knowledge of the life history and biology of individual species and rely on the role and ecological dynamics that they play in a given system. For instance, abundance thresholds of particularly competitive species in a high-nutrient environment as *Phaeocystis* sp. in the UK (Devlin et al., 2007) and *Dactyliosolen fragilissimus* in the Baltic Sea (Andersson et al., 2006) are used as indicators and predictors of a stress condition in the system potentially leading to foams and/or anoxic events. It follows that appropriate knowledge of both the study area and the species biology and characteristics are essential to validate the indicators and effectively track environmental changes.

Nevertheless, given the tremendous diversity and variability of both phytoplankton and the environments that they populate, generalizable indicators based on single species are quite complex to extrapolate (Gowen et al., 2011). For this reason, in recent years a successful conceptual approach has been to associate species that have similar biology and similar ecological roles and functions in the environment in so-called functional groups or life-forms. As already explained above, both taxonomic and biological information is lost when species are grouped into wider categories, nevertheless life-forms allow to keep the ecological information associated with certain species (e.g., species with the same biochemical properties, trophic strategies, size classes) to provide a generalizable scheme. On these bases and considering phytoplankton sensitivity to environmental changes, Paul Tett conceived that the state of the environment at a certain moment could be described as a function of a set of representative life-forms of the system (Tett et al., 2007). This kind of approach is called a state-space approach, where the space is the ecological system and the state is defined by state variables represented by life-forms which, given their sensitivity to environmental disturbances, define the state of the environment itself (Fig. 1.6). The combined use of this approach on long-term observations of diverse life-forms can help to investigate ecosystem functioning from different perspectives and tell a lot about the evolution of a system and possibly the nature of its changes. For example, by comparing both the abundance and biovolume (as a proxy of size) of different life-forms including pelagic diatoms, autotrophic/mixotrophic dinoflagellates, heterotrophic dinoflagellates and ciliates, Whyte and colleagues (Whyte et al., 2017) traced and quantified the evolution of the pelagic system in Loch Creran (UK) associating it to changes in the precipitation patterns of the region.
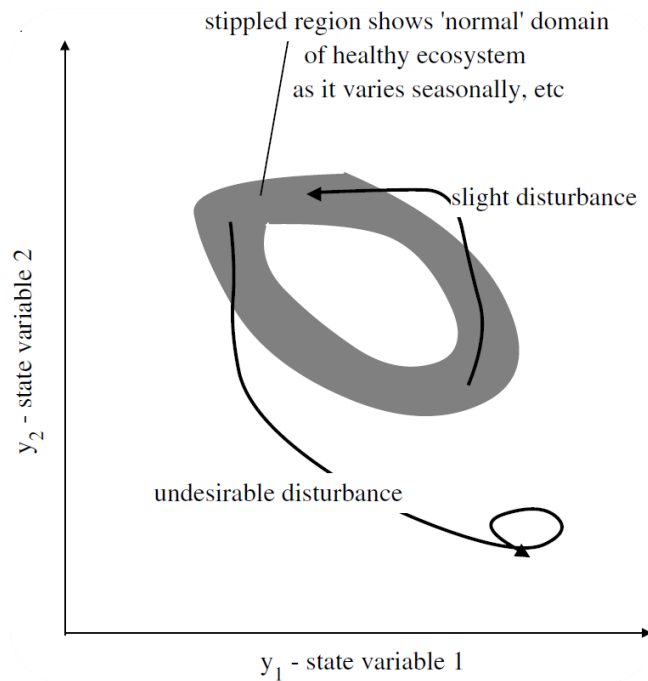
Figure 1.6 State-space diagram describing the ecological state of a system by two state variables. In normal conditions, state variables define a set of trajectories producing a characteristic 'doughnut' shape. Disturbances can divert trajectories outsides of the doughnut region as much as to define a new state. Picture taken from Tett et al. (2007).

The possibility of describing and tracing environmental changes is one of the most important issues of modern ecological science also from a societal point of view. Indeed, the relevance of phytoplankton abundance, biodiversity, composition and structure as sensitive indicators to both small and large-scale disturbances have been formalized in The Marine Strategy Framework Directive (Directive of the European Parliament, 2008). Furthermore, given the impellent need to trace the pace and magnitude of climate change there is a growing interest in phytoplankton long-term data, with the dual purpose of describing the current changes in the environment, and interpreting the present by identifying reference conditions by past information (Karl et al., 2010).

# 1.4 The long-term ecological research

Variability is a recurrent property of every natural system. In the sea, depending on the phenomena investigated, variability scales from less than millimetres to thousands of miles in space, and from less than seconds to centuries in time. In order to investigate the complexity of natural phenomena, science has developed theories, approaches and technologies targeted to match the scale of variability of specific natural processes. The division of a cell takes place in about one hour and is observed and interpreted through fluorescence microscopy techniques whereas the migration of marine mammals occurs in a solar year and is described through geolocation tracking techniques. So, if we want to investigate phenomena such as the succession of species and evolution of biological communities, in addition to applying the right technologies, we would also need several years to correctly describe and interpret these phenomena as they occur on relatively long-time scales. John J. Magnuson (1990) elegantly described the complex of phenomena and processes that occur too slowly for our perception as the 'invisible present', that is, that reality made of slow changes, causal relationships and lag effects that can be revealed and understood only if placed in the right temporal context provided by temporally sustained observations.

Misinterpretation is likely to occur if certain phenomena are not framed in a long-term perspective and approach. For example, Tilman (1987) investigated the effect of nitrogen fertilization on three plants over five years. In the first year of the experiment, the treated perennial ragweed showed an important abundance increase compared to control whereas bluegrass and blackberry did not respond to the treatment. During the following years, the treated perennial ragweed returned to the control levels whereas the other plants responded gradually to the treatment. If only the results of one year had been available, researchers would have erroneously concluded that fertilizing treatments favoured the perennial ragweed with no influence on other plants.

The long term ecological research (LTER) has the aim to unveil and understand the 'invisible present' mentioned by John J. Magnuson by conducting integrated studies over several years of sampling. Nonetheless, long-term biological time-series have played the role of 'Cinderella' in Marine Research (Koslow and Couture, 2013) until recently, when their importance has been widely acknowledged. The ongoing anthropogenic climate change has brought up new scientific challenges and is increasingly pushing the scientific community towards multiscale and integrative approaches. How can we evaluate and judge environmental changes or the state of an ecosystem or a population at a given moment? Ecological time-series are crucial for answer to these questions for many reasons.

As commented by Boero et al. (2015), ecological systems are historical systems governed by both constraints and contingencies. While constraints given by physical laws can be predicted and modelled, contingencies like species interactions, evolutionary patterns or unpredictable events can be only recorded and described in a probabilistic way. The collection of contingencies over time shapes the history of an ecosystem, and the study of that history can provide scenarios and predictions (by detecting regularities and patterns) on the future, as well as a tool to set the baseline to distinguish the 'normal' background variability of a process from possible signals of disturbance or degradation. As more observations are collected and integrated, new processes and new phenomena become apparent. Long-term research is thus the irreplaceable tool that integrates ecological observations with the right temporal context to give them significance and to translate information into knowledge and ultimately, into future predictions. Apart from the intuitive argumentation on the potential of time-series in producing reliable projections in a rapidly changing world, quantitative experiments have revealed that the predictability of a historical process increases with the increasing length of the time-series (Giron-Nava et al., 2017), highlighting longer time-series as indispensable to correctly understand ecological dynamics (White, 2019).

The potential to generate reliable future projections is one of the ultimate goals of LTER and it is what from a political and decision-making point of view enhances and valorise long-term research. For these reasons, studies based on long-term programs have a relatively greater scientific impact compared to short-term ones and are more frequently cited and promoted in policy-making and social contexts (Hughes et al., 2017). Furthermore, long-term programs have historically operated as attractors for further parallel research interests and as boosters for a synergistic and integrative scientific approach (see Franke et al., 2004; Karl et al., 2010; Zingone et al., 2019), stimulating new research lines and proposing new scientific and societal advancements. Paradoxically, many long-term projects around the world suffer from a lack of funding (Hughes et al., 2017), and many were interrupted although as never before there is the need to strategically set new long-term programs and, at the same time, update the existing ones.

# 1.4.1 Oceanographic time-series

The scientific interest in oceanographic long-term observations has at least two independent origins: weather prediction related to efficient transoceanic aviation, and management of commercial fisheries. A huge network of ocean-weather ships was established during World War II to aid transoceanic aviation and defence activities (Dinsmore, R.P. 1996). After the war, most of the ocean weather stations were dismissed, but at least 13 kept to collect oceanographic data, mostly temperature and salinity. As time passes, and with the growing interest in marine sciences, many ships converted from support to aviation to support oceanography and ecosystem research, while biological parameters were started to be collected in addition to physical ones. Examples are the Ocean station PAPA located in North-East America and Station M in the Norwegian Sea.

Other oceanic ecosystem time-series programs had their origins as commercial fishery investigations. The most emblematic cases are the California Cooperative Oceanic Fisheries Investigation (CalCOFI) and the Continuous Plankton Recorder Survey (CPR) in the North Atlantic. CalCOFI started in 1949 with the main aim of investigating the recruitment variability of the Pacific sardine and its collapse in the California Current (Ohman & Venrick 2003), whereas the CPR survey was designed to monitor the distribution of plankton in the North Sea in relation to fish stocks. The combined effort of different time-series around the globe has contributed to identifying and highlighting oceanographic phenomena of significant importance. Indeed, the combined analysis of oceanographic chemical data from DYFAMED (Dynamique des Flux Atmospheriques en MEDiterranee) in the Mediterranean sea, ESTOC (European Station for Time-series in the Ocean) in Canary Island, MOTS (Munida Microbial Observatory Time -Series) in New Zealand Subtropical Frontal Zone, HOT (Hawaii Ocean Time-series) in central North Pacific, Ocean Station Papa and KNOT (Kyodo North Pacific Ocean time-series) in the North Pacific, and BATS (Bermuda Atlantic Time-series Study) and CARIACO (CArbon Retention In A Colored Ocean) located in the central Atlantic reported an increase in the partial pressure of $CO_2$ in the last decades (Tanhua et al., 2015), and therefore an increase in acidity in many ocean basins on a global scale. Similarly, an integrated analysis performed on data from different platforms and measuring instruments has brought to light important insights on the trends of different descriptors of the biogeochemical status of many ocean regions, such as the decline of oxygen content in both

open ocean and coastal waters (Breitburg et al., 2018) and global rates of marine primary production (Behrenfeld et al., 2006) and chlorophyll (Boyce et al., 2010), both obtained by combining *in situ* and satellite data.

While the combined use of different measuring instruments and platforms scattered across the oceans has contributed to the identification and quantification of temporal processes of global importance, the gradual communication and integration of time series originally established for different disciplines have provided an additional level of knowledge, aimed at a more holistic cognition of the complexity of the marine system dynamics. The synergy between long-term meteorological and biological observations contributed to elucidating the close coupling between large-scale climatic phenomena and biological processes, the reported biological response to large-scale climate fluctuations as El Niño-Southern Oscillation (ENSO) and the AMO represent case studies of this synergy. In the first case, it was found that negative anomalies in the intensity of trade winds cause a weakening of the upwelling along the western coasts of Latin America, leading to an accumulation of relatively warmer and nutrient-poor waters. This atmospheric process is followed by lower production of phytoplankton (Barber & Chavez, 1983; Chavez et al., 1999; Chavez et al., 2010), which involves a cascade effect along with the whole trophic network, and up to the whole fish stock of the area on which many coastal countries of western Latin America depend on. Referring to the transversality of these findings, Chavez et al. (1999) cite in their abstract: '*The description and explanation of these dynamic changes would not have been possible without an observing system that combines biological, chemical, and physical sensors on moorings with remote sensing of chlorophyll.*'

The integration of different time-series has allowed to record and document large changes in the structure and functioning of ecosystems, commonly referred to as regime-shift. Indeed, an integrated analysis of time-series physical-chemical data, concentrations of algal pigments, primary production and mesozooplankton biomass in relation to atmospheric variability at ALOHA station returned a very detailed and complete picture of the influence of climate fluctuations (El Niño-Southern Oscillation and North Pacific Index) on the North Pacific system (Bidigare et al., 2009), from nutrients fluxes to primary producers and up to consumers. Similarly, the integration of time-series of different nature from CPR surveys has led to the identification of strong coupling between climatic decadal fluctuations as the

Atlantic Multidecadal Oscillation (AMO) and the North Atlantic Oscillation (NAO) and the variability of the pelagic system in the North Atlantic (see Harris et al., 2014).

Among the first established time-series, the Continuous Plankton Recorder program (CPR) was probably the first to take the shape of a real modern scientific program on plankton (phytoplankton, zooplankton and ichthyoplankton). The CPR program has led to over 900 publications (Reid et al., 2003), which have contributed significantly to the development of wide environmental policy programs as the EU Water Framework Directive. It was established by Sir Alister Hardy in 1931 (Hardy, 1953), who intuited the potential value of sampling over extended periods to better understand and interpret ocean systems processes. The variables considered include phytoplankton biomass (Phytoplankton Colour Index, PCI) and the relative abundance of up to 500 different phytoplankton and zooplankton taxa (Warner & Hays, 1994). Several monitoring programs were integrated into the CPR program over time, adding new physical and chemical parameters to pre-existent data. The elaboration of almost 50 years of plankton records highlighted some of the most significant effects of climate change on the marine system, examples of which are the potential of global warming to affects marine species phenology with impacts on the trophic synchrony between phytoplankton and zooplankton (Chivers et al., 2020; Edwards & Richardson, 2004), and the biogeographical shift observed in copepod populations due as a result of water surface temperature increase (Beaugrand et al., 2002).

Time-series represent the only set of tools able to explore the causal mechanisms between the complex components of the marine ecosystem and to distinguish between anthropic and natural forcing as this effort requires several decades of observation (Henson et al., 2016). Since natural phenomena occur on different temporal and spatial scales, we can understand and make predictions on a certain process only by adapting the appropriate scale of investigation (Fig. 1.7), and this makes time-series a fundamental tool for societal and policy needs (Frost et al., 2006) besides the merely scientific value.
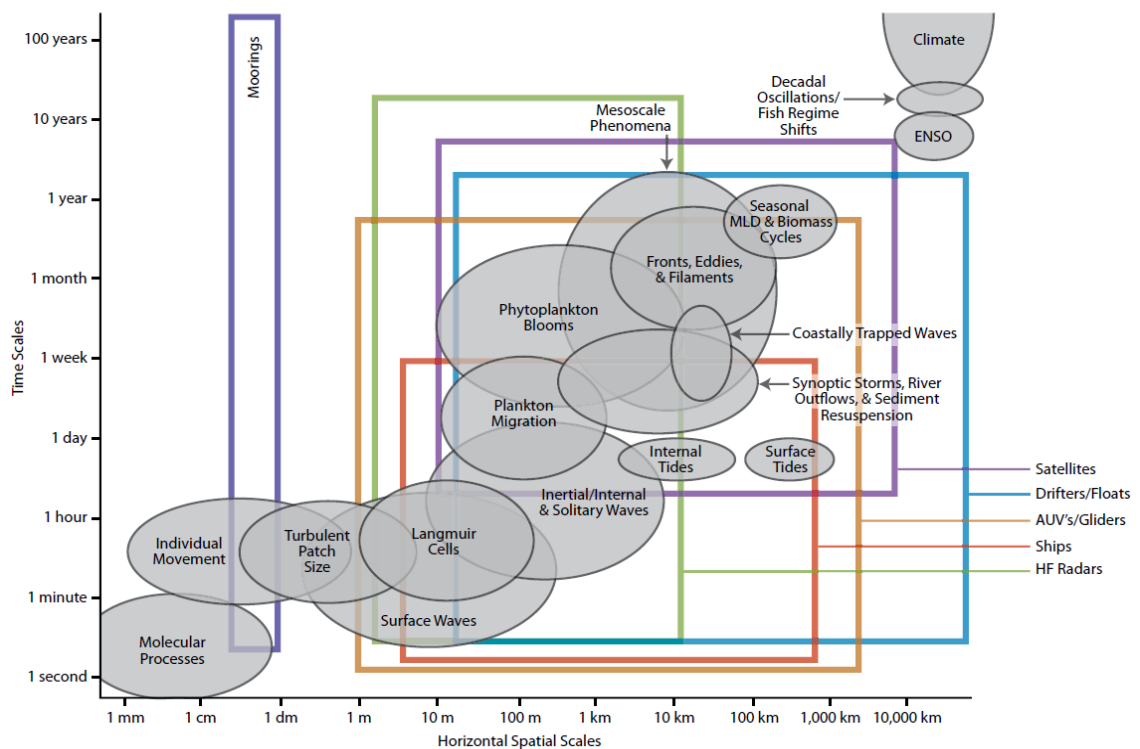
Figure 1.7 Adaptation of Stommel diagram by Dickey (2003) defining the appropriate temporal and spatial scales of observation of biological and physical processes occurring in the marine environment. Coloured polygons define the observation scale of the platforms and sampling systems typically used in oceanography. Picture taken from Karl et al. (2010).

## 1.4.2    Phytoplankton time-series

Modern time-series specifically addressing phytoplankton diversity arose according to different times and aims. Helgoland Roads, one of the richest marine long-term programs on phytoplankton was born following the first observations of plankton in the pelagic environment of the German Bight in the North Sea by Johannes Müller, Ernst Haeckel and Viktor Hensen (Franke et al., 2004). Although a wide range of organisms was regularly sampled and studied (plankton, lobsters, macroalgae and benthic invertebrates) the main aim was to understand the dynamics of fish stock variability of the area. A real scientific and interdisciplinary monitoring program started more recently, in 1962, with qualitative and quantitative analyses on phytoplankton performed on a workday frequency. With the specific aim of understanding the temporal and successional dynamics of phytoplankton, Ted Smayda and David Pratt began to regularly sample phytoplankton and environmental parameters in Narragansett Bay, then formally establishing a weekly time-series of almost 40 years (NABATS, 1959-1997). Many phytoplankton time-series programs born on specific needs. For instance, a huge long-term monitoring program was established in the Baltic Sea (HELCOM) in order to monitor the environment in response to climate change (Backer et al., 2010). Moreover, the HELCOM program includes different sampling stations, which allow obtaining more generalizable results by discriminating between local and regional phenomena (Jaanus et al., 2011; Wasmund et al., 2011). Similarly, Chesapeake Bay (Florida, USA) is the subject of an extensive temporal and geographical monitoring program, where phytoplankton species together with abiotic parameters are sampled monthly since 1984 to monitor the area and investigate the effect of anthropogenic and climate forcing on the large net of water ecosystems present in the region. Other phytoplankton time-series and time-series programs werevborn as a result of health and economic needs. The Department of Fisheries and Oceans (DFO) in Canada initiated the Harmful Algae Monitoring Programme (HAMP) in 1989 to investigate and contrast the effects of HABs on both public health and commercial exploitation of some marine species. Similarly, The French Phytoplankton and Phycotoxin Monitoring Network (REPHY) was instituted in 1987 with the double aim of enhancing the knowledge of coastal phytoplankton communities and protecting public health and the marine environment. The REPHY program includes long-term records of phytoplankton and environmental data sampled up to twice a month in multiple stations located around the French coast.

The topics studied in the different time-series covered different levels of biological scale, from single species to communities. In recent years, many time-series have been queried to contribute to the knowledge of the effects of climate change on phytoplankton communities. Indeed, the L4 station located in the Western English Channel is part of the larger Western Channel Observatory (WCO) which integrates weekly sampling of biological and abiotic data as well as satellite surveys and has an excellent tradition in phytoplankton studies. A long-term study on the L4 station revealed species-specific responses of the phytoplankton community to environmental variability and highlighted the taxa most involved in large-scale environmental forcing over 15 years of sampling (Widdicombe et al., 2010). As a result of the increase in water surface temperature, Widdicombe and colleagues reported a gradual long-term reduction in the abundance of many species of diatoms and *Phaeocystis*, while the heterotrophic component together with coccolithophores and *Prorocentrum minimum* increased. A comparative study on 3 phytoplankton time-series locates in the same area (English Channel), reported similar signals to those observed at L4, with a general increase in the proportion of abundance of dinoflagellates compared to diatoms (Hernández-Fariñas et al., 2014). Changes in nutrient load, salinity and temperature indexed by large-scale climate oscillations the North Atlantic oscillation (NAO, Jones et al., 1997) and the Atlantic multidecadal oscillation (AMO, Enfield et al., 2001) were correlated with a steep increase of the dinoflagellates Gymnodium-Gyrodinium spp. and a decrease of diatoms taxa belonging to *Guinardia*, *Coscinodiscus* and *Stellarima* clades. In the northern Adriatic in the Mediterranean Sea, observations of phytoplankton species abundances together with environmental parameters highlighted the reorganization process of the phytoplankton community over almost 30 years (Totti et al., 2019). Climatic and anthropogenic processes linked to changes in the hydrological conditions and nutrients loads have been associated with important changes in the composition of the community and the behaviour of representative species of the pelagic system. Indeed, *Skeletonema marinoi*, which typically bloomed in January has been seen extending its temporal window into late winter. The authors observed changes in phytoplankton assemblages in each season, from a winter assemblage characterized by *P. delicatissima* complex and *E. huxleyi* to one characterized by larger species as *Thalassiosira* spp., *Dytilum brightwellii* and *Lauderia annulata*, and from summer a community characterized by relatively large species as *C. pelagica*, *P. alata* and *G. flaccida* to a community characterized by diverse representatives of *Pseudo-nitzschia* and *Rhizosolenia* genera. Unlike the two other studies taken as a

reference, in the last described study the changes in phytoplankton community did not follow a linear trend but phases characterized by common elements along the interannual scale were rather recognized, an important information that emerges only by covering a relatively long period.

The ecological information associated with long-term changes in abundance or presence/absence of a certain species in a certain area is generally difficult to export to other systems. However, studies more focused on the ecology of individual species are building the basis for achieving this goal. Indeed, the Helgoland Roads dataset, by taking advantage of a daily sampling frequency and more than 50 years of sampling was investigated in order understand the ecological behaviour of many species using different methods and different ancillary data (Scharfe & Wiltshire, 2019; Schlüter et al., 2012; Wiltshire et al., 2015). The quantification of the different performance of groups as *Chaetoceros*, *Thalassiosira* and *Guinardia* to environmental and biological constraints (Wiltshire et al., 2015) provide valuable information not only on the ecology of the pelagic system in the German Bight, but they represent precious information for understanding the ecology of these wide-spread groups and to potentially predict their response to environmental changes.

The ecological properties of individual species have been successfully used to answer important scientific questions. Indeed, the CARIACO time series located in the Cariaco basin (Venezuela) was analysed to investigate the adaptive patterns of phytoplankton subject to a linear increase in temperature and reduction of the nutrient budget over 15 years (Irwin et al., 2015a). By calculating the realized niche of 67 species, the authors reported a substantial adaptation of phytoplankton on the long-term scale with important consequences on the debate on the impact of climate change on marine systems (Brun et al., 2015a; Irwin et al., 2015b). Similar results on the adaptive abilities of individual phytoplankton species were also found in a study conducted on Port Hacking coastal station in eastern Australia (Ajani et al., 2018) and, using both spatial and temporal data in the North Sea (Chivers et al., 2017). Other studies have used an approach based on the ecological concept of the niche to explain the ecological dynamics associated with potentially HABs-forming species. An example is that of some species belonging to the genus *Phaeocystis*, which can produce large gelatinous colonies that during bloom collapse can accumulate and altering both benthic and pelagic compartments (Blauw et al., 2010). Through the construction of the ecological niche of some species belonging to *Phaeocystis*, Karasiewicz

et al. (2018) showed that the optimal condition for these species to reach high biomasses was strictly influenced by biological interaction with other species such as some *Skeletonema* spp., *Thalassiosira gravida* and *Thalassionema nitzschioides.*

Availability of taxonomical information is a prerequisite for a more robust and coherent view of the ecological dynamics of phytoplankton. Many studies using aggregate phytoplankton measurements tend to focus on the most evident temporal signals, generally the spring bloom and biomass reduction in winter. However, analyses based on species' scale revealed that phytoplankton can reach high biomass and show a complex ecological activity even in generally thought unfavourable periods, as in the case of winter at LTER-MC station in the Gulf of Naples (Mediterranean Sea), where complex patterns of variability generated by the interaction between environmental forcing and species' life cycles were detected (Zingone et al., 2010a), highlighting the current limitations in marine phytoplankton paradigms.

Overall, phytoplankton time-series with a taxonomic resolution at species level offer the possibility to identify the subjects really involved in a process compared to bulk and aggregated measurements (such as entire functional groups or chlorophyll), and have the considerable potential of delimiting the scale of investigation of a process facilitating and optimizing the implementation of management measures (Boero et al., 2015; McQuatters-Gollop et al., 2017). Nevertheless, phytoplankton time-series with detailed taxonomy are relatively few compared to those characterized by aggregate measures of chlorophyll or functional groups despite their importance. In some of those still active, the characterization of phytoplankton diversity generally done by microscopy is being accompanied by new molecular techniques for improved taxonomic identification, opening a new dimension of knowledge to be integrated with the classic ones (Piredda et al., 2017; Zingone et al., 2019).

Currently, the long-term research programs on phytoplankton have made important progress following their original 'mission' and, through high-resolution taxonomic data have achieved a high level of knowledge on the behaviour of phytoplankton in their reference areas. However, very little has been done in comparative terms between these different time series. We have excellent examples of meta-analyses on phytoplankton biomass (Cloern & Jassby, 2008; 2010; Winder & Cloern, 2010), whereas we have very few studies comparing the dynamics of individual species in different habitats. This is not surprising as phytoplankton species data are relatively complex to standardize, and above all, because data of different series are obtained from different procedures and instruments (Zingone et

al., 2015). Furthermore, while we have achieved a suitable temporal coverage for the study of decadal patterns, adequate geographical coverage is lacking since most of the phytoplankton time-series are located in the northern hemisphere and temperate areas (fig. 1.8), and, with the notable exception of the CARIACO time-series and the Australian Phytoplankton Database, long-term data in the tropical environment are scarce.

Some efforts to integrate data and knowledge from different phytoplankton time-series has been made by the SCOR WG (Scientific Committee on Oceanographic Research Working Group) first, and nowadays by the IOC WG TrendsPO (IOC Working Group to Investigate Climate Change and Global Trends of Phytoplankton in the Oceans), a specific scientific commission formed to investigate climate change and global trends of phytoplankton in the oceans.
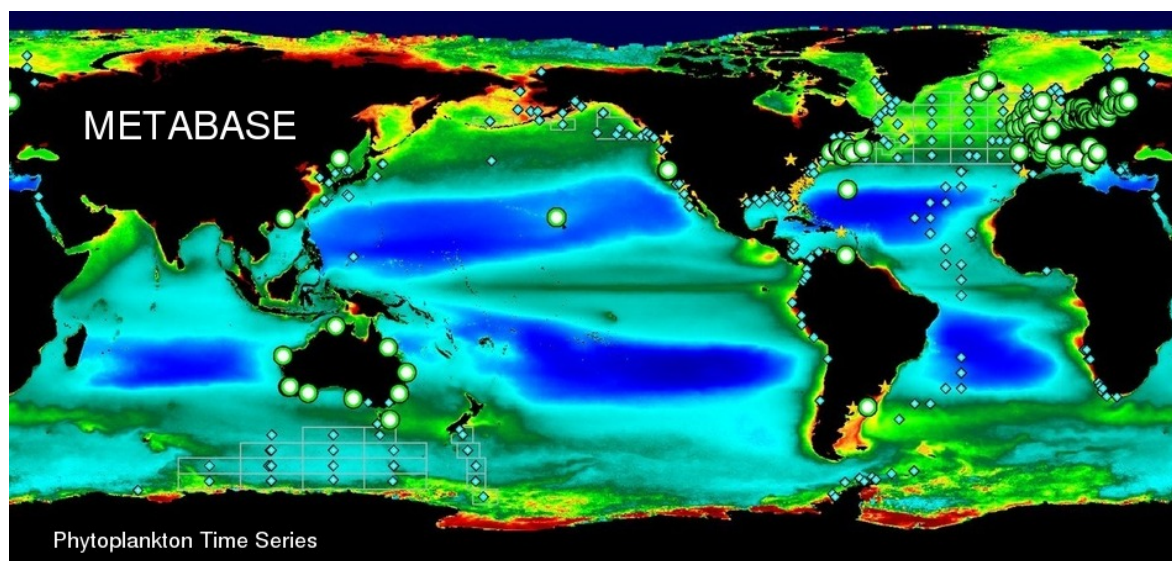


Figure 1.8 Map of phytoplankton time-series around the world (green circles). Picture taken from https://www.st.nmfs.noaa.gov/copepod/time-series/index-pts.html.

# 1.5 Long Term Ecological Research station MareChiara (LTER-MC)

## 1.5.1    The Mediterranean Sea and the Gulf of Naples

The Mediterranean Sea is the largest semi-enclosed basin on earth. Its location and physiography, together with the high urbanization level along the coastline, make it a unique marine system in the world. The Mediterranean Sea is characterized by distinctive physical features derived from a complex geological history that has testimonies in the richness and peculiarity of its marine biota. This basin can be represented as a crossroads of biogeographical features between cold temperate biota and subtropical species (Lejeusne et al., 2010) and, given its unusual and higher biological diversity compared to other temperate basins, it is considered a hotspot of biodiversity (Bianchi & Morri, 2000; Coll et al., 2010). Also, due to its processes of dense water formation and its well-defined thermohaline circulation, the Mediterranean Sea is considered as 'miniature ocean', and described as an ideal laboratory for the study of biotic and abiotic marine processes in a rapidly changing world (Bethoux et al., 1999; Siokou-Frangou et al., 2010), a window on the possible evolutions of the current human-driven changes.

The Gulf of Naples is a coastal embayment located in the middle of the Mediterranean Sea, in central Tyrrhenian. Its coastline measures 195 km and has an extension of 870 km² and an average depth of 170 m. Due to its complex geomorphology and hydrography, it is one of the most diversified areas of the Mediterranean Sea which hosts a rich biodiversity, and it is historically one of the most studied are of the Mediterranean Sea in the context of marine biology (Russo, 1995). The foundation in 1872 of Stazione Zoologica Anton Dohrn (SZN) contributed to attracting many researchers who left enormous scientific contributions in many branches of the study of marine life and oceanography. First studies about the plankton of the area date back to even before the foundation of the SZN (e.g., Costa 1838), while other studies were carried out later and focused on the diversity and distribution of copepods (Giesbrecht 1892; Hure & Scotto di Carlo 1974), and on the phenology of

microplankton (De Angelis, 1958; Indelli, 1945). More interdisciplinary studies aimed at describing and linking the biotic and abiotic dynamics of the pelagic system of the Gulf of Naples were made from the 80s onwards (Carrada et al., 1980; 1981; Marino et al., 1984; Scotto di Carlo et al., 1985), and shed light on the general functioning of the area and provided the basis for the establishment of long-term research projects such as the LTER-MC project.

## 1.5.2    LTER–MC

The LTER-MC station is located two nautical miles off from the coast (40.81°N, 14.25°E, Fig. 1.9), on a depth of ca 76 m, in an area characterized by quite peculiar hydrological dynamics. In fact, despite the proximity to the coastline, the LTER-MC station is strongly influenced by the offshore waters of the Tyrrhenian Sea. LTER-MC station is indeed characterized by the coexistence and alternation of two different subsystems: a eutrophic coastal system, strongly affected by land runoff, and an oligotrophic system characterized by the intrusion of offshore waters (Carrada et al., 1980). The boundaries and the extension of these two different subsystems depend mainly on physical factors and vary over the seasons (Carrada et al., 1981; Marino et al., 1984). Previous studies on the area highlighted that human activities along the coast (more than 4 million people surround the Gulf) have a strong influence on the distribution of nutrients and salinity on the water column, which is composed of two main layers: a surface layer (0-10m) and a deep layer (10-80m). Land runoff is the main source of new nutrients for the surface layer, which is generally accompanied by relatively fresher waters compared to the deep and that gives to surface specific characteristics during all seasons, even during the winter, when most of the water column is homogeneous (Scotto di Carlo et al., 1985). These characteristics influence the phytoplankton community composition and its successional patterns and make the Gulf of Naples a very productive area (Cianelli et al., 2017; Ribera d'Alcalà et al., 2004; Zingone et al., 1990) without however triggering too severe eutrophication processes or relevant dystrophies.
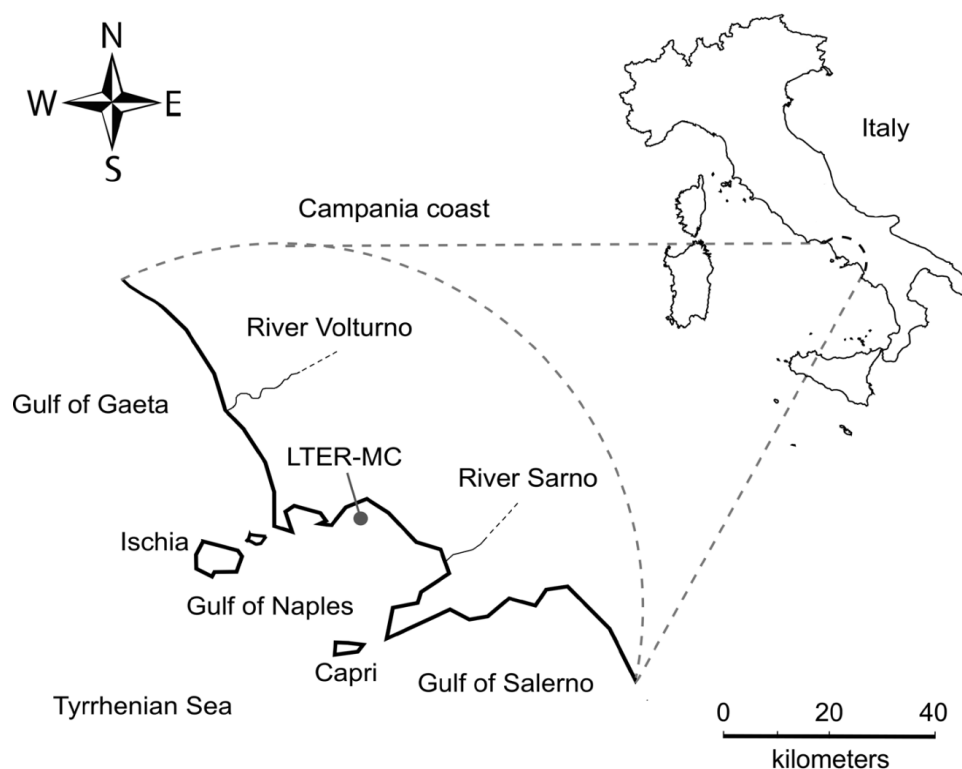
Figure 1.9 Map of the Gulf of Naples and the location of LTER-MC station (taken from Zingone et al., 2019) .

Regular fortnightly sampling at LTER-MC started in 1984, was interrupted in 1991 and then restarted with a weekly scale from 1995 to date. The site became part of the Italian, European and international LTER network in 2006. Data collected include physical, chemical and biological samples along the water column. Biological samples include chlorophyll, microphytoplankton and mesozooplankton. Other variables were added in the sampling routine as pigments through by High-Performance Liquid Chromatography (HPLC) since 1996 and, since 2007, bacteria and picoplankton by flow-cytometry and mesozooplankton carbon and nitrogen content. Also, an HTS-environmental DNA metabarcoding dataset was produced for the period 2011-2013 (Piredda et al., 2017). Other data include microzooplankton (March 1984–March 1985, September 1996–December 2009) and primary production (1984–1989 and 2007). The research activity is mainly directed towards the understanding of the diversity and the ecology of protists. The site is used as a natural laboratory in which specific temporal periods or particular events can be analyzed using the biotic and abiotic variety of data to highlight the mechanisms underlying life-cycles, foodweb processes and biodiversity of protists (see Zingone et al., 2019).

Although the first investigations on the composition and seasonal cycle of phytoplankton at LTER-MC were conducted before and immediately after the start of the regular sampling at LTER-MC (Carrada et al., 1980; 1981; Scotto di Carlo et al., 1985), a comprehensive descriptive study of phytoplankton at LTER-MC was conducted in Ribera d'Alcalà et al., in 2004. In terms of abundance, the surface phytoplankton community was found to be largely dominated by small phytoflagellates and by diatoms all the year. A slight increase in coccolithophores and dinoflagellates abundances was reported during the onset of spring and autumn bloom respectively. Winter and early spring communities were characterized by the presence of large and colonial diatoms as *Pseudo-nitzschia delicatissima, Thalassionema bacillaris and several Chaetoceros species,* while from the onset of stratification in late spring throughout the summer, large and colonial diatoms were generally substituted by small-sized and non-colonial species as *Skeletonema pseudocostatum*, *Chaetoceros tenuissimus*, and *Chaetoceros socialis.* In autumn, bimodal species have a second peak while other species (especially coccolithophores) were observed only during this period. Generally, few species dominated the community such as *Chaetoceros tenuissimus*, *Leptocylindrus danicus* and *Chaetoceros socialis*, which succeed one another following a relatively regular pattern. Besides, it has been noticed that some species, especially those among the most abundant, had a wide temporal distribution while others occurred within narrow temporal windows. Previous investigations conducted reported a similar successional pattern (Scotto di Carlo et al., 1985), and additional samples taken along the water column reported that except for summer, when small phytoflagellates dominated the deeper layers (10-70m), the composition community was mostly homogeneous in the whole water column.

A first attempt to investigate phytoplankton compositional patterns in relation to the abiotic context was conducted for the winter period of the series (Zingone et al., 2010a). Biomass and phytoplankton community data were analysed to explain the biomass accumulation (bloom) during winter, a period generally perceived as an unfavourable season for autotrophic growth. Conditions of relatively low salinity were associated with a reduced turbulent mixing and higher light availability for microalgae, which exploited such transient conditions producing relatively high biomass. Specifically, diatoms seemed to be particularly favoured under these conditions, whereas a flagellate-dominated community was characteristic of saltier and turbulent conditions. The variability of the phytoplankton

community in LTER-MC in relation to the environment was analysed also over an entire solar year. By integrating phytoplankton community data and wind speed and direction, Cianelli et al. (2017) reconstructed the main patterns of community variability and diversity in relation to physical forcing. As highlighted for the study on the winter cited above, the phytoplankton community responded actively to small scale environmental forcing all the year, mainly traced by changes in salinity. It was observed that phytoplankton composition at LTER-MC was highly variable and was strongly affected by physical and hydrodynamics processes at a weekly scale and that such forcing played an important role in maintaining and enhancing the biodiversity of the community.

Environmental forcing was also explored focusing on the trophic structure of the overall planktonic component at LTER-MC (D'Alelio et al., 2015). Planktonic trophic dynamics were described under a eutrophic condition, characterized by low salinity and high chlorophyll-a' concentration, and an oligotrophic condition identified by salty and chlorophyll-a-poor offshore waters, namely green and blue phases respectively. Although both phases were characterized by the same species, the results showed a complex dynamic of reorganization and plasticity in the structure of the planktonic trophic web, resulting in an optimization of the efficiency of the flows of matter and energy. Based on the general trophic condition, primary and secondary consumers selectively predated different plankton size-classes, stabilizing the delivery of organic matter along the trophic web, from picoplankton to fishes.

Overall, these works have been conducted investigating different periods and hypotheses highlighting that phytoplankton responses to environmental forcing involved different properties of the same: species composition, diversity, biomass and trophic web structure. Nevertheless, there is no information yet on the role of each environmental factor in relation to the single species and to wider temporal community patterns.

## 1.5.3    Data

The phytoplankton dataset from LTER-MC consists of records of species diversity and abundance obtained on more than 1500 samples collected in surface waters (Niskin bottle at 0.5m) at fortnight scale in the first years and at weekly scale since 1995. The data are characterized by a quite deep taxonomic resolution, with abundance data of more than 370 taxa (mostly classified at species level) and presence record of about 800 taxa. Cell counts are performed using an inverted light microscope at 400x magnification on a volume of seawater ranging from 0.02 to 1.52 mL. The identification is checked at an electron microscope for selected species. In addition to cell counts, carbon content is calculated routinely from mean cell biovolumes using the formula introduced by Strathmann (1967).

Samples for salinity and nutrients are routinely samples at ten fixed depths (0, 2, 5, 10, 20, 30, 40, 50, 60 and 70 m), seven of which (0, 2, 5, 10, 20, 40 and 60 m) are also sampled for chlorophyll-a. The temperature was sampled with reversing thermometers in the first part of the series (1984-1991) whereas from 1995 it was sampled with multiparametric profilers along with salinity and pressure. Until 2002 salinity was determined using a salinometer (Beckman mod. RS7C and subsequently Autosal Guildline Instruments). Since 2002 one CTD multiparametric profiler (Sea-Bird Electronics, 9-11 plus V2) has been used to measure temperature, salinity and pressure as well as biogeochemical (fluorescence and dissolved oxygen) and optical (Photosynthetically Available Radiation, PAR) parameters. Inorganic nutrients (ammonia, nitrates, nitrites, phosphates and silicates) are routinely collected from Niskin bottles into 20 ml polyethylene vials and immediately frozen and concentrations are determined following Hansen & Grasshoff (1983). In order to determine the chlorophyll-a concentration, a variable volume of seawater is filtered under a low vacuum and then extracted in 10 ml of neutralized acetone.  All the data are routinely subject to quality check protocol, a complete technical description of the sampling and quality check protocol is found in Sabia et al., (2019).

# 1.6 Thesis aims and chapters summary

The aim of my thesis is to explore the main mechanisms underlying phytoplankton variability at seasonal, interannual and decadal-scale mainly based on the data collected in the Gulf of Naples over a time series of observations started in 1985. To this aim, I have proposed and integrated a variety of analytical methods, and investigated temporal phytoplankton patterns at the organization levels of species, functional groups and the whole community, highlighting the role and the hierarchy of environmental factors in regulating the observed variability. Also, 10 global phytoplankton time-series were analysed to investigate the ecology of 10 widespread coastal phytoplankton species following a niche-based approach.

- The aim of Chapter 2 is to provide an overview of the trends and seasonal patterns of the whole time-series and compare them with previous results obtained on shorter fragments. Moreover, I wanted to test whether individual taxa showed periodic patterns in one of the most urbanized coasts of Europe characterized by high environmental variability and also, how the community as a whole responded to short and long-term environmental perturbations.

- Chapter 3 explores the dynamics related to climate change and its impact on the coastal system of the Gulf of Naples, addressing the issue of the responses of the abiotic and biotic components of LTER-MC in relation to the seasons.

- Chapter 4 takes up the most relevant results and conclusions from Chapter 2 and provides new conceptual approaches and a further level of detail to investigate the phenology of individual taxa in relation to environmental variability. I have addressed the relation between species phenological behaviour and environmental variability hypothesising that species bloom initiation and development are strongly dependent on species-specific annual temporal windows (temporal niches), which prevail to species response to environmental variability.

- The effort of Chapter 5 is addressed to a better understanding of the adaptive/conservative dynamics of phytoplankton. I used a unique set of 10 worldwide-distributed coastal phytoplankton time-series to test whether regionally-different morpho-species share the same ecological niche, and to clarify whether they conserve a common dynamic/organization during comparable environmental conditions from different regions.

# 2 Stability and recurrence of LTER-MC phytoplankton community

## 2.1 Introduction

Given the tremendous importance that phytoplankton have in the functioning and health of the oceans, one of the challenges of the modern scientific community is to understand and quantify the responses of phytoplankton to a rapidly changing environment. The impact of physical and chemical forcing on biological communities has been changing rapidly in the last decades (Harley et al., 2006; Hoegh-Guldberg & Bruno, 2010) and is also predicted to increase and become more severe in the next years due to the human-induced increase in global average temperature (Rahmstorf & Coumou, 2011).

Changes in physical and chemical factors in the marine environment can affect phytoplankton dynamics acting directly on species physiology (Rivkin, 1989; Toseland et al., 2013) or by changing the physical habitat of the autotroph communities (Doney, 2006), resulting in modifications of light and nutrients availability by which phytoplankton is ultimately governed (Sommer et al., 1986)

The comprehension of the mechanisms underlying the relationships between the environment and phytoplankton is particularly important in coastal areas. Land-sea interface areas represent one of the most productive types of environment (Falkowski et al., 1998) and are among the most ecologically and socioeconomically important systems on the planet (Harley et al., 2006). Half of the world's population lives along the coasts (Vitousek et al., 1997) and is thus directly linked with human well-being through sea-food availability, overall environmental quality and ecosystem services that coastal environments provide (Zingone et al., 2010b). Unlike open oceans, where phytoplankton variability is argued to be mostly driven by the annual cycles of solar radiation and atmospheric heat input (Sverdrup 1953; Cushing 1959), the presence and the influence of land in coastal systems translate into very complex ecological dynamics (Cloern & Jassby, 2008). Although challenging, understanding which factors are involved in phytoplankton variability is crucial since the

pace of change in coastal-estuarine areas is accelerating (Cloern et al., 2016), and aberrant events like eutrophication and HABs are occurring more and more often (Rabalais et al., 2009; Heisler et al., 2008).

Many studies have shown the relationship between environmental fluctuations and phytoplankton in terms of variability in the seasonal cycle (Romagnan et al., 2015), short-term disturbance events (Guadayol et al., 2009) and long-term variability processes (Brito et al., 2015; Cabrini et al., 2012; Hernandez-Farinas et al., 2013; Widdicombe et al., 2010; Zingone et al., 2010a). Different factors may have different importance among places in modifying the phytoplankton community, given the incredible diversity of coastal environments (Zingone et al., 2010b). Indeed, nutrient load and temperature fluctuations in the Baltic Sea are considered the main responsible factors altering the temporal structure of the community (Suikkanen et al., 2007; Wasmund et al., 2011). In other systems, especially the shallow ones, turbidity can modify the light environment perceived by phytoplankton and can affect the community composition (Barbosa et al., 2010; Cloern et al., 1987). Long-term observations. In Ilha Grande Bay (Brazil), Barrera-Alba et al. (2019) showed that the stability of the annual cycle and the microalgal composition were shaped by atmospheric conditions, such as rain and wind patterns. In other cases, no strong links have been found between environmental conditions and changes in the phytoplankton community despite important interannual changes in the environmental context (Brito et al., 2015), suggesting that other factors such as trophic processes may play a more important role. Collectively, these studies have highlighted an impressive complexity of ecological dynamics and the multidriver nature of environmental change and, not least, the technical challenges and limitations faced by the acquisition and comparability of long-term high-quality data (Zingone et al., 2015).

However, despite the high degree of variability in the planktonic habitat (and coastal environments in particular), there is a common and widespread temporal regularity that encompasses many levels of biological organization, from species successional patterns (Smayda, 1980) and biological associations (Teeling et al., 2016, Ward et al., 2017), to massive biological events as blooms (Carstensen et al., 2015). Seasonality and recurrence of marine autotrophs are typical of mid and high-latitude systems (Cloern & Jassby, 2010; Winder & Cloern, 2010;), where the annual astronomical cycle of solar radiation and day length trigger and shape the times for several biological dynamics (Morin, 2009; Tonkin et

al., 2017). As phytoplankton is at the base of food webs in marine environments, its temporal patterns set the pace for the dynamics of high-trophic levels and biogeochemical processes, and it follows that modifications in temporal properties of phytoplankton are closely coupled with the stability and the general ecological processes in marine systems (Edwards & Richardson, 2004).

Seasonal and interannual patterns of phytoplankton variability have been usually investigated based on relatively easy-measurable parameters as bulk and aggregated indicators such as chlorophyll-a and functional groups which, although being very useful in identifying changes in wide spatial and temporal scales (Boyce et al., 2017; Vantrepotte & Mélin, 2009) can provide a solid interpretative framework only if integrated with taxonomic data (McQuatters-Gollop et al., 2017). As a matter of fact, due to the scarcity of marine long-term programs (Koslow & and Couture, 2013) and also to a growing scarcity of taxonomic experts (McQuatters-Gollop et al., 2017), little is known about the temporal dynamics of individual taxa and even the quantification of their recurrence and periodicity is lacking.

Molecular-based approaches have shown that bacterioplankton communities are characterized by a high degree of seasonal re-occurrence (Auladell et al., 2019; Cram et al., 2015; Gilbert et al., 2012; Ward et al., 2017) and were highly predictable by ocean conditions suggesting the pivotal role of environmental changes in shaping the abundance and diversity of communities (Bunse & Pinhassi, 2017; Fuhrman et al., 2006; Fuhrman et al., 2015; Galand et al., 2010). Similar approaches were also performed on molecular time-series of eukaryotic phytoplankton, which highlighted very regular temporal patterns at the seasonal scale (Brannock et al., 2016; Countway et al., 2010; Piredda et al., 2017). In coastal areas the recurrence of surface phytoplankton taxa was associated with day length and temperature (Genitsaris et al., 2015; Giner et al., 2019; Lambert et al., 2019), nevertheless, a high degree of temporal variability in the community remained unexplained, and according to a recent study (Giner et al., 2019), not all the taxa followed periodic patterns suggesting that other factors besides environmental parameters can play a crucial role in driving the seasonal occurrence of eukaryotic protists.

In the Gulf of Naples, a certain temporal recurrence of planktonic assemblages was reported for phytoplankton in the first period of the LTER-MC time-series (1984-2000) (Ribera d'Alcalà et al., 2004), and for copepods populations (Mazzocchi et al., 2012). Moreover, Ribera d'Alcalà and colleagues highlighted some long-term signals of the LTER-MC' system, including a decrease in the autotrophic biomass over 15 years. Nevertheless, a more extensive analysis focused on seasonal and interannual phytoplankton' variability has not been done yet. Here, I used phytoplankton data from one of the longest, high-resolution coastal time-series in Europe in order to investigate the individual and community temporal dynamics over 25 years of high-resolution sampling. The sampling site (LTER-MC) is located in the Gulf of Naples in an area strongly influenced by the offshore waters of the Tyrrhenian Sea where two different subsystems coexist: a eutrophic coastal system, strongly affected by land runoff, and an oligotrophic system characterized by the offshore waters (Carrada et al., 1980). Specifically, my aim is to provide an overview of the trends and seasonal patterns of the whole time-series and compare them with previous results obtained on shorter fragments. Also, I wanted to characterize and assess the coupling between phytoplankton community' turnover and environmental variability and, by using high-resolution taxonomic data, provide a hierarchy of the environmental factors involved in the temporal recurrence of phytoplankton species.

## 2.2 Materials and Methods

## 2.2.1    Environmental variability

The seasonal variability of surface (0-10m) and deep (10-70m) physical (temperature and salinity, mixed layer depth), chemical (total inorganic nitrogen, phosphates and silicates) and biological parameters (chlorophyll-a) was described plotting the corresponding weekly means and standard deviations. The interannual variability of these parameters was analysed by performing a Mann-Kendall test (Mann 1945; Kendall 1975) implemented in package 'wq' (Jassby & Cloern, 2015) to statistically assess the presence of monotonic upward or downward seasonal trends using surface monthly averaged data.

## 2.2.2    Phytoplankton composition

In order to describe the general characteristics of phytoplankton community composition, the taxa abundance and carbon content data of the LTER-MC dataset (see 1.5.3) were summarised in functional groups composed of diatoms, dinoflagellates, phytoflagellates and coccolithophores, and were visualised at a monthly scale. Similarly, I represented the climatology of both abundance and carbon content of the most representative taxa of diatoms in order to highlight the main temporal patterns of phytoplankton species at LTER-MC.

## 2.2.3    Taxa periodicity and seasonal succession

In order to quantify the seasonal variability associated with phytoplankton taxa at LTER-MC, I used Lomb-Scargle periodograms (Lomb 1976; Scargle 1982) on abundance data. Periodograms are mathematical tools used to search and characterize periodic properties in a generic signal. Their application has initially received much credit in astrophysics (revealing to be a good tool to intercept the periodicity of signals emanating from celestial bodies) while in recent years their application has been extended to other areas, including time-series of high-frequency biological data (Legendre et al., 1981; Ruf, 1999; Vasseur et al., 2005; Lambert et al., 2019). As periodograms are quite sensitive to heterogeneous data, they were performed on weekly species abundance data of the second part of the LTER-MC series (1996-2015). Therefore, although I did not take into account the whole time-series length, the 19 years of the second part of the series was considered a period enough long to robustly quantify the periodicity of the taxa.

Frequency and abundance thresholds, along with expert judgement by taxonomists (Adriana Zingone and Diana Sarno) were used to select the taxa to be used in the analyses. To limit the spectral analysis to taxonomically unambiguous entities and focus on biological entities that were taxonomically as close as possible to species level, a first selection was made excluding groups composed by the aggregated abundance of multiple unidentified taxonomic units (e.g. undetermined phytoflagellates and dinoflagellates, centric or pennate diatoms), heterotrophic flagellates and life cycle stages cysts and spores were also removed. Then, relative frequency thresholds were used in order to take into account only taxa that were less subject to misclassification. Specifically, I have set a proportion of occurrence of 4% in order to include all the taxa that had been virtually recorded at least 2 times per year. Four 4 taxa with a lower frequency (*Bacteriastrum furcatum*, *Skeletonema tropicum*, *Lioloma* spp. (corrected to sp.), and *Umbilicosphaera sibogae*) were retained because their identification was considered reliable.

The taxa passing the selection (93 taxa, 86 classified at the species level and 7 at genus) were considered statistically and taxonomically reliable and representative of the pelagic system at LTER-MC. Their abundance was log-transformed and analysed using the periodogram by the 'lsp' function from the 'lomb' R package (Ruf, 2013) to 1) distinguish periodic from non-periodic taxa, 2) quantify taxa periodicity and 3) assess the modality of taxa's temporal

distribution (unimodal or bimodal). A general periodogram output shows how the total variance of a signal is distributed over a range of frequencies and returns both the period and the significance of a frequency (expressed by normalised power). Therefore, given the weekly frequency of sampling, taxa that peaked once or twice a year were supposed to show a significant peak in the periodogram at frequencies associated with a period around 52 (average number of weeks in a year) and 26 respectively. Thus, significant peaks found at frequencies associated with a period around 52 and 26 were considered indicative of unimodal and bimodal species respectively.

The seasonal succession of the phytoplankton community was investigated using the Indicator Value (*IndVal*, Dufrêne & Legendre, 1997). IndVal is a measure that ranges from 0 to 1 that synthesizes the fidelity and specificity of a biological entity versus a specific category, and is calculated according to the following formula:

$$\text{IndVal}_{ij} = 100 \times A_{ij} \times B_{ij}$$

where $A_{ij}$ represents the specificity, measured as the proportion of the individuals of species $i$ that are in the category $j$, and $B_{ij}$ represents the fidelity, that is, the proportion of observations in the category j that contain the species i. Here, in order to describe phytoplankton species succession at LTER-MC I have calculated the specificity and fidelity of species versus each month of the year.

## 2.2.4    Long-term community patterns

In addition to the periodicity of individual taxa, I have investigated the seasonal and long-term variability of the turnover of the selected taxa using a Bray–Curtis pseudo-autocorrelation. Bray–Curtis pseudo-autocorrelation is performed averaging the similarity of the communities (indexed by Bray-Curtis distance) for each possible time-lag among all the months in the time-series, and it is visualized on a bidimensional space representing the differences in community composition as a function of the time lag between the samples. The final output is an index (ranging between 0 and 1) of the community's similarity at all possible time lags of the series. Such methodology is successfully applied in the analysis of microbial time-series (especially molecular ones) since it summarizes both the seasonal and the interannual compositional variability of a community (Fuhrman et al., 2015). I performed the Bray–Curtis pseudo-autocorrelation on both the entire length of the time-series (1984-2015) and on the second part of the time-series (1996-2015) and compared their interannual variability using a Mann-Kendall test.

In order to assess the importance of environmental factors in driving the temporal variability of the phytoplankton community, I performed a discriminant function analysis (DFA), a multivariate technique used to quantify how well a certain combination of continuous variables discriminate between two or more levels of a categorical variable. The idea behind the use of DFA is to create a single multivariate index that describes the compositional variability of a community over time and to analyse such an index in relation to environmental factors (Fuhrman et al., 2006). In order to obtain this single multivariate index, the DFA was performed to predict the time of sampling (the month of the year) starting from monthly averages of phytoplankton abundances, assuming that given a certain taxa distribution and abundance, it would be possible to predict the sampling month. The DFA generates n-1 discriminant functions (with n = n° of months), the first of which (DF1) provides the most overall discrimination among the groups (months), thus, the first discriminant function (DF1) represents a multivariate index that summarizes the variability of the composition and abundance of the phytoplankton community in the LTER-MC time-series.

 In order to investigate the relationship between environmental factors and community variability, a multiple linear regression was performed using DF1 as a response variable and

environmental parameters as independent variables (day length, temperature, salinity, total inorganic nitrogen (hereafter TIN), silicates, phosphates and chlorophyll-a). In order to check any collinearity issues among predictors, I have calculated the variance inflation factor (VIF), a measure of how much the variance associated with a predictor is influenced (and inflated) by its interaction with the other independent variables, a circumstance potentially generating lower reliability of the parameters estimates.

Then, I have estimated the contribution of predictors to the model's total explanatory power. One of the problems when decomposing the variance in regression models is that each order of the regressors generates a different decomposition of the sum of squares. Therefore, the contributions were calculated according to Lindeman, Merenda and Gold's method (LMG; Kruskal, 1987; Lindeman et al., 1980) with the function '*calc.relimp*' implemented in the '*relaimpo*' R package (Groemping & Matthias, 2018). The LMG method is based on the sequential sums of squares of all regressors and takes into account the dependence of the order of the regressors in the decomposition process and despite being computationally expensive, it provides reliable results also when dealing with multicollinearity among predictors (Bi, 2012; Grömping, 2006; Tian, 2013).

## 2.2.5 STATICO analysis

In order to investigate the relationship between interannual environmental fluctuations and variability in the phytoplankton community, I have applied the STATICO method (Thioulouse et al., 2004) on the whole period covered by the LTER-MC sampling program. The STATICO method is an implementation of the Partial Triadic Analysis (Thioulouse & Chessel, 1987) based on co-inertia objects (Dray et al., 2003), and is designed to quantify and describe visually the relationship between pairs of tables associated with different years, here corresponding to yearly environmental and biological matrices. The main aim of STATICO is to search for a common structure in the species-environment relationship (usually mentioned as 'Compromise') and assess the temporal stability of such structure by taking advantage of repeated samplings (26 years in this case). Here, data were organized in yearly pairs of tables corresponding to surface monthly-averaged values of physicochemical parameters (temperature, salinity, TIN, phosphates and silicates) and surface monthly-averaged counts of the taxa selected using periodograms.

The flow chart of the STATICO method is displayed in Fig. 2.1 and proceeded according to the following steps:

1) Yearly pairs of tables (species and environmental matrices of each year) were crossed producing a single cross-covariance table for each year. This operation is performed by a co-inertia analysis (Dray et al., 2003) aimed to maximize the covariance between the observations of the two clouds of points (species and environmental matrices). According to the schemes proposed by Dray et al. (2003) a principal component analysis (PCA) was performed on the euclidean distances of the scaled environmental matrix, whereas a principal coordinate analysis (PCoA) was performed on the square-root transformed Bray-Curtis distances (Bray & Curtis, 1957) of the biological matrix. The choice to square-root transform the Bray-Curtis distances was dictated by the fact that PCoA can produce negative eigenvalues when is computed on non-Euclidean distances as the Bray-Curtis one, potentially impeding a full euclidean representation (and a correct interpretation) of a set of objects. Solutions to this problem include adding a constant to the squared distances among objects (Lingoes correction, Lingoes, 1971) or to the distances themselves (Cailliez correction, Cailliez, 1983), or to square-root transform the distances, as suggested by Gower & Legendre (1986), Legendre & Legendre (2012) and Borcard et al. (2018).

2) A partial triadic analysis was used to analyse the sequence of cross-covariance (co-inertia matrices). A set of RV coefficients (a measure between 0 and 1 describing the similarity between the two matrices (Escoufier, 1973)) were computed for each couple of cross-covariance tables producing a matrix of vectors' correlations between the different years. Then, an eigenanalysis (PCA) was performed on the RV coefficients' matrix to quantify the similarity of the different tables (years) in a visual configuration mentioned as 'Interstructure'. The Interstructure resulted in an ordination of the different years, showing the proximity of the vectors associated with each year on a bidimensional space, thus highlighting similarities and divergences between years' tables.

3) A new table was computed as the weighted mean of all the tables of the series using the components of the first eigenvector of the Interstructure as weights. This is the Compromise, a configuration outlying the common structure of the species-environment relationship over the different years.

4) Each table (year), was projected in the Compromise map in the form of trajectories in order to highlight the similarity/divergence of the biological-environmental dynamics versus the stable structure of the overall species-environment relationship at LTER-MC (the Compromise).
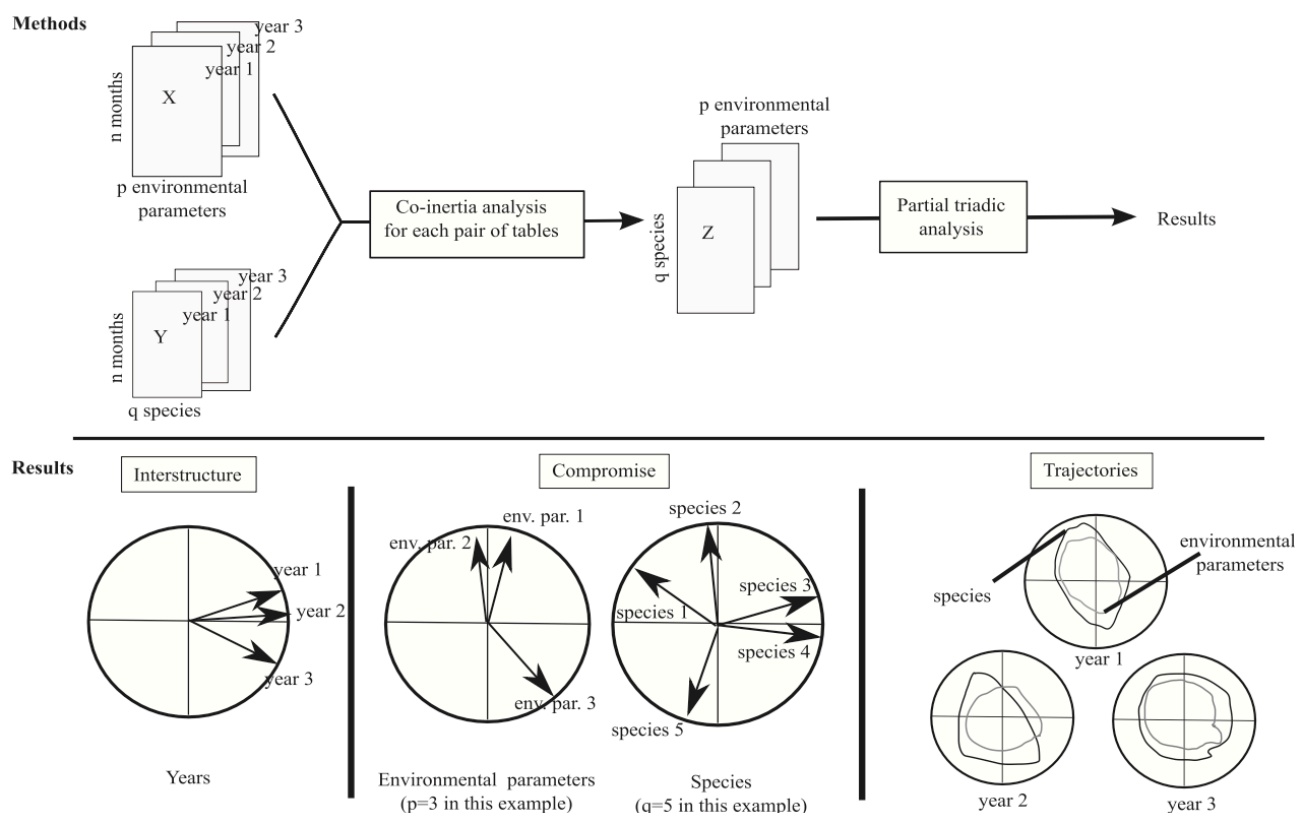
Figure 2.1 Flow chart taken from Mazzocchi et al. (2012) of the STATICO' methodological steps (upper panel) and results (lower panel). The data consist of a sequence of pairs of environmental and species matrices (X and Y respectively). In this example, p environmental parameters and q species were sampled n months for 3 years. The first step consists to perform a co-inertia analysis for each pair of tables producing the cross-tables Z. Then, a partial triadic analysis is used to analyze the sequence of cross-tables. The three main results produced are the Interstructure, the Compromise, and the Trajectories. The Interstructure shows the proximity between each pair of tables (3 years in this example). The Compromise analysis results in an ordination space representing the stable part of the species-environment relationship across the years. The Trajectories projected on the compromise summarise the reproducibility of the structure across the years. Taken from Mazzocchi et al. (2012).

# 2.3 Results

## 2.3.1     Seasonal environmental variability

Surface water temperature followed a well-defined seasonal cycle characterized by minimum values in winter and maximum in summer (Fig. 2.2A). The lowest average values were recorded in February and March of 2006 (13.13 ± 0.58 °C and 13.41 ± 0.20 °C respectively) while the maximum average values were recorded in July 1998 (27.79 ± 0.76 °C) and in August 1988 (27.69 ± 0.57 °C). During winter, deep and surface temperature tended to have the same values, while from mid-spring to the beginning of autumn deep layers reached temperatures significantly lower than those recorded on the surface. Salinity at LTER-MC (Fig. 2.2B) showed wide seasonal fluctuations characterized by the highest values generally recorded in autumn when increasing winds contribute to higher evaporation of surface water compared to other periods of the year.  By contrast, the lowest salinity values, ranging between 37.5 and 36, were typical of late spring and early summer. At greater depths, salinity followed the same pattern as in surface but the values were characterized by a lower overall variability around the mean compared to the surface. The annual cycle of Secchi depth followed a cycle substantially coupled with that of salinity, with maximum values in autumn and winter and lowest recorded during spring and summer (Fig. 2.2D). The mixed layer depth (Fig. 2.2C), mainly driven by temperature, shallowed out quickly between the $10^{th}$ and $20^{th}$ week of the year (from mid-March to the end of May) and settled around 10-m depth until the beginning of September, after which it gradually widened comprising the entire water column.

With the exception of phosphates (Fig. 2.2H), nutrient concentrations in the water column followed a seasonal cycle characterized by wide fluctuations. Both silicates and TIN (Total Inorganic Nitrogen: nitrates + nitrites + ammonia) showed the highest concentrations during winter followed by a gradual decline towards the end of winter (Fig. 2.2G and Fig. 2.2F respectively). Since late winter- beginning of spring, surface chlorophyll-a concentrations reached gradually their maximum values (generally in May), while in summer nutrients showed the minimum values (Fig. 2.2E). Nutrient concentrations in the deep layers (10-70 m) were typically lower than those observed at the surface for most of the annual cycle except for the summer period. The average TIN concentrations at 10-70 m were quite

comparable with those of the surface, while deep-layers silicates concentrations were typically higher than those recorded at the surface during the stratified period. Interestingly, both surface silicates and TIN concentrations showed a rapid and marked decrease between the 15th and 18th week of the year and then returned to values following their characteristic cycle. The breaking of the thermocline occurred in late summer and early autumn. During the latter season, a second bloom generally occurred but surface chlorophyll-a concentrations were generally lower than the spring ones.
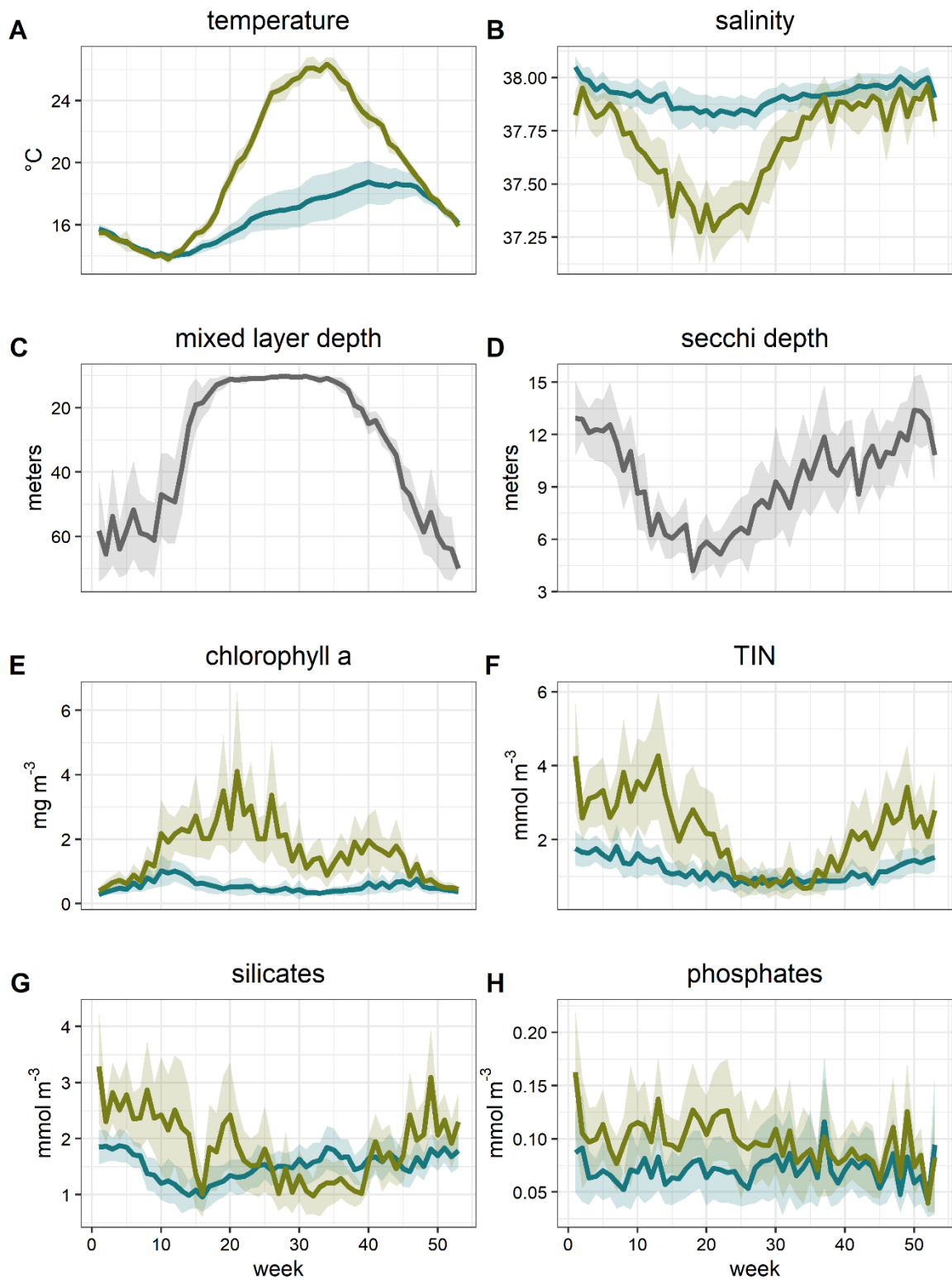
Figure 2.2 Climatology of the main environmental parameters, nutrient and Chlorophyll *a* concentration at LTER-MC during the period 1984-2014. The green lines (average) and shaded areas (0.95 confidence interval) refer to the surface layer (0-10m), the blue ones to the layer 10-70m.

## 2.3.2    Interannual environmental variability

Except for winter, the surface temperature at LTER-MC generally increased over all the seasons (Fig. 2.3A). Results of the Mann-Kendall test reported a significant average increase of 0.03, 0.07 and 0.04 °C per year in April, July and September respectively (Fig. 2.3B). Conversely, surface salinity abruptly decreased at the interannual scale, and the Mann-Kendall test reported a significant downward trend in most of the spring and summer months and in October. Moreover, the local regression performed on the series revealed a pluriannual cyclical behaviour that defined alternative fresher and saltier states of surface salinity with a period of around 5 years. Inorganic nutrient concentrations in the surface layer were generally higher in the first part of the series, especially for phosphates, whereas at the beginning of the 90s, phosphates experienced a gradual but significant decline at LTER-MC. The decline involved all the months of the year as supported by the results of the Mann-Kendall test. In contrast, total inorganic nitrogen (TIN) and silicates remained quite stable over the years, except for a significant long-term increase of TIN during February and a silicates' decrease in August. In parallel with the main nutrients, surface chlorophyll-a concentrations were generally higher during the first part of the series, when they reached by far the highest peaks of the whole series. On average, the period 1997-2003 was characterized by the lowest concentrations of phytoplankton biomass, after which chlorophyll-a' levels gradually increased, resulting in a significant positive trend in April and November.
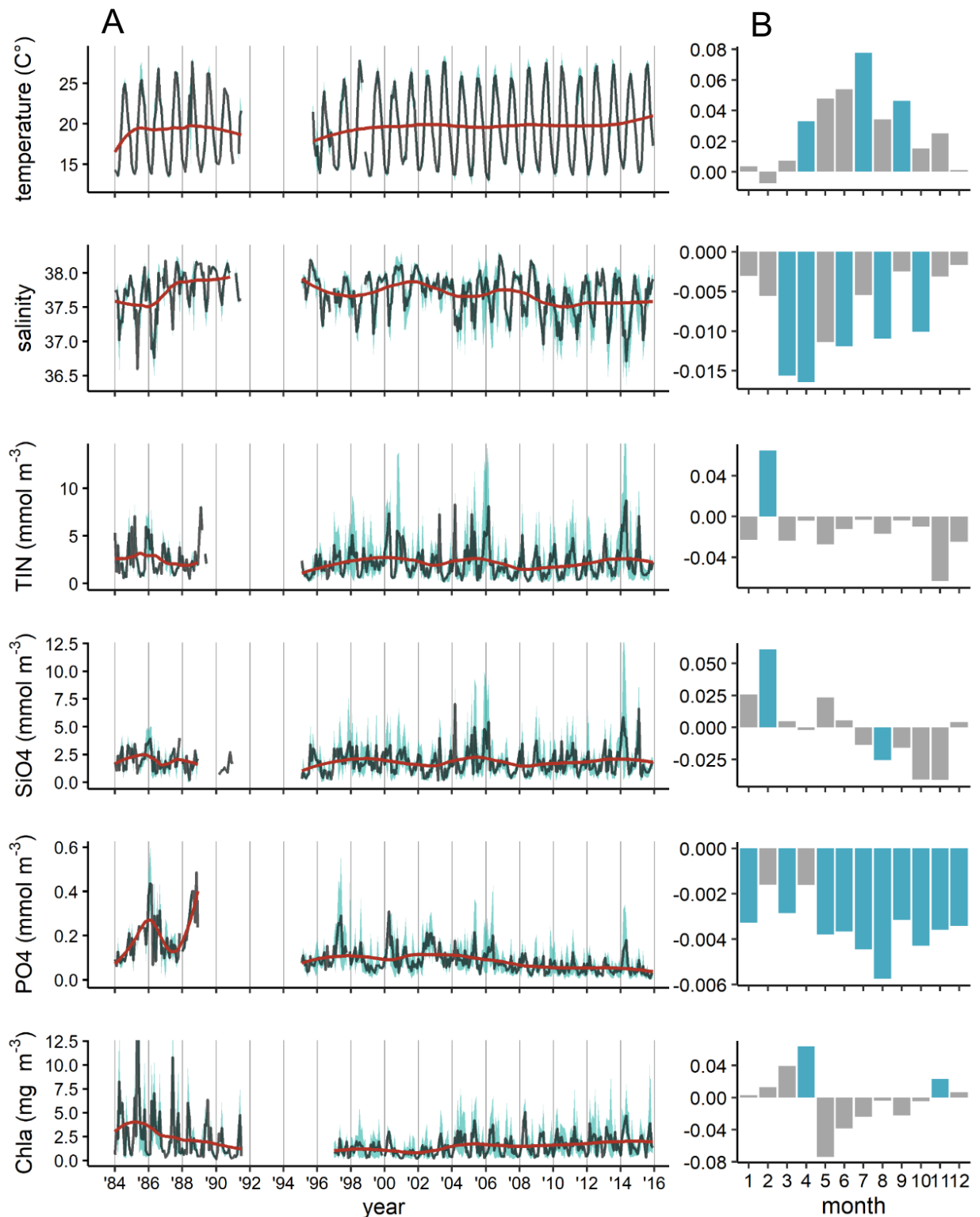
Figure 2.3 (A) Time-series of monthly averaged values of environmental parameters and chlorophyll *a* concentrations in 1984-2015 at the sampling site LTER-MC. Local polynomial regression for each time-series is represented by the red line while the green shaded area around values represents the dispersion range given by minimum and maximum values around the mean. (B) Trends calculated of individual months of each parameter for the period 1984-2015. Grey bars refer to a pvalue higher than 0.05, blue bars are significant trends at pvalue < 0.05. Vertical axes values correspond to the average change per year for each variable.

### 2.3.3 Phytoplankton seasonal cycle

The average abundance values were the highest in late-spring and early-summer and the lowest during the winter periods (Fig. 2.4A). The community was largely dominated by small phytoflagellates and small diatoms for almost all seasons. The conversion from abundance to carbon content made for each taxon revalued the high abundances of phytoflagellates and highlighted the important contribution of dinoflagellates, together with diatoms, to the total biomass produced in spring and summer (Fig. 2.4B). The average seasonal cycle was characterized by a first and minor peak of diatoms around April, followed by a more conspicuous one between May and June, during which also small phytoflagellates and dinoflagellates reached their highest concentrations, defining an almost continuous, unimodal pattern. Coccolithophores at LTR-MC occasionally reached high abundances during summer (mostly due to *Emiliania huxleyi* bloom) while they occurred more regularly in autumn (Fig. 2.5).
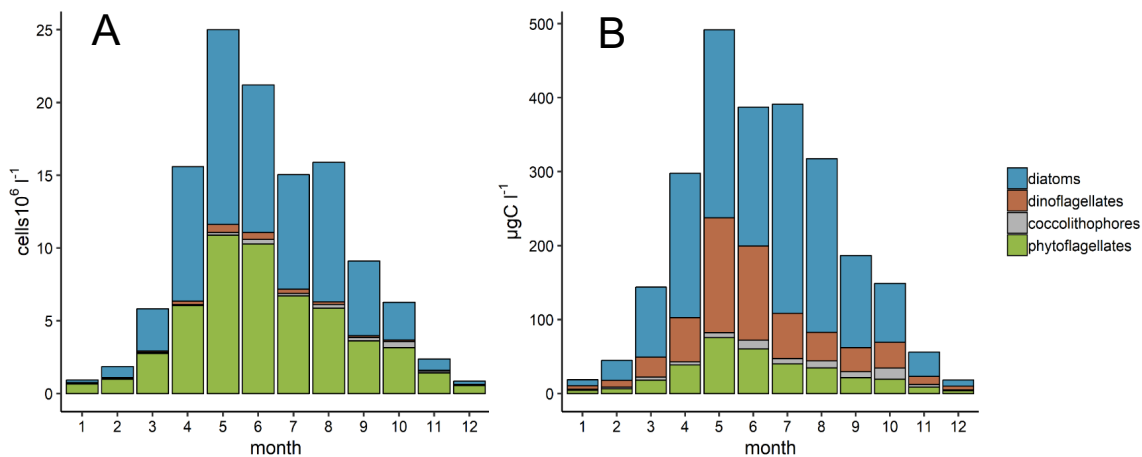


Figure 2.4 Monthly average values of 4 phytoplankton groups (diatoms, phytoflagellates, dinoflagellates and coccolithophores) at LTER-MC during 1984–2015 for abundance (a) and carbon content (b).

With the exception of small phytoflagellates (<10 μm) that were by far the most abundant group, the winter community was mainly dominated by species belonging to the genus *Chaetoceros*, in particular by *Chaetoceros socialis* and *Chaetoceros curvisetus*. During the same period, the coccolithophore *Emiliania huxleyi* represented an important part of the total abundance.
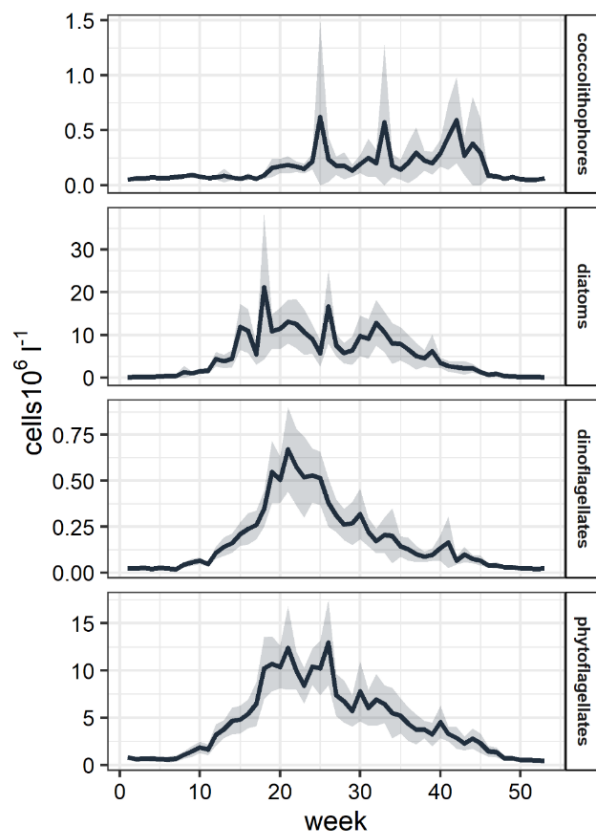


Figure 2.5 Weekly average values of the main 4 phytoplankton groups (coccolithophores, diatoms, dinoflagellates and phytoflagellates) at LTER-MC during 1984-2015. The shaded area around each line represents 0.95 confidence interval.

With the beginning of the water column stratification, small phytoflagellates and *Chaetoceros* spp., reached their highest densities. Together with *Chaetoceros* spp., *Thalassiosira* and *Bacteriastrum* species were also found to reach the highest densities in surface waters. The chain-forming species *Skeletonema pseudocostatum* was among the most abundant species of the spring period and showed regular, high-density peaks between March and June whereas it was virtually unrecorded during winter. Several *Pseudo-nitzschia* species (*P. delicatissima*, P. *pseudodelicatissima and P. galaxie* 'small morphotype') also contributed significantly to the total abundance of the community, especially during the last 10 years of the series. Among dinoflagellates, small naked and thecate dinoflagellates (<15 μm) reached the highest abundances during spring and early summer. Together with *Protoperidinium* spp., *Prorocentrum triestinum* and *Heterocapsa niei*, they contributed significantly to the total biomass peaks reached by the phytoplankton community at LTER-MC.

In summer*, Leptocylindrus danicus*, generally abundant over the entire seasonal cycle, reached the highest abundances together with some species characteristic of the typical summer at LTER-MC like *Cerataulina pelagica*, *Chaetoceros simplex* and *Cylindrotheca closterium*. Occasionally, *Emiliania huxleyi* and other undetermined coccolithophores contributed to summer blooms. During the autumn, several spring species showed a second peak (*Chaetoceros tenuissimus*, *Chaetoceros socialis*, *Pseudo-nitzschia delicatissima*, *Leptocylindrus danicus*) whereas other species were recurrently found mainly in this period (*Skeletonema menzelii, Tenuicylindrus belgicus*)*.* Coccolithophorids (*Emiliania huxleyi*, *Calciopappus caudatus*) were also relatively more abundant in autumn.

The 8 most abundant diatom species (*Chaetoceros tenuissimus, Leptocylindrus danicus, Cylindrotheca closterium*, *Chaetoceros socialis*, S*keletonema pseudocostatum, Pseudo-nitzschia delicatissima, Pseudo-nitzschia galaxiae and Skeletonema menzelii)* (Fig. 2.6A) constituted the 34.02 ± 12.27% of the total diatom abundance on a mean annual basis. Some of these species (*C. tenuissimus, C. socialis, L. danicus, S. pseudocostatum and P. delicatissima*) were also included among the most important diatoms in terms of relative contribution to the biomass of the system (Fig. 2.6B). Others as *T. rotula*, *D. blavyanus* and *C. pelagica* were among the largest diatoms collected and contributed significantly to the total diatoms' biomass despite their relatively low abundance.
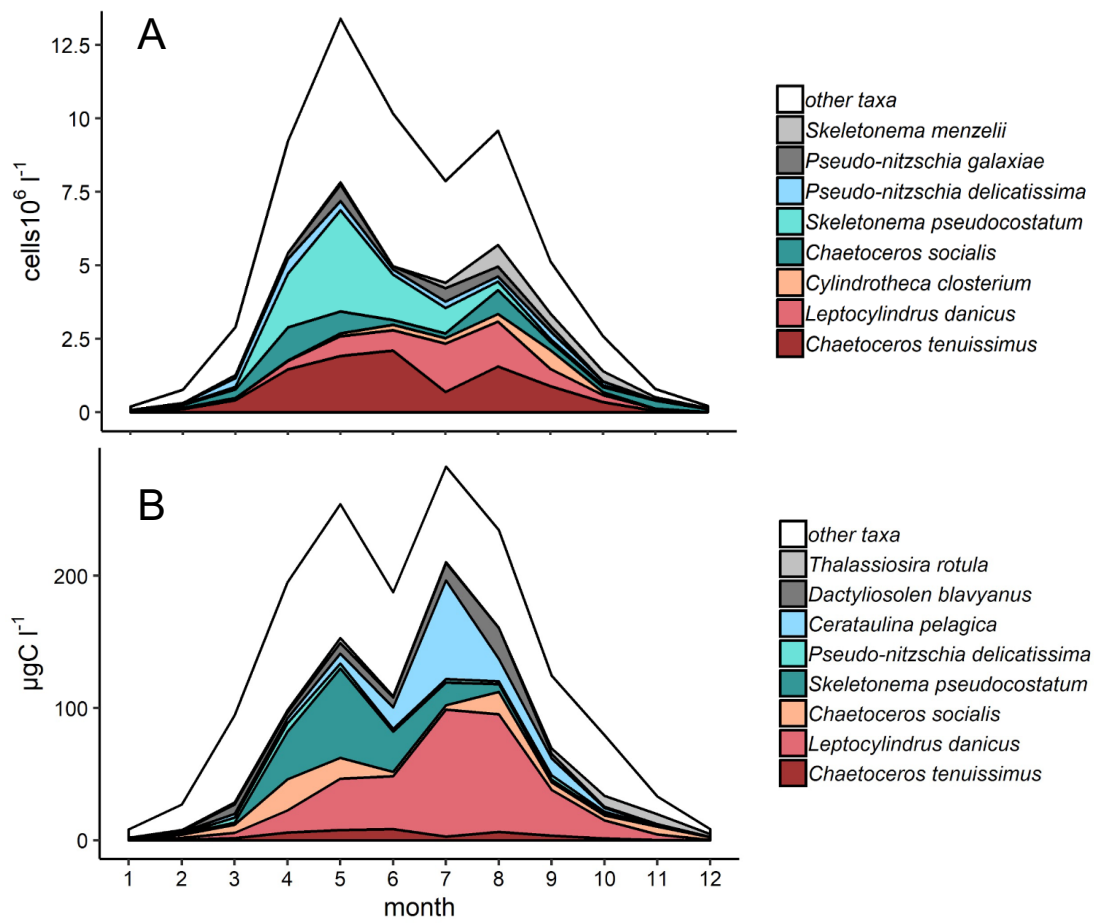
Figure 2.6 (A) Contribution of the 8 most abundant diatom species to the mean seasonal cycle of total diatom concentrations at LTER-MC during 1984-2015. (B) Monthly average of the 8 most carbon-contributing diatom species to the mean seasonal cycle of total diatom biomass at LTER-MC 1984-2015.

## 2.3.4 Periodicity and seasonal recurrence

On a total of 96 representative taxa selected by experts' judgment and frequency thresholds, more than half were diatoms (58 taxa) while the remaining 38 taxa were divided among 17 phytoflagellates, 7 dinoflagellates and 11. The periodograms' output, together with the relative frequency of each taxon, are summarised in Fig. 2.7 where, on a total of 96 taxa investigated, 73 were periodic and 20 non-periodic. The most frequent taxa of the series were associated with a more significant periodicity than the others although there were several exceptions. Indeed, both the phytoflagellate *Meringosphaera mediterranea* and the coccolithophore *Rhabdosphaera clavigera* showed an irregular pattern with several peaks scattered throughout the solar year (Fig. 2.8) despite their relatively high occurrence in all the samples (relative frequency of 25.23% and 13.46%, respectively). Conversely, the diatom *Leptocylindrus mediterraneus* and the phytoflagellate *Dinobryon coalescens* showed a very regular pattern over time (Fig. 2.8) although both of them were characterized by low occurrence values (relative frequency <7%).
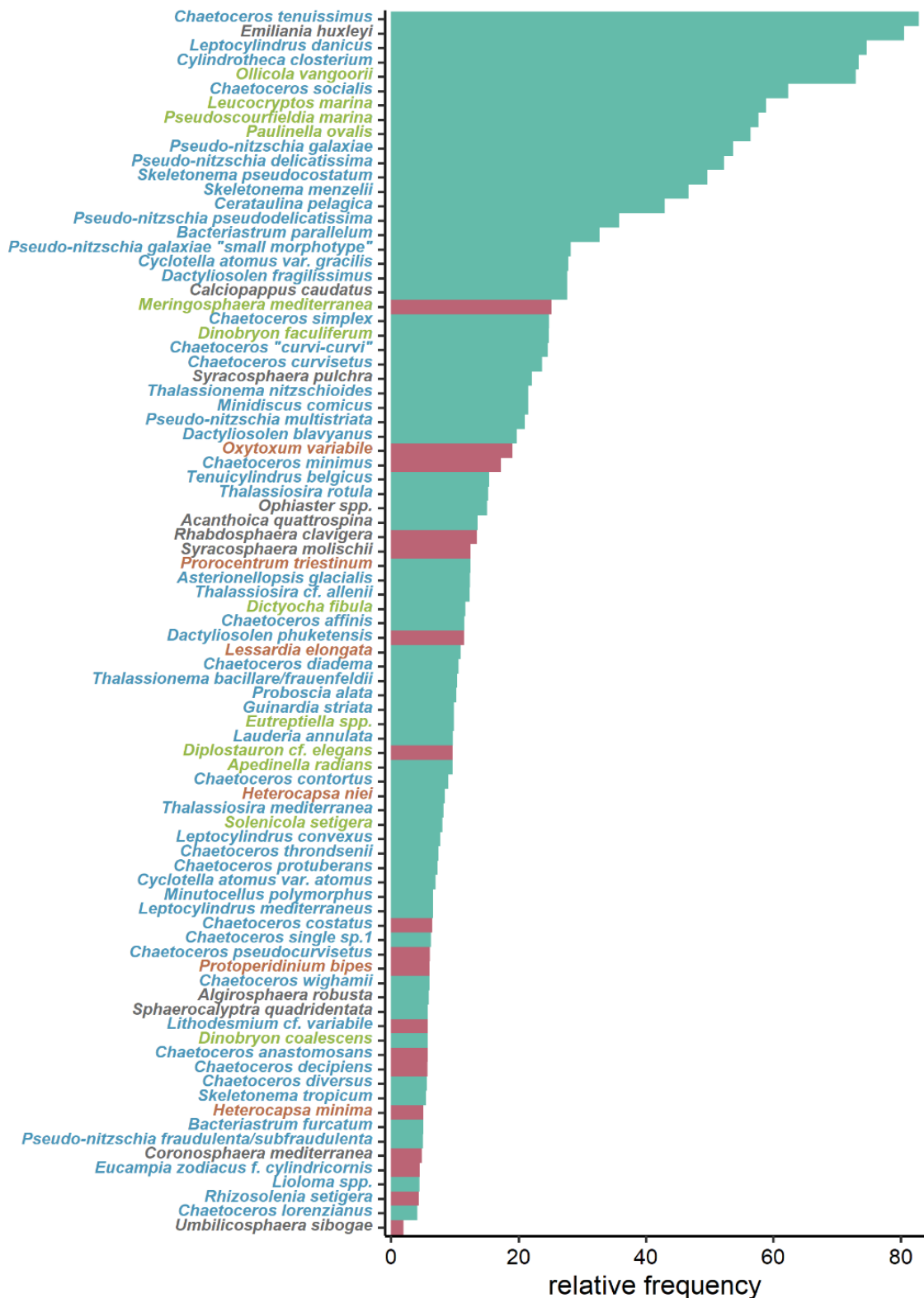
Figure 2.7 List of the most frequent taxa identified at the species level collected at LTER-MC ranked by their relative frequency: green, red, black and blue denote phytoflagellates, dinoflagellates, coccolithophores and diatoms, respectively. Periodic and non-periodic taxa are represented with green (78 taxa) and red bars (23 taxa) respectively
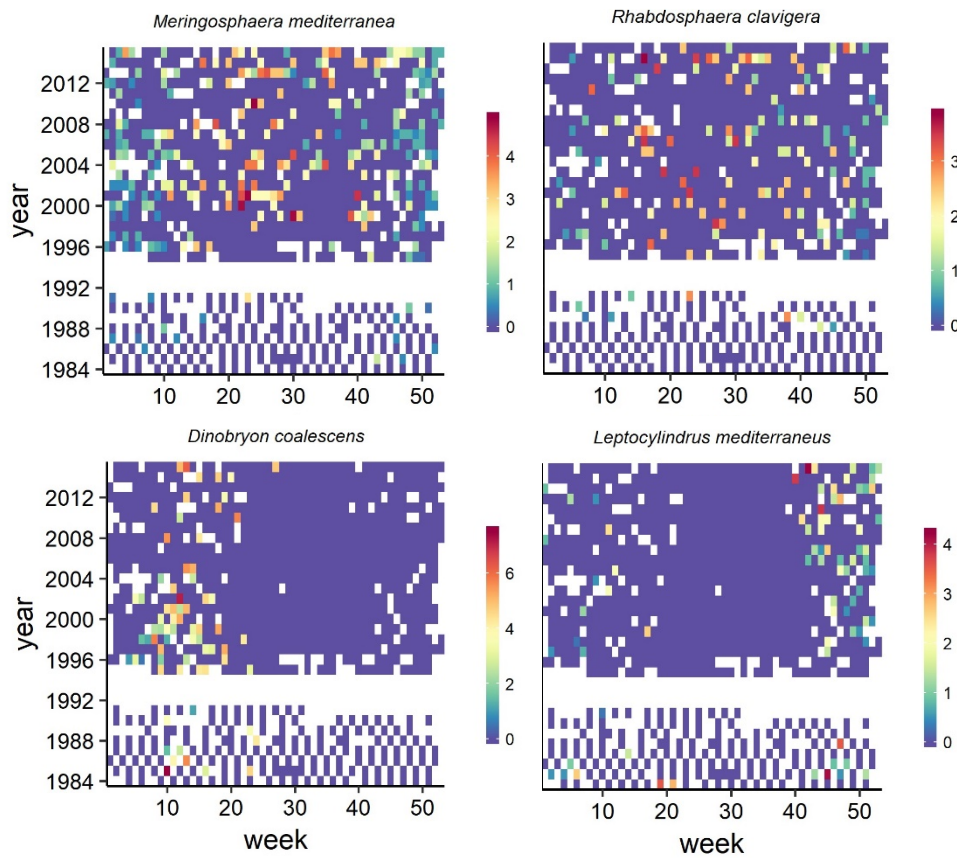
Figure 2.8 Heatmaps of some examples of non-periodic (top panels, *Meringosphaera mediterranea*, *Rhabdosphaera clavigera*) and periodic taxa (bottom panels, *Dinobryon coalescens*, *Leptocylindrus mediterraneus*). Lateral vertical bars represent log abundance of cells (cells ml−1).

Most of the periodic taxa were characterized by a unimodal distribution (around 52 weeks), while only 10 over 73 taxa showed a period of ca. 26 weeks, i.e., were bimodal. Among the latter, one was the coccolithophore *Syracosphaera pulchra* and the other 9 taxa were diatoms that spanned from relatively rare species as *Minutocellus polymorphus* and *Thalassiosira* cf. *allenii* to some of the most abundant and representative of the LTER-MC system such as *Chaetoceros socialis* and *Pseudo-nitzschia delicatissima*.

The IndVal calculated for each taxon versus each month over the series highlighted a strong association of phytoplankton taxa with well-defined temporal windows and outlined a clear successional pattern in which they succeed and overlapped each other following a predictable order (Fig. 2.9). Although most of the taxa were diatoms, it was possible to identify a certain regularity in the succession also at the level of functional groups. Indeed, several coccolithophores (*Algirosphaera robusta*, *Ophiaster* spp.*, Acanthoica quattrospina*, *Umbilicosphaera sibogae*, and *Coronosphaera mediterranea*) had their maximum IndVal in association with winter months while phytoflagellates and dinoflagellates taxa were mostly associated with late spring and summer months, whereby diatoms were distributed on all seasons with a lower density during summer.
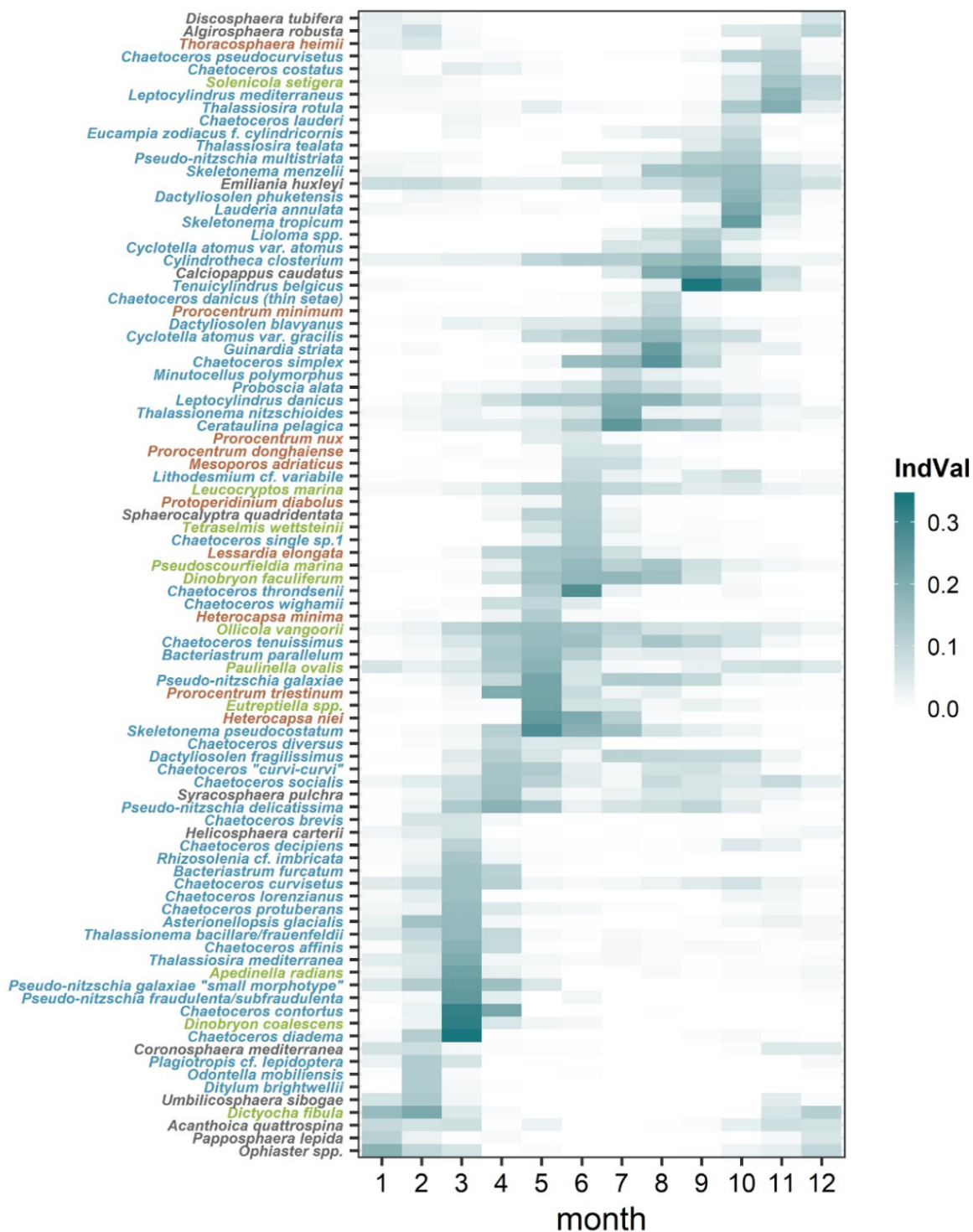
Figure 2.9 IndVal calculated crossing each taxon with each month of the year. The list of taxa was sorted in order to highlight the seasonal succession through the year. Green, red, black and blue font on the vertical axis denote phytoflagellates, dinoflagellates, coccolithophores and diatoms respectively.

Bray–Curtis pseudo-autocorrelation performed on the whole time-series and its second part (1984-2015 and 1996-2015, respectively) highlighted a clear seasonality in the similarity of community composition (Fig. 2.10A, 2.10B). Phytoplankton communities sampled at a time distance of 12 months and their multiples (24, 36, and so on) were more similar than those sampled at a distance of 6, 18 and 30 months apart. Such recurrent pattern in the similarity of the community was kept along all the length of series and highlighted the periodic nature of the community turnover over many years of sampling. While the seasonal recurrence of the community in both series appeared to be a property that was preserved throughout the whole length of the series (the period of the series), the community similarity considered in the whole series (1984-2015) seemed to show a gradual decrease over time with respect to seasonality, a property traced by the trend of the series and indicative of a temporal decay in the community similarity over time. Indeed, the slope of the trend was negative in both the series, but the Mann-Kendall test reported a significant downward monotonic trend only for the whole series (pvalue<0.001), indicating that the community of period 1984-1991 was quite different from the rest of the series, while the second part of the series had higher similarity values over time.
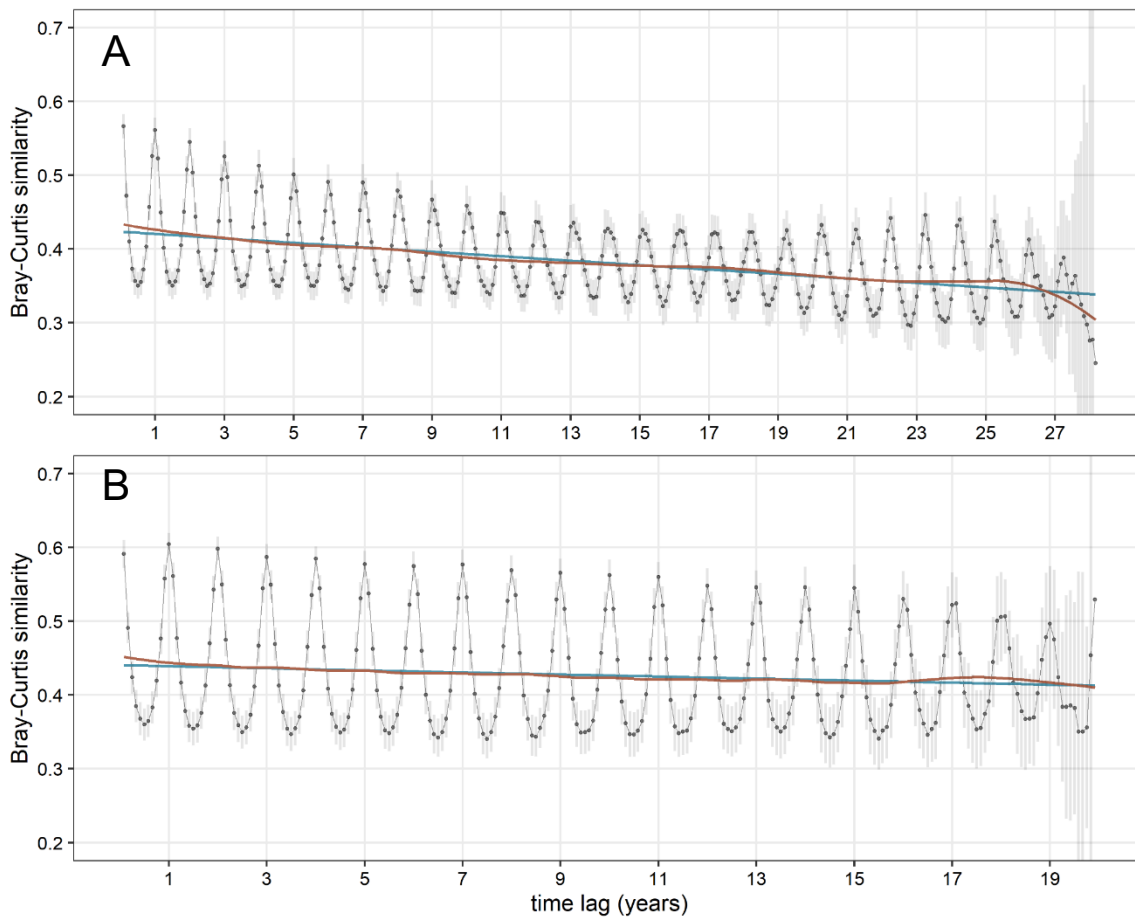
Figure 2.10 Average similarity of community composition between samples paired at different temporal lags (months) of the series. The grey vertical bars represent the 95% confidence interval. The first point represents the mean and the 95% confidence interval associated to the Bray-Curtis similarity calculated among all the communities sampled 1 month apart. Similarly, the point at year 3 represent the average of the community similarity among all the communities sampled at a temporal distance of 3 years (lag of 36 months).

# 2.3.5    Relationship between phytoplankton communities and environmental factors

The discriminant function analysis (DFA) correctly predicted 89% of the sampling months (301 on a total of 339, see confusion matrix in Fig. 2.11), meaning that, based on taxa abundance and composition it was possible to predict correctly the sampling month. The first discriminant function (DF1) explained 48% of the overall predictive power of the DFA and was used as a single multivariate index synthesizing the temporal variability of the composition and abundance of the phytoplankton community (Fig. 2.12A). As the analysis of the similarity pattern shown in the previous section, the autocorrelation function performed on the DF1 (Fig. 2.12B) showed phytoplankton community following a sinusoidal and continuous cyclic pattern, with correlation maxima and minima at 12, and 6 lag intervals respectively. These results suggest that phytoplankton composition at a given time (indexed by DF1) was similar to that of adjacent months, while it changed during the annual cycle, reaching the highest dissimilarities between the communities with a temporal lag half a year away (6 months), and then gradually returning to be similar to that of the subsequent same month.

For several authors, the VIF threshold to avoid multicollinearity must be less than 10, while others rely on more conservative thresholds (<5) (O'Brien, 2007). In my study, the analysis of the collinearity of the predictors returned values lower than 4 (Tab. 1), and hence a threshold widely considered satisfactory. Multiple linear regression results indicate that phytoplankton temporal composition was highly predictable from environmental factors as the model explained 92% of the total temporal variability of phytoplankton composition (Fig. 2.12C). According to the LMG method (Lindeman et al., 1980) day length and temperature explained most of the model's variance accounting for 43.73% and 23.83% respectively, whereas, excluding TIN which accounted for 11.43% other factors returned values lower than 10% (Tab. 1).

Table 1 Variance inflation factor (VIF) and percentage of explained variance (LMG method) of the regressors used in the multiple linear regression model ($R^2$=0.92, pvalue<0.001).

| regressor | variance inflance factor (VIF) | explained variance (%) |
|---|---|---|
| day length | 3.362 | 43.731 |
| temperature | 2.299 | 23.835 |
| TIN | 3.130 | 11.436 |
| salinity | 2.494 | 9.659 |
| chlorophyll-a | 1.866 | 6.284 |
| silicates | 2.142 | 4.240 |
| phosphates | 1.283 | 0.816 |
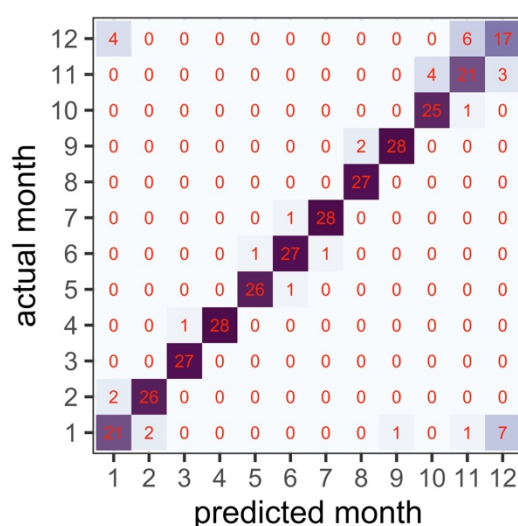


Figure 2.11 Confusion matrix of the discriminant function analysis (DFA) classyifing months based on phytoplankton community abundance and composition. The diagonal elements represent the number of instances for which the predicted month is equal to the actual month, whereas off-diagonal elements are those misjudged by DFA.
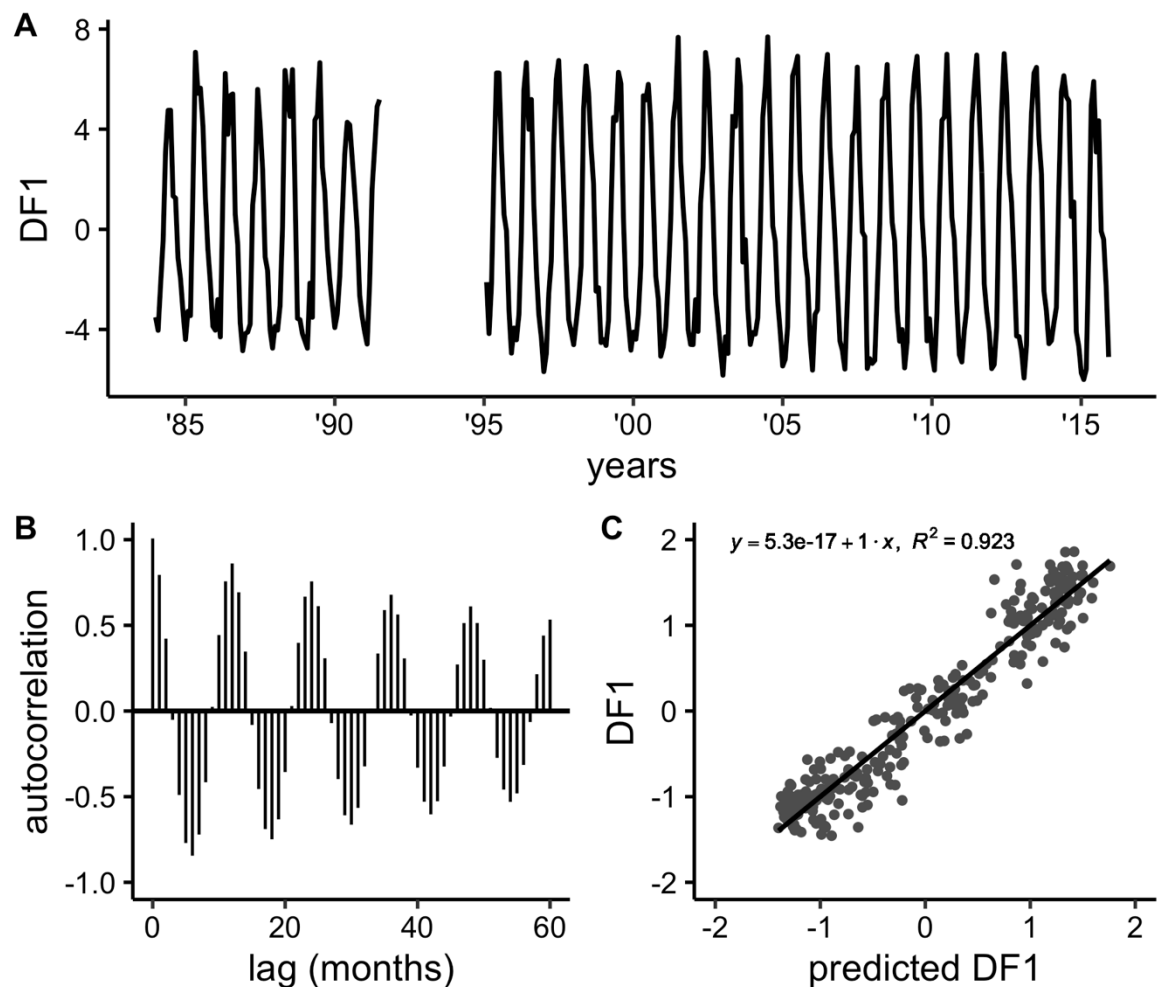
Figure 2.12 Time-series of the phytoplankton community variability at LTER-MC (1984-2015) indexed by the first discriminant function (DF1) and derived from the discriminant function analysis (see methods). B. Autocorrelation function performed on DF1 covering a 60-months' lag. C. DF1 values and its predicted values derived from a multiple linear regression model (p-value: <0.001, Adjusted $R^2$: 0.92). The set of regressors used for the model were day length, temperature, salinity, TIN, silicates, phosphates and chlorophyll-a.

The Interstructure configuration calculated using the STATICO approach explained 40% of the total inertia and showed the similarity of the biological-environment structure among the different years of the LTER-MC time-series. The first years of the series (1984-1988), clearly separated from the others (Fig. 2.13), clustered in the lower part of the factor map and showed lower loadings compared to the other years of the series (a median 0.14 and 0.21 respectively). As the calculation of the Compromise space was based on the loadings of the Interstructure, the years of the first part of the series contributed less to the building of the Compromise and their interpretation on the trajectories map was considered unreliable.
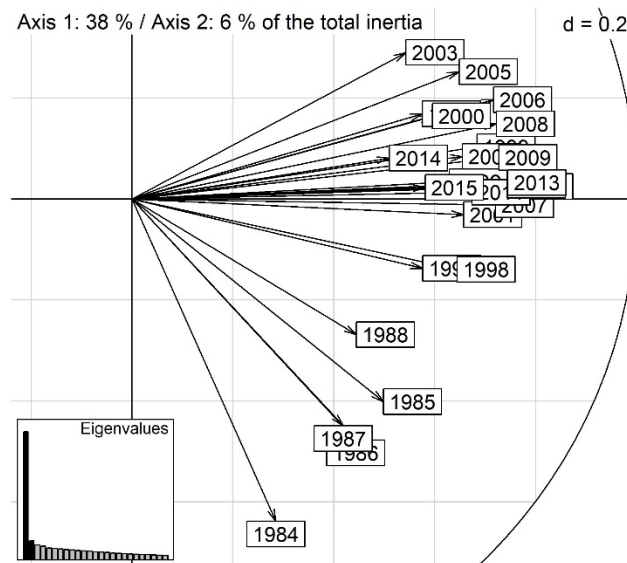
Figure 2.13 Intestructure map of the STATICO analysis on LTER-MC sampling years. The length of each arrow indicates the importance of each year in the compromise space while the angles formed by the arrows represent correlations among the years. Same-direction arrows indicate similar years in terms species-environment structure. The inset on the bottom-right part shows the eigenvalues diagram while the scale of the graph given by the grey grids is indicated by the term 'd' in the right-upper part of the graph.

The first component of the Compromise factor map explained 82.5% while the second component accounted for 14.6% of the total inertia (Fig. 2.14). The Compromise map outlined an environmental gradient driven by temperature and salinity in the first and second axis respectively. Considering the seasons, the bottom-left part of the map represented the winter and late-winter period as characterized by relatively high salinity, high-nutrient concentrations and low-temperature values. The spring and late-spring period of the series covered the central bottom part of the map where nutrients were still relatively high but get lower approaching towards the bottom-right part of the map. Indeed, the latter part marked the transition toward summer, with the highest temperatures and lowest of nutrient (upper-right part of the map). The upper-left area of the Compromise map included samples characterized by high salinity and relatively high nutrient concentration, a condition typical of the autumn at LTER-MC.
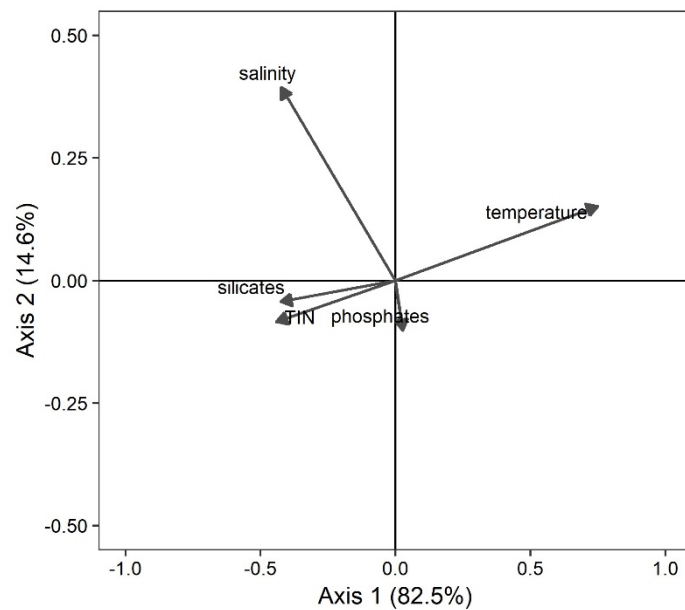
Figure 2.14 Compromise factor map of the surface water environmental parameters chosen for the analysis (0–5m average).

Community and environmental trajectories in each year are represented in Fig. 2.15. Each trajectory represents the chronological projection of each month of a given year for both the phytoplankton community and the environment in the stable part of the species-environment relationship (the Compromise map). With the exception of the years of the first part of the series (1984-1988) - characterized by low loadings values and thus unreliable for any interpretation, as said above - phytoplankton communities were characterized by a consistent regularity on the interannual scale compared to the environment. Environmental trajectories showed several divergences among the years, particularly evident in late winter and spring, where pulses of nutrients conferred trajectories peculiar shapes observable during the periods 2003-2006 and 2008-2009. In addition to these marked differences in late winter and spring, yearly environmental trajectories appeared quite diverging in both their shape and breadth compared to phytoplankton ones (Fig. 2.16), suggesting that the environmental context perceived by the phytoplankton community is highly mutable from year to year. It is noteworthy that the turnover of the community during the annual cycle was not homogeneous, it showed two seasonal phases coinciding with the transition from winter to spring and from summer to autumn where it changed more rapidly.
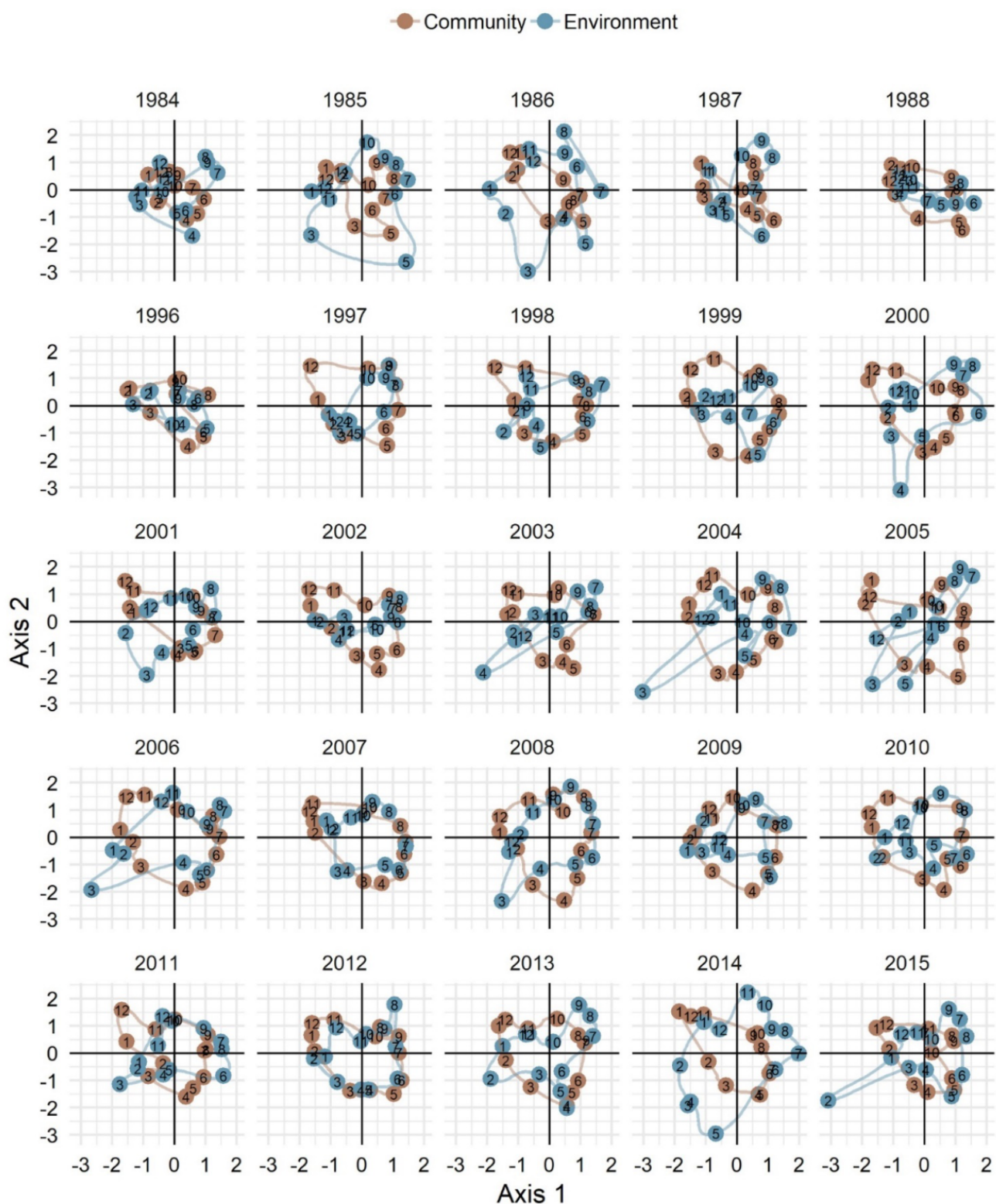
Figure 2.15 Projection of the monthly coordinates of each year on the compromise space. Phytoplankton community coordinates are in orange while the environment ones are in blue. Numeric labels in each trajectory represent the month. In order to improve the graphical visualization, the lines connecting monthly coordinates were smoothed using a periodic cubic spline interpolation.
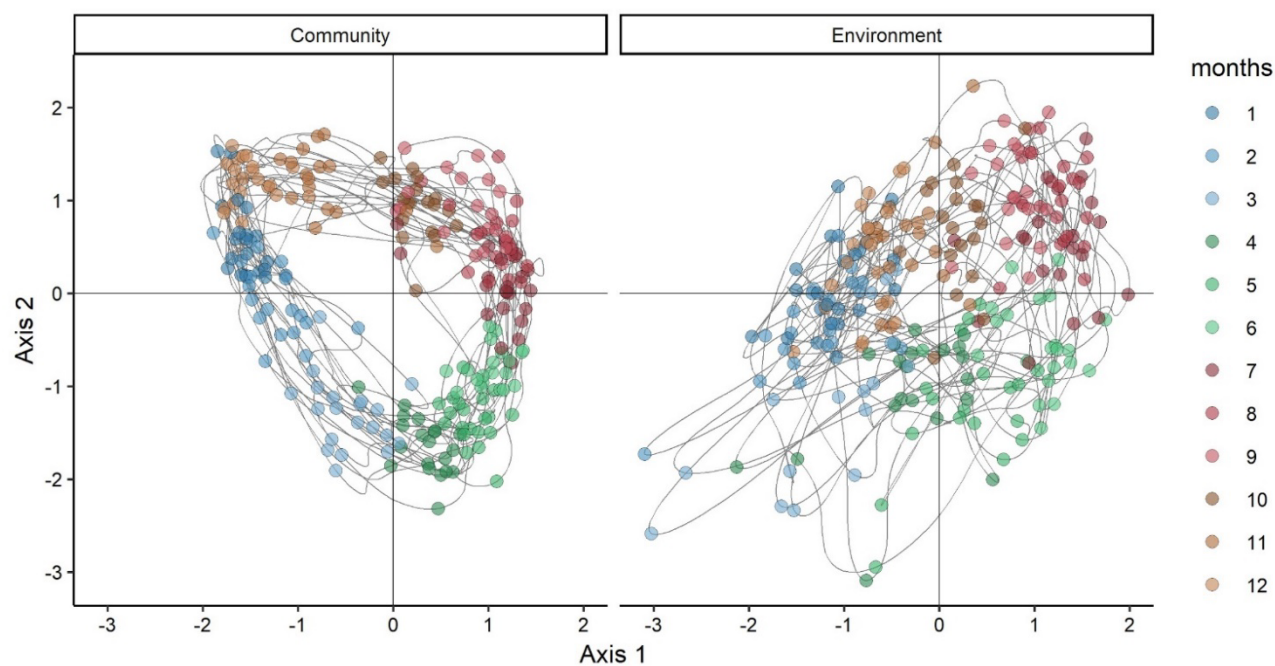
Figure 2.16 Representative map of the monthly coordinates for both phytoplankton community and environment on the compromise space for all the years analysed except those characterised by low loadings (1984-1988). Filled, coloured circles refer to the months of the years.

# 2.4 Discussion

Compared with previous investigations on the same area (Ribera d'Alcalà et al., 2004), this study revealed that the most representative taxa and the bulk of the community remained relatively unchanged over time, whereas there were some environmental changes taking place. Salinity exhibited a very marked downward trend in all the months and defined more or less regular cycles of 5 years, which were also detectable in the main nutrients. Temperature, although not linearly, showed an increase in the long-term in line with the widespread sea-surface warming, and was particularly marked during the summer. Consistent with these results, a previous analysis comparing the two major components of the chlorophyll-a temporal variability among 84 coastal sites showed that LTER-MC variability resulted to be mainly associated with the interannual component and to a slightly lesser extent to the seasonal component (Cloern & Jassby, 2010). Therefore, LTER-MC would fall among the areas characterized by a seasonal temporal dynamic but strongly influenced by climatic shifts and processes of anthropic origin at the interannual scale. Such dynamics and the presence of one of the most urbanized coasts of Europe make the LTER-MC pelagic system an extremely dynamic area subjected to a strong anthropic pressure (Tornero & Ribera d'Alcalà, 2014; Zingone et al., 2019), and decadal regular high-frequency sampling at LTER-MC revealed that environmental variability occurred at different temporal scales, in the form of isolated events, seasonal and cyclical fluctuations, and trends.

The analysis of the temporal recurrence over more than 25 years of sampling at the coastal site LTER-MC revealed an impressive regularity in the annual occurrence of the phytoplankton community and individual taxa, despite the highly variable environmental context. The taxa analysed in this work constituted a pool of biological entities characterized by a wide spectrum of differences in terms of phylogeny, sizes, shapes and functions and even different representativeness in the study area (in terms of occurrence and abundance). Despite such a remarkable overall diversity among these tiny organisms, I have recorded a clear temporal regularity, common to the majority of the analysed taxa (78 taxa over 101 were periodic) and extended to the community level.

Although a certain recurrence had already been observed in some species in the period 1984-2000 (Ribera d'Alcalà et al., 2004), it had never been assessed and quantified before. Therefore, what is surprising, or at least, unexpected, is that such temporal regularity is predominant for the majority of the species and the whole community.

Under a bottom-up perspective, phytoplankton successional patterns in temperate zones are argued to be strongly determined by the seasonal nature of the chemical-physical environment, whereby light, temperature, nutrients and turbulence are the factors that have historically been associated with the control of phytoplankton growth (Reynolds, 2006; Sommer, 1989). The seasonal interplay of these parameters would cyclically shape the environment creating seasonal predictable conditions resulting in a gradual selection of certain species' niche or, in a broader view, a specific phytoplankton assemblage. The STATICO analysis allowed us to explore the environmental variability at LTER-MC for almost 3 decades of samplings and compare it with that of the phytoplankton community.

Comparing the factorial map of the compromise with the annual trajectories of the environmental regime, I have recorded considerable inter-annual variability in all factors included, and among these, the oscillations of salinity and nutrients emerged in a particular way. The magnitude and the frequency of nutrient supplies were unpredictable and variable from year to year and were seen as nutrients 'pulses' both in the STATICO space and in the interannual variability plot. The structure of the phytoplankton community during these nutrient pulses remained mostly stable suggesting a limited role of these nutrient pulses in shaping the phytoplankton community at this coastal site. Despite a seasonality in nutrient supply patterns, previous studies conducted on the same area covering the period 1984-2004 revealed that the water column was very seldom depleted in nitrates and silicates even in summer (Ribera d'Alcalà et al., 2004), a condition that would also explain the large dominance of high nutrient-requiring and fast-growing species as diatoms during summer (Zingone et al., 1990). Moreover, as I did not record any significant decrease in silicates and nitrates after the period analysed by Ribera and colleagues (2004), I assumed that the system has maintained a non-limiting environment for the phytoplankton community even in the years that followed to 2004. Therefore, additional nutrients load even of strong intensity as in the period 2003-2006, in 2008 and during 2014-2015, seem to have a marginal role in shaping phytoplankton successional dynamics.

An in-depth study of the phytoplankton community in relation to the eutrophic and oligotrophic regimes recorded at LTER-MC revealed that the temporal intermittence of such regimes played an important role in promoting the community diversity and regulating the phytoplankton successional dynamics (Cianelli et al., 2017). Indeed, autogenic control (sensu Reynolds, 2006) was predominant during long-lasting eutrophic states, whereas the dilution caused by the intrusion of off-shore oligotrophic waters (allogeneic control) was responsible to dampen the impact of dominant species in the community. The intermittency of these trophic regimes seems to define a particular trophic situation in which nutrients are very seldom limiting but not enough to substantially restructure the phytoplankton community as it has been reported in other coastal areas (Guadayol et al., 2009; Suikkanen et al., 2007; Zhou et al., 2008) and in particular in the North Baltic (Andersen et al., 2017). This does not imply that nutrients, from a broad perspective, are irrelevant in regulating the succession processes. I would rather emphasize that our study system is characterized by peculiar hydrological dynamics that allowed us to unveil the underlying species-specific biological processes for individual species, and, in a broader perspective, for the whole community. Autogenic succession is the result of several endogenous biological attributes such as species-specific life-cycle, physiology and growth rates (Reynolds, 2006) and the peculiar conditions at LTER-MC allowed to detect the result of these processes as a temporally regular signal.

Visual inspection of the trajectories over months revealed that community annual change was not continuous but experienced two phases where it changed more rapidly. These 'jumps' coincided with the transition from summer to winter conditions and vice versa and discriminated winter and summer communities from the other periods. Such regular switching states were recently documented for the eukaryotic community in the oligotrophic Blanes Bay (Giner et al., 2019) and also for the bacterioplankton communities in Beaufort (North Carolina, USA) and the Western English Channel (Gilbert et al., 2012; Ward et al., 2017). In temperate areas as those cited below, spring and autumn are the periods where light duration and physical parameters experience a directional change faster than other periods of the year and are likely to exert a prominent hierarchical control on the succession and structure of the community. Nevertheless, the temporal regularity of individual taxa together with the stability of the entire community in the long term suggests

that more important internal regulation processes may play a key role and need to be considered in the understanding and interpretation of these processes.

The fact that day length accounted for most of the temporal community variability (indexed by DF1) in the multiple regression analysis is indicative in this sense. Indeed, Eilertsen et al., (1995) observed that the timing of diatom bloom in Norwegian coastal waters varied little although environmental conditions varied from year to year, and hypothesized that the onset of the bloom was related to day-length-regulated germination of spores. Most species (mostly belonging to the genus Chaetoceros) showed a distinct photoperiod response while others did not appear to exhibit photoperiodism recalling associated terms of terrestrial botany, LDP (long-day plants) and DNP (day length- neutral plants) respectively. The bloom progress would be regulated by the prevailing condition of light and physical status of the water column, but the species succession would be under tight control of photoperiod changes which differentially affect LDP and DNP species (Eilertsen et al., 1995). I think that this explanation could apply to what I observed at LTER-MC. A more extensive study based on samples distributed over a larger area of the North Atlantic revealed that the timing and amplitude of several coastal *Chaetoceros* spp. and *Skeletonema costatum* (highly abundant at LTER-MC) were controlled both by the physical processes described by the Sverdrup model (1953) but also by internal dynamics of phytoplankton life-history strategies, specifically by day-length-regulation germination of spores (Eilertsen & Wyatt, 2000). Further evidence of the close relationship between phytoplankton dynamics and photoperiod have also been found in studies of growth rates of individual phytoplankton taxa, where the dependence between photoperiod and carbon assimilation in different species was highlighted (Rost et al., 2003; Rost et al., 2006). In a whole-community study as well, day length explained a large fraction of phytoplankton growth rate variability while a lack of a clear relation was found with inorganic nutrients (Gutiérrez-Rodríguez et al., 2011). Seasonally recurring patterns in phytoplankton autotrophs associated with day length were also found in more recent studies using modern molecular approaches in a large proportion of archaea and bacteria, despite variable environmental conditions similar to LTER-MC, in terms of irregular inter-annual nutrient supply and salinity levels (Lambert et al., 2019). Similarly, spring-bloom timing remained relatively unchanged at Helgoland monitoring station during 30 years of daily sampling despite significant shifts in nutrients regimes, light conditions, wind speed and temperature (Wiltshire et al., 2008). Signals of comparable

resilience were also observed at Plymouth L4 Site in the western English Channel by analysing the distribution of 57 plankton taxa during 25 years and investigating the sensitivity of their temporal patterns in relation to environmental fluctuations, particularly on the long-term increase of temperature in the area (Atkinson et al., 2015). The resilience and the temporal stability of the pelagic communities would be supported by the idea of the regulation of the phytoplankton succession based on the direct control of the photoperiod.

Other factors linked to the astronomic settings at our study site, notably temperature and irradiance, also explain partially the variability of phytoplankton community composition over the year. However, the prominent role of photoperiod suggests that this variable, rather than directly influence species physiology and growth rates, could act as a signal for phytoplankton species to set their timing, thus growing in certain periods of the years that are most favourable to their success. Environmental conditions would hence act at an evolutionary level as distal rather than proximal drivers, selecting populations set to grow in those periods. Like in terrestrial plants, biological rhythms driving phenology in these microbes could include a genetic component (Forrest & Miller-Rushing, 2010) which would be entrained by the signal provided by light and modulated by other environmental factors, which would explain a certain amount of the phenological variability normally observed for phytoplankton. As a matter of fact, the diversity of phenological patterns of phytoplankton communities as well as of individual species covers the whole year, while it would be more plausible to expect that light availability under longer daylength should be favourable for all species, or at least for diatoms (Rost et al, 2006).

Nevertheless, long-term molecular data from the Blanes Bay (NW Mediterranean Sea) revealed a wide non-recurrence of most of the pelagic community although the photoperiod, along with temperature, was the major driver of the seasonal variability of the community (Giner et al., 2019). Accordingly, I also found non-periodic taxa and some periodic taxa that 'disappear' for short periods. To explain this behaviour, I might consider 3 main hypotheses. The first concerns genetic variability, nowadays widely documented in phytoplankton populations. There is an impressive amount of evidence of genetic diversity among the same morphological types that imply important divergences in physiological and phenological behaviour and, consequently, ecological ones (Degerlund et al., 2012; Smayda, 2011). Secondly, if biological regularity is an adaptive strategy allowing a species to anticipate and 'be ready' to exploit the cyclic environmental variation (Rosbash, 2009),

biologic unpredictability due to irregular temporal patterns would represent a life-strategy too which would dampen the top-down forcing of periodic grazers (Wyatt, 2014). And, thirdly, it has been described how in pelagic systems, phylogenetically diverse organisms can significantly shape the community through ecological interactions (Needham et al., 2017), and that these dynamics can have greater importance than environmental parameters in regulating phytoplankton temporal succession (Needham & Fuhrman, 2016). In this context, a study focused on the temporal analysis of primary consumers a LTER-MC revelaed a remarkable recurrency pattern of the zooplankton community (Mazzocchi et al., 2012) although less pronounced than that presented for phytoplankton in this study. The idea of a translation of the temporal regularity from consumers to producers through a top-down mechanism was widely discussed in terms of overall biomass (see Sommer, 2012), whereas very little is known at the level of interspecific trophic relationships. An in-depth analysis on LTER-MC' pelagic trophic web suggested that both phytoplankton and zooplankton species' composition remain almost stable relatively to the astronomic season, whereas different trophic links among grazers and potential preys set up based on the overall availability of resources (D'Alelio et al., 2015 - 2016).

In conclusion, several studies revealed the importance of light regimes in the dynamics of specific sets of taxa but as yet it is not clear to which extent such observation is reflected in wider communities in the natural environment. Several molecular studies showed that regularity in taxa occurrence of both bacterioplankton and phytoplankton communities translated in regular patterns of the whole community at an annual scale, and it is noteworthy that the major source of community variability was found to be a temporally-constant signal such as photoperiod, although these studies covered different areas and used different statistical methods. This study has shown similar patterns of temporal recurrence over almost 3 decades in an extremely dynamic environment in which taxa, rather than passively follow environmental fluctuations showed a quite marked degree of resilience by regularly occupying defined temporal niches. Noteworthy, these results are consistent with those obtained by Piredda et al. (2017), which in the same site showed that differences in environmental parameters explained a minor part of the variance among HTS samples collected in different seasons.

Considering that many biogeochemical and trophic processes in the pelagic system are regulated by phytoplankton temporal patterns, it is crucial to implement our understanding

of these dynamics through the continuous collection of data from long-term research programs and to account. Moreover, a greater effort in taking into account phytoplankton life-history strategies (Eilertsen & Wyatt, 2000), as well as the widespread imprint of biological rhythms in the marine environment (Mat, 2018), can enforce our perception of observing phytoplankton temporal patterns and also improve our ability to interpret both the signals of change and those of resilience.

# 3 Long-term patterns and impact of climate fluctuations on phytoplankton at LTER-MC

## 3.1 Introduction

The impacts of climate change are now recognized in virtually every ecosystem on the planet, and there is a vast literature that highlights how these impacts are transversal to all scales of biological organization, from the molecular level up to community dynamics (Scheffers et al., 2016). The increase in greenhouse gas emissions is considered the main factor of the gradual alteration of the chemistry of the different components of the ecosphere and at the same time the main factor responsible for the increase in the world average temperature of about 1 °C since preindustrial times (Rahmstorf et al., 2012; Smith et al., 2015). Concurrently, an intimate link exists between the increase of atmospheric temperature and the variability of the general climate system, which is manifested in alterations of the atmosphere-ocean system, in the increase in frequency and intensity of extreme weather phenomena and modifications in wind and precipitation regimes (Stocker et al., 2013). Given the multifactorial nature of climate change, the use of large-scale climate indices has been very successful in recent years. These indices capture climate variability through the measurement of defined meteorological observations and act as proxies for local climatic and oceanographic conditions, which in turn influence biological and ecological processes (see Stenseth et al., 2003).

Coastal marine ecosystems are among the most important systems on the planet from both a socio-economic and ecological points of view. It is estimated that half of the world's population lives along the coast (Vitousek et al., 1997) and hence is directly or indirectly connected with the evolution and the health status of coastal systems. Historically, human populations settled in coastal areas to take advantage of a set of ecosystem services that nearshore waters provide and exploit the proximity of water as an enhancing factor for the development of transport and commercial trades. In recent years, the demographic explosion and the increase of industrial activities are threatening the functioning and

stability of coastal ecosystems and made these important biogeographic areas particularly vulnerable (Halpern et al., 2008); overfishing (Jackson et al., 2001), wild-habitat reduction and destruction (Jones et al., 2018) and alteration of water quality through nutrient enrichment and discharge of pollutants (Islam & Tanaka, 2004) are representative examples of the effects of direct anthropogenic actions on coastal systems. Furthermore, local threats as those described before (although globally widespread) are in the recent decades concurrent with anthropogenic climate change, which acts on a more extensive temporal and spatial scale (Harley et al., 2006) and engages transversally different levels of biological organization (Brierley & Kingsford, 2009). Therefore, as never before the scientific community has been called upon to provide reliable and sensible tools useful to trace, decipher and interpret signals of alteration and modification of the state of coastal biological communities.

In the pelagic marine environment, phytoplankton represent a key group of organisms to address regarding the impacts of climate change, both because they respond quickly to environmental fluctuations and because they represent the primary food source in the marine habitat (Winder & Sommer, 2012). Climate fluctuations exert an important control over phytoplankton from various perspectives. For instance, temperature can both affect the physiology and the physical environment of phytoplankton. At the physiological level, the increase in water temperature was observed to directly affect the rate of proteins' translation (Raven & Geider, 1988; Toseland et al., 2013), a core element of cellular metabolism. Moreover, Toseland and colleagues (2013) showed how the temperature increase would impact cellular resource allocation, altering the N:P stoichiometry with potentially large consequences on global biogeochemical cycles.

Although these physiological processes have not been observed at a pluriannual scale yet (at least to the best of my knowledge), the effects of the long-term increase of temperature on the physical habitat of plankton are widely documented. The most supported hypothesis predicts that the gradual rise of SST determines a more marked stratification of the upper layer of the ocean, thus requiring more energy to mix with the deep and nutrient-rich waters (Doney, 2006). Together with light, nutrients support primary production, so that the projected result of a more marked stratification in the oceans would lead to a gradual decline of marine phytoplankton biomass, a condition that has been already proposed for the past century (Boyce et al. 2010), despite some controversies on data interpretation

(McQuatters-Gollop et al., 2010). New evidences based on time-series of primary production also support the hypothesis of a decline in photosynthetic activity as a result of the effects of increased stratification (D'Alelio et al., 2020; Fu et al., 2016, Roxy et al., 2016).

In addition to changes in such bulk properties as primary production and chlorophyll-a levels, Long-term studies have provided several pieces of evidence of climate-driven changes in size and composition of the phytoplankton community. Conceivably, a nutrient-depleted environment as a consequence of increased stratification would favour smaller taxa over larger ones because of larger surface-area-to-volume ratio and hence higher capacity to assimilate nutrients (Litchmann et al., 2007), with important consequences for both the functioning of the trophic networks and global biogeochemical processes. Studies performed at geological time-scales on both diatoms and dinoflagellates have also supported the hypothesis of a temporal shift in phytoplankton size governed by geological variations along the temperature gradients (Finkel et al., 2005, 2007).

Based on empirical observations, Margalef (1978) described the dynamics of phytoplankton community composition in relation to the interplay between nutrient availability and turbulence. According to his model, alterations of the water-column mixing dynamics induced by variability in wind and temperature regimes would favour distinct phylogenetic morphotypes. Specifically, community composition is shaped by the alternation between fertile conditions (high turbulence and high nutrients) favouring r-strategist and fast-growing taxa as diatoms, and conditions of high stratification and nutrient limitation that would promote k-strategist taxa, i.e., slow-growing and mobile forms like dinoflagellates and phytoflagellates. Although this paradigm cannot explain the enormous heterogeneity of phytoplankton community dynamics among the different marine habitats, there are several pieces of evidence of climate-induced shifts in phytoplankton community composition coherent with the Margalef model. An average increase of 0.5 °C over the past 50 years in the English Channel (Smyth et al., 2010) was considered as the main driver of important changes in the abundance of several taxa and functional groups and consequently of the general phytoplankton composition (Widdicombe et al., 2010). Phytoplankton community tended to shift towards a status dominated by dinoflagellates and characterized by the concurrent temporal decrease of other group abundances, mainly diatoms and *Phaeocystis* spp. Similarly, in the North Baltic, the proportion of dinoflagellates increased from 10% up

80% over 40 years of records as a result of variability in winter weather conditions (Klais et al., 2011; Spilling et al., 2018).

Climate change has an important influence on phytoplankton phenology as well. The interplay between the biology of the species and the abiotic environment shapes the growth of phytoplankton populations which, in favourable conditions, show a growth that overwhelms the losses, that is, a bloom. In temperate systems, the variability of the abiotic component of the pelagic habitat is closely related to the solar cycle, and phytoplankton blooms generally occur regularly over the seasons following this cycle. Rising temperatures can advance bloom formation, as in the case of the Western Scheldt estuary (Kromkamp & Van Engeland, 2009) and Baltic Sea as a result of a shift to a warmer NAO phase (Smayda, 2004). As several ecological processes, as reproduction and prey-predator interactions are strongly regulated on definite temporal windows, shifts in the timing of phytoplankton blooms are predicted to have tremendous consequences for higher trophic levels (Edwards & Richardson, 2004).

Nevertheless, there is an intrinsic complexity in phytoplankton dynamics in coastal habitats that in most cases hampers the understanding and interpretation of the data. While in the open ocean the most representative effect associated with climate change is the ongoing and predicted warming trend in sea surface temperature, the transitional nature of marine coastal areas makes phytoplankton variability strongly linked to many other factors. The specific hydrography, the shape of the coastline, precipitation regimes as well as human interventions can act synergistically to shape the dynamics of the autotrophic pelagic communities (Zingone et al., 2010b). The effects of climate change on coastal phytoplankton communities are strongly influenced by land-water connectivity processes as atmospheric fluctuations that characterize different climatic eras of precipitation and dry periods, or different regimes of wind speed and direction (Cloern et al., 2016). In this context, repeated observations over time become crucial to unbundle the different sources of variability and lead to a better interpretation of phytoplankton variability at both short and long-term scales. Overall, time-series have provided broad evidence that coastal systems are changing at an unexpected magnitude and pace and are supposed to change even faster in the future (Cloern et al., 2016).

For example, nutrient levels and phytoplankton biomass were found to be very sensitive to variations in regional climatic conditions of wind intensity and direction, precipitations and

air temperatures along the Atlantic and Mediterranean French coasts (Goberville et al., 2010). Both organic and inorganic nutrients and chlorophyll-a were relatively low during calm atmospheric conditions and low precipitations regimes compared to wetter periods, when precipitations intensified the nutrient runoff from the nearby lands.

Furthermore, studies conducted at a more detailed taxonomic level revealed that the effects of climate variability propagated differently in different phytoplankton groups. Indeed, in a 14-years record of weekly observations in the Bedford Basin (Canada), different regimes of precipitations exerted a strong control on diatoms biomass through changes in the stratification degree of the water column, whereas only a weak correlation was evident for other groups such as dinoflagellates and small-sized groups (pico and nanophytoplankton) (Li & Harrison, 2008). Biotic and abiotic data of an extensive dataset covering more than 50 years of monitoring in the Chesapeake Bay confirmed the strong sensitivity of phytoplankton to climatic variations measured as both aggregated parameters (chlorophyll-a) and functional groups. Wet and dry periods related to precipitation regimes influenced the annual freshwater flow from the Susquehanna River and the amount of nutrients that entered the bay resulting in alternating periods of high and low biomass (Harding et al., 2015, Harding et al., 2016). Diatoms and chlorophytes were more responsive than other groups to the climatic phase changes, whereby wet years favoured large-sized cells in both spring and summer. In a global analysis based on 126 coastal phytoplankton time-series in relation to fluctuations in precipitation patterns, there was a generally positive relationship between phytoplankton biomass (chlorophyll-a) and precipitation, but the main functional phytoplankton groups responded differently in terms of abundance, and their response was more pronounced depending on the season (Thompson et al., 2015). Chlorophytes appeared to be significantly advantaged in wetter conditions, whereas diatoms showed a negative correlation with precipitations, especially in spring.

Overall, these long-term studies suggest that precipitations, by influencing water quality, water turbidity, retention time and stratification processes, represent an important driver for the variability of coastal phytoplankton communities and that the impact of climate change in coastal systems are readily quantifiable as measures of phytoplankton abundance, composition and structure. Because geographic patterns of precipitation regimes are predicted to change in the next years (Trenberth, 2011), it is essential to 'map' the past and

ongoing effects of precipitation variations at long-term coastal stations, and to provide a forecast on the future changes.

In this Chapter, I use data from one of the most complete long-term plankton monitoring programs to investigate the effects of climate fluctuations on the physical, chemical and biological components of the pelagic ecosystem at a Mediterranean coastal site. Specifically, the aims are 1) Describe the relationships among long-term signals of physical, chemical and biological components of the pelagic system of the Gulf of Naples, 2) to assess and propose a model of the impact of climatic fluctuations on the system, and 3) to assess the seasonal dependence of climate fluctuations on the system.

# 3.2 Materials and methods

## 3.2.1    Dataset

I used monthly averaged values of surface chlorophyll-a and total carbon content (calculated from mean cell biovolumes) of diatoms, dinoflagellates, and other phytoflagellates to describe variations of phytoplankton biomass and composition in relation to environmental variability during 1984-2015. The physical parameters used in this study included monthly mean data from CTD profiles of temperature and salinity while chemical data included monthly mean concentrations of surface (average 0-5m) $SiO_4$, $NO_3$, $PO_4$ and particulate nitrogen. Particulate nitrogen was modelled by a regression equation using surface particulate nitrogen and chlorophyll-a collected in the period 2007-2014 ($R^2$ = 0.61). Total nitrogen was obtained by aggregating TIN ($NO_3$+ $NO_2$+ $NH_4$) to particulate nitrogen.

Time-series of precipitation, atmospheric pressure, air temperature at 2 meters, and relative humidity covering the period 1984-2015 were obtained from the second Modern-Era Retrospective analysis for Research and Applications (MERRA-2) (Gelaro et al., 2017). The MERRA-2 project includes modelled atmospheric parameters since 1980, and it represents a reanalysed and corrected version of the previous MERRA project (Rienecker et al., 2011). These data are based on a spatial resolution of 0.5 x 0.5 degrees centred on the LTER-MC station' coordinates (40.485 N - 14.15 E). The MERRA-2 precipitation data were validated using a non-continuous dataset of 8-years length (from 2002 to 2012) of in situ precipitations data obtained from the Italian regional agriculture agency (Agrometeo Regione Campania (http://agricoltura.regione.campania.it/home.html).

 In order to validate MERRA-2 precipitation data, I performed a set of linear regressions using weekly and monthly cumulative precipitations data from MERRA-2 and those from meteorological stations in Forio, Ischia Island (40.716 N - 13.87 E) and Cuma, Naples (40.866 N - 14.06 E), which resulted in $R^2$ values higher than 0.70 and 0.75 for cumulative weekly and monthly data respectively.

Monthly Mediterranean climate indices were obtained from the Climatic Research Unit of the University of East Anglia, while other indices were downloaded from the NOAA National Weather Service (https://www.cpc.ncep.noaa.gov/products/precip/CWlink/pna/nao.shtml).

## 3.2.2    Long-term trends

In order to describe the overall long-term of LTER-MC system, I identified the low-frequency signals (trends) of the physical, chemical and biological surface variables. The choice to work with seasonally adjusted data was justified by the fact that most of the temporal variability of the pelagic system at LTER-MC is associated with the seasonal component (see Chapter 2, Ribera d'Alcalà et al., 2004; Zingone et al., 2019). In order to extract low-frequency signals, '*Seasonal and Trend decomposition using Loess'* (STL, Cleveland et al., 1990) was performed on each parameter. The two main parameters to be chosen when using STL decomposition are the seasonal and trend windows, which control how rapidly the seasonal and trend components can change (the smaller the parameters, the more the component vary). In order to achieve a good balance between overfitting the seasonality, but at the same time keeping a certain degree of variability, I have set the parameters of seasonal and trend windows to 13 and 25 respectively (Cleveland et al., 1990). Two independent decompositions were made on the first (1984-1988) and on the second part of the LTER-MC time-series (1997-2015) because of the interruption between 1991 and 1994. The years 1989-1991 and 1995-1996 were excluded from the analysis because of many missing observations which could have affected the robustness of the time-series' decomposition process. Phytoplankton group (diatoms, dinoflagellates and flagellates) biomass and surface chlorophyll-a values were log-transformed to stabilize the variance. To characterise the main covarying patterns among environmental and biological parameters at LTER-MC, the residuals extracted through the time-series decomposition process were correlated using the Spearman correlation. Also, the Sen-slope estimator (Sen, 1968) was calculated by the trend components for each variable to characterise the magnitude and direction of the long-term trends.

### 3.2.3    Precipitation–salinity relationships

Under the hypothesis that precipitation affected water-column stratification patterns by influencing surface salinity, I have further investigated the relationship between precipitation and salinity' variability. Because the variability of surface salinity is related to both whole water-column forcing (i.e., circulation patterns) and water-surface forcing (precipitation, winds, runoff), I have considered the difference between integrated deep salinity (50-70 m) and surface salinity (0-2 m) values as a suitable descriptor (hereafter Δsal) of the complex of forcing acting on the surface layer of the water column.

Seasonal regressions were performed to assess the relationships between precipitation and surface salinity fluctuations (indexed by Δsal). Seasons were identified based on the vertical dynamics of the water column as winter (December, January, February, full mixing), spring (March and April, early stratification), summer (May to August, marked stratification) and autumn (September to November, mixed layer deepening). Moreover, I assessed the interannual variability of long-term trends of Δsal, surface and deep salinity using Sen's slope method (Sen, 1968) on seasonal averaged data for each year.

### 3.2.4    Response of the planktonic system to precipitation

In order to assess the response of the LTER-MC planktonic system to precipitations, I have analysed the statistical distribution of physical, chemical and biological parameters in relation to the precipitation gradient using a cumsum-oriented approach. Cumsum approaches are widely used in ecological and oceanographic time-series analyses in recent years, in studies spanning from climate change and regime-shifts (Beaugrand et al., 2003, Briceño & Boyer, 2010, Conversi et al., 2010, Grbec et al., 2015) to changes in the biological components (Adrian et al., 2006). An effective transformation, useful to highlight anomalous phases and change-points when analysing time-series, consists of normalizing the time-series under investigation before performing the cumsum, i.e., subtracting the overall mean of the time-series and dividing each sample by the standard deviation. As argued by Regier

et al. (2019), the cumulative sum of the scaled deviations from the mean highlights hidden temporal properties of a generic time-series, facilitating the identification of prolonged phases of positive or negative anomalies, or of any change-points occurring along a time-series. I have investigated the influence of precipitation on LTER-MC system modelling driver-response curves based on a novel cumsum approach proposed by Regier et al. (2019). Driver-response plots were modelled using two variables, one of which assumed to be the driver (monthly cumulative precipitation), and the other the potential response. Driver and response variables are paired and ordered so that the driver variable is ranked in ascending order in such a way to create a continuous gradient, and the response variable is scaled by subtracting the mean and dividing each observation by the standard deviation. Such a procedure allows easier identification of any underlying relationship between driver and response variable, recognizable by changes in the slope of the response curve (Regier et al. 2019). The figure below (Fig. 3.1) shows the relationship between precipitation and salinity using the original data (a) and the transformed data (b) according to Regier et al., (2019). The untransformed data give little information about the relationship between the two parameters, while the transformed data reveal the change in salinity along the precipitation gradient. Furthermore, the transformation provides other important information from an operational point of view. Salinity ranges from below average to above average around a precipitation threshold of 120.

I have used cumulative monthly precipitation data as the driver variable for several descriptors of the LTER-MC pelagic system: surface salinity, total nitrogen, phytoplankton biomass (carbon content of diatoms, dinoflagellates and other flagellates) and surface chlorophyll-a. In order to quantify and to statistically assess the effect of precipitation on response variables, the precipitation thresholds and the significance of the change in the slope of response curves along the precipitation gradient were assessed using the Davies' test (Davies, 1987). The Davies' test tests the null hypothesis that both slopes are equal by computing k test statistics for the difference-in-slope (here I used k=100). Then, it returns the best estimate (change-point and pvalue) for the rejection of the null hypothesis. As I expected a different effect of precipitation in different seasons, the driver-response curves were performed independently for each season.
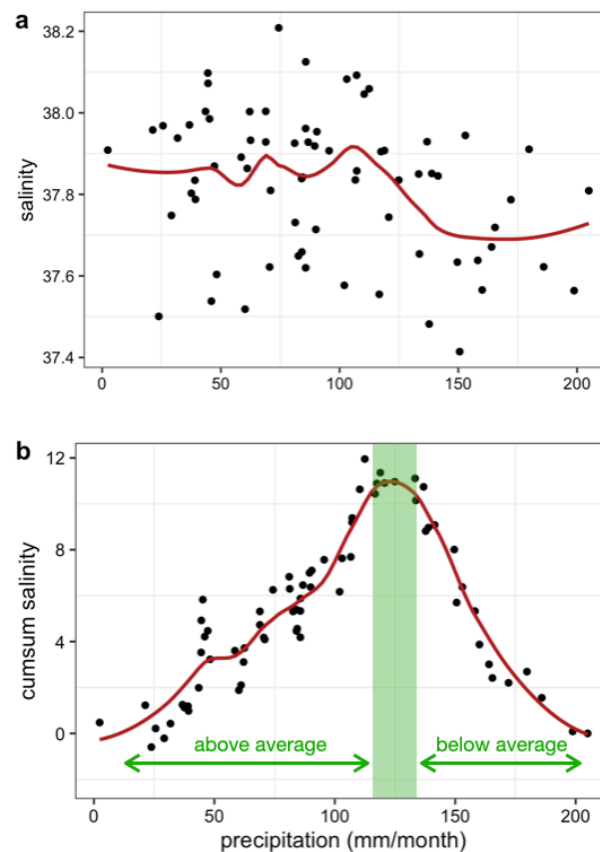
Figure 3.1 An illustrative example of the relationship between salinity and precipitation in winter using raw data (a) and cumsum-transformed data according to Regier et al. (2019) (b). The green elements in plot b show the change of salinity along the precipitation gradient which spans from values above average to values below average around the threshold of 125 mm/month.

In order to assess phytoplankton community variability during low and high precipitation phases, a non-metric multidimensional scaling (nMDS) was performed on Bray-Curtis distances of log-transformed and monthly-averaged abundance data for each season. Low and high precipitation phases were defined according to the seasonal precipitation thresholds corresponding to the change in the slope of salinity response curves. I performed a permutational analysis of variance (PERMANOVA, Anderson, 2001) to test for significant differences in the abundance and composition of the phytoplankton community between high and low precipitation regimes in each season. PERMANOVA is a non-parametric test used to compare differences between groups of objects and test the null hypothesis that

the centroids of the groups are equivalent for all the groups. As the results of PERMANOVA can be biased when there is a significant within-group variation (Anderson, 2001), I have integrated the analysis with a test for significant differences in the dispersion between the groups performing a permutation analysis of multivariate dispersion (PERMDISP, Anderson, 2006a).

## 3.2.5    Climate variability at LTER–MC

The overall climatic variability at LTER-MC was investigated integrating several atmospheric parameters through a principal coordinate analysis (PCA). Specifically, I developed a PCA starting from monthly anomalies of precipitation, ait temperature at 2 meters, atmospheric pressure, relative humidity and wind speed. The extracted principal components were used as a proxy of LTER-MC climate variability and correlated with large-scale climatic oscillations indices to provide insights into interannual climate variability at LTER.MC. I have selected three mid-latitude teleconnections indexes that could potentially influence the climate variability at LTER-MC, namely Mediterranean Oscillation (MO, Conte et al., 1989), Western Mediterranean Oscillation (WeMO, Martin-Vide et al., 2006), the North Atlantic Oscillation (NAO, Jones et al., 1997) and the Atlantic Multidecadal Oscillation (AMO, Enfield et al., 2001) indexes.

# 3.3 Results

## 3.3.1    Long-term signals

Phytoplankton biomass showed a general tendency to increase on the interannual scale (Fig. 3.2). According to the results of the trends' test, chlorophyll-a, diatoms and dinoflagellates (Figs. 3.2 A-B-C respectively) showed a significant upward trend that was particularly pronounced for chlorophyll-a and diatoms as suggested by their high values of the slopes (Tab. 2). On the contrary, phytoflagellates (Fig. 3.2 D) did not show any significant trend despite fluctuations over the interannual scale. All environmental parameters but nitrates and silicates showed a significant interannual trend (Fig. 3.3). Both salinity and phosphates experienced a significant decrease during the last 18 years whereas surface temperature, together with precipitation and total nitrogen increased.

The correlations among the residuals (derived from the STL decomposition) of the decomposed time-series highlighted consistent patterns in the interannual variability among several parameters (Fig. 3.4). Chlorophyll-a was mostly associated with diatoms as shown by their high correlation ($R^2$=0.72), and in minor measure to dinoflagellates and phytoflagellates ($R^2$=0.58 and $R^2$=0.49 respectively). Nutrients were generally correlated to each other and both the total nitrogen and phosphates were related to salinity, with low salinity associated with higher nutrient concentrations. Lower salinity was also significantly related to higher phytoplankton biomass considering both the chlorophyll-a and the functional groups. Both temperature and precipitation residuals showed no significant correlation with any of the other parameters.

Apart from linear trends, some parameters showed characteristic multiannual fluctuations. These were particularly evident in the interannual variability of salinity and precipitation, which were characterized by overlapping cyclical phases of increase and decrease which alternated throughout the second part of the time-series, despite the lack of significant correlation between the residuals (Fig. 3.5A). Similar wave-like patterns were also observed for chlorophyll-a (Fig. 3.5B) and diatoms biomass (Fig. 3.5C), which roughly matched the interannual trend of salinity.

Table 2 Trend significance and slopes for the selected chemical, physical and biological parameters in the period 1997–2015 at LTER-MC.

| parameter | tau | slope | pvalue |
|---|---|---|---|
| chlrophyll *a* | 0.617 | 0.00200 | <0.001 |
| diatoms | 0.472 | 0.00238 | <0.001 |
| dinoflagellates | 0.231 | 0.00093 | <0.001 |
| phytoflagellates | 0.079 | 0.00035 | 0.075 |
| precipitation | 0.438 | 0.15496 | <0.001 |
| salinity | -0.403 | -0.00131 | <0.001 |
| temperature | 0.363 | 0.00242 | <0.001 |
| total nitrogen | 0.220 | 0.00432 | <0.001 |
| nitrates | 0.015 | 0.00013 | 0.734 |
| silicates | -0.050 | -0.00047 | 0.264 |
| phosphates | -0.588 | -0.00036 | <0.001 |
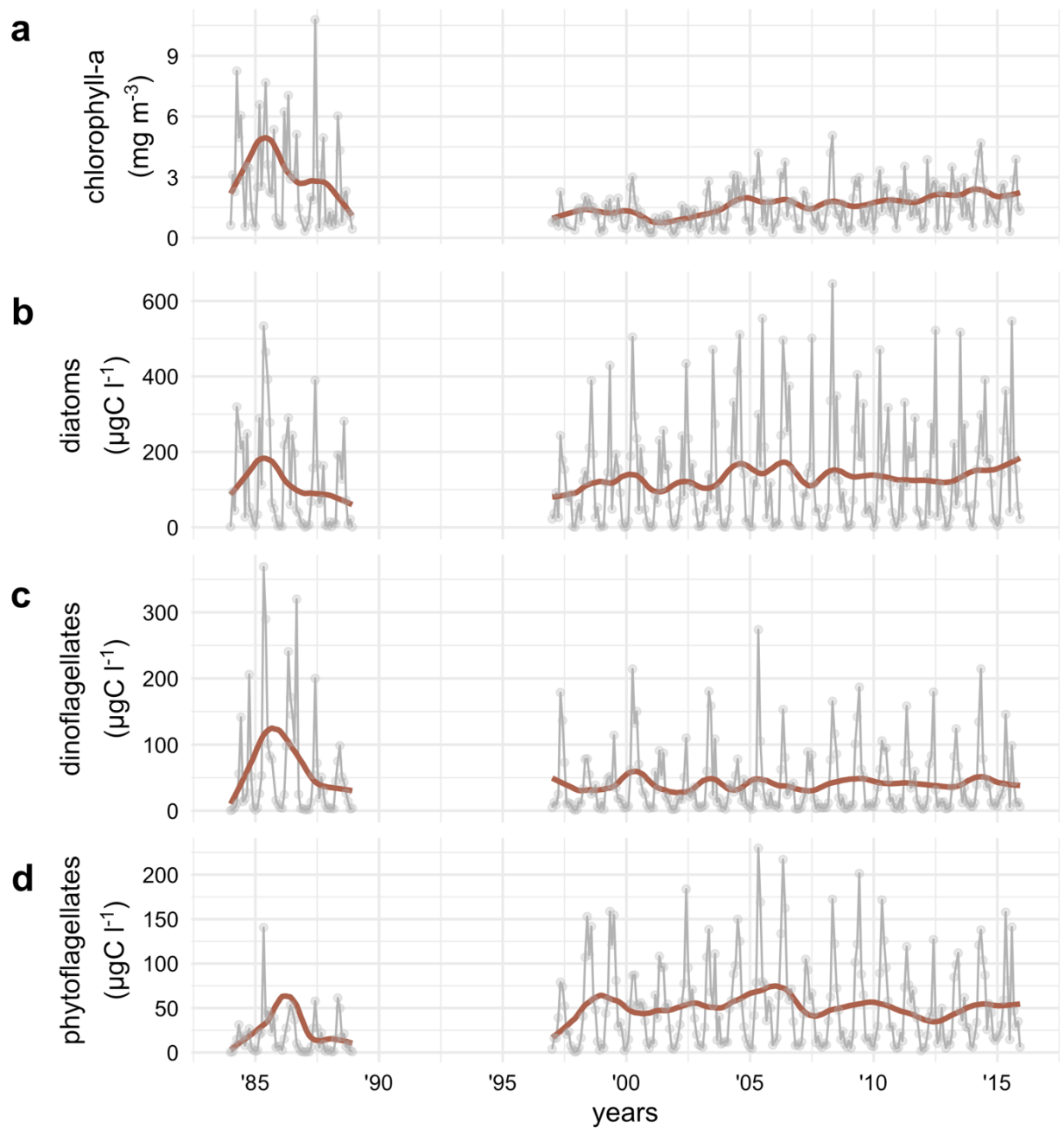
Figure 3.2 Interannual variability of suface phytoplankton biomass at LTER-MC. Grey lines are monthly averaged values of biomass, as a) chlorophyll–a and b),c), and d) biovolume-derived carbon data for diatoms, dinoflagellates and phytoflagellates, respectively,  over the years 1984-2015. Red lines are the trend components obtained through Seasonal and Trend decomposition using Loess' (STL, Cleveland et al., 1990).
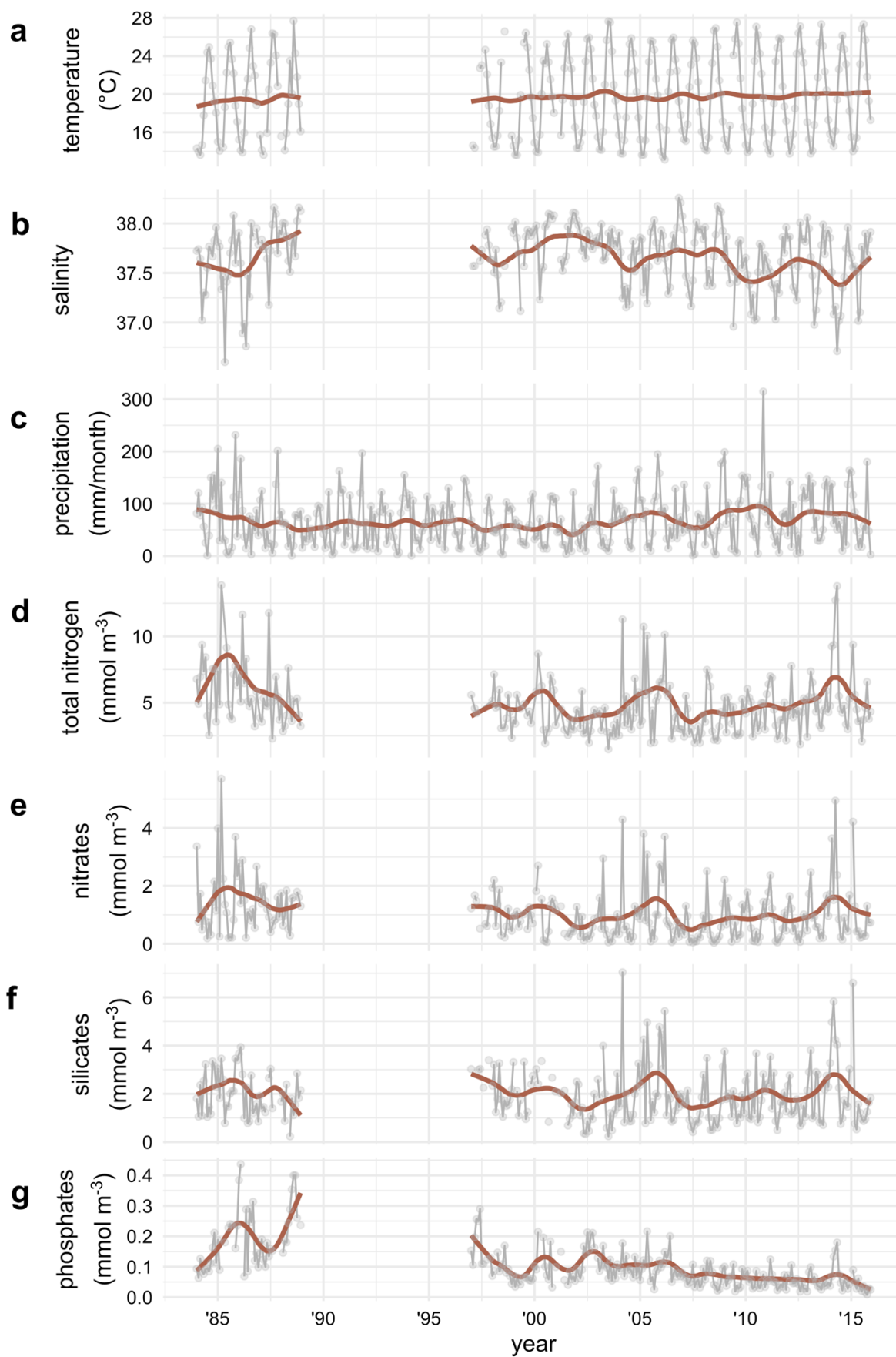
Figure 3.3 Interannual variability of abiotic variables at LTER-MC. Grey lines are monthly averaged values of environmental parameters. Red lines are the trend components of the temporal variability as derived from the 'Seasonal and Trend decomposition using Loess' (STL, Cleveland et al., 1990).
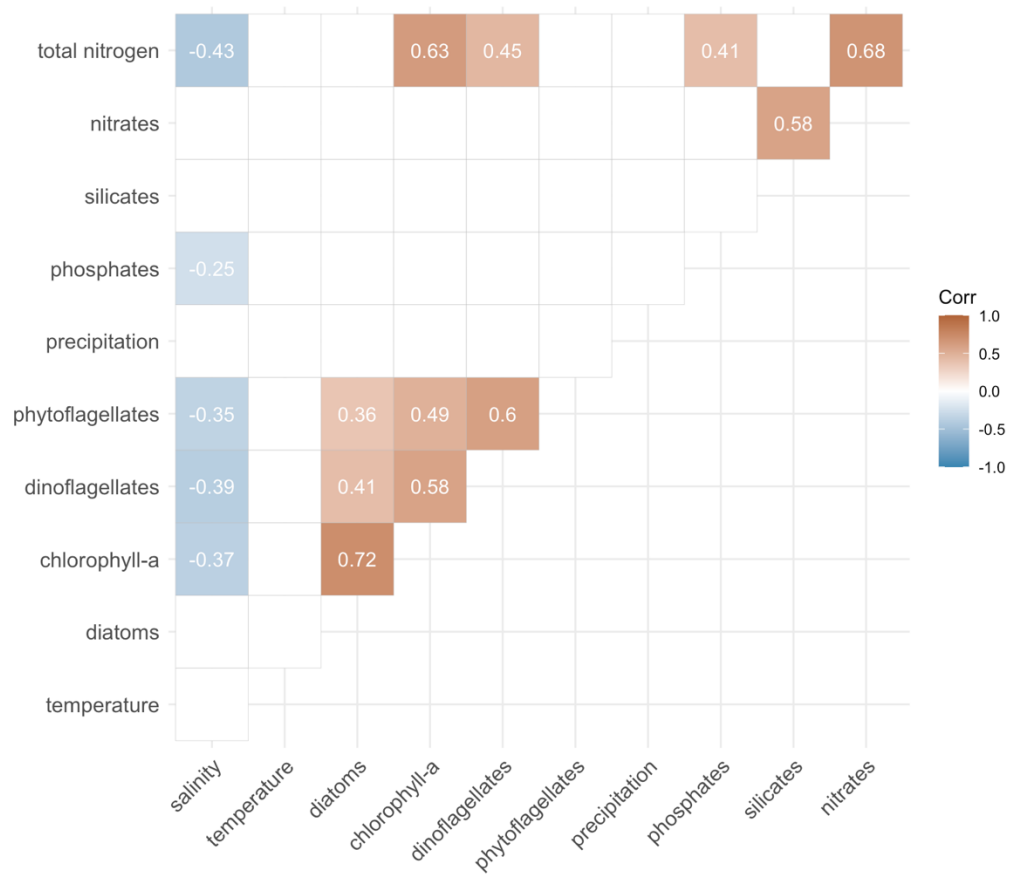
Figure 3.4 Spearmann correlation matrix of the residual components from the 'Seasonal and Trend decomposition using Loess' (STL, Cleveland et al., 1990) of physical, chemical and biological variables at LTER-MC. Significant correlation values (p-value <0.05) are highlighted in orange (positive) and blue (negative) nuances. The correlation measures the strength and direction of monotonic association between the variables.
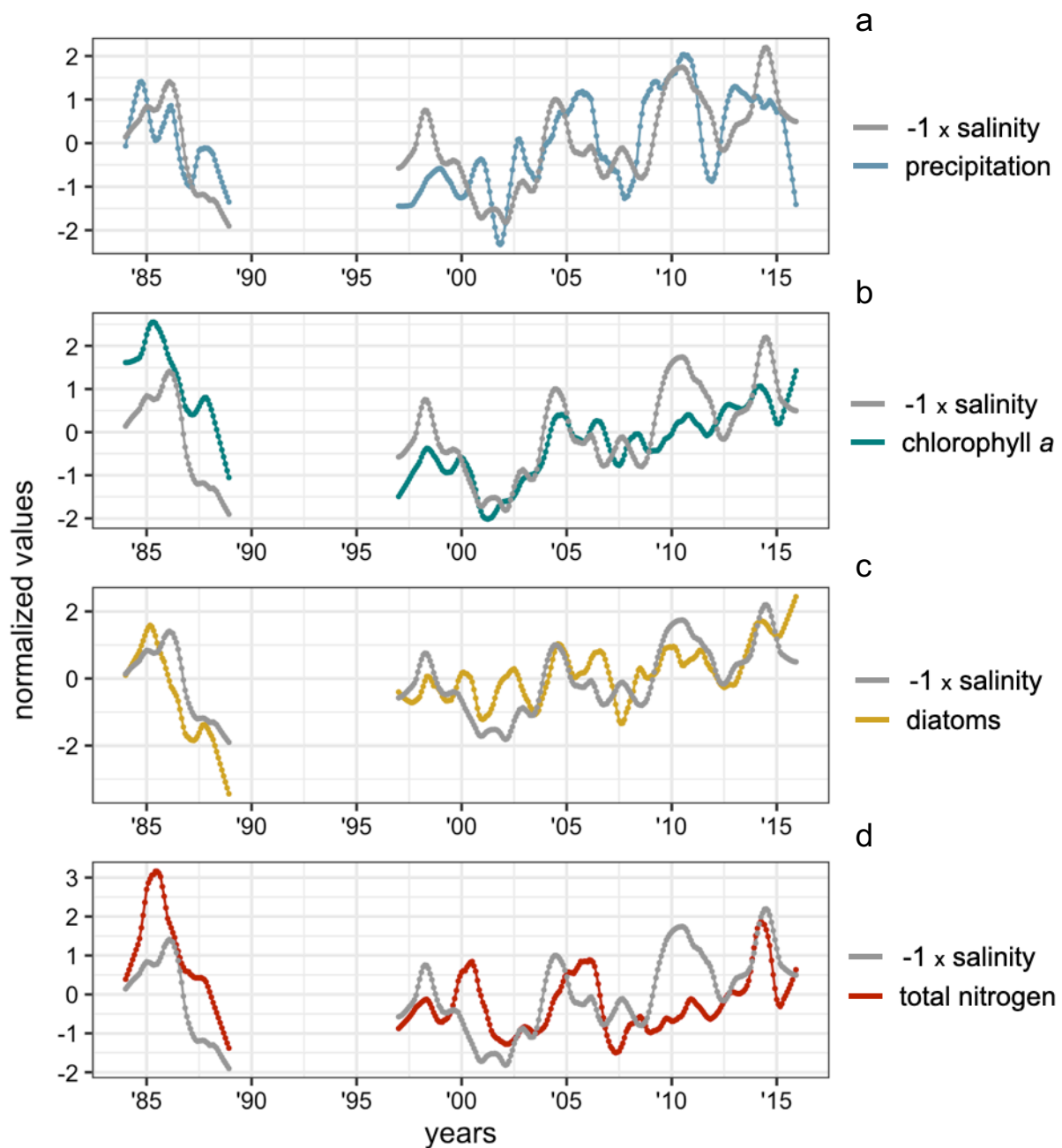
Figure 3.5 Trend components of the temporal variability at LTER-MC of monthly normalized values of cumulative precipitation (A), chlrophyll-a (B), diatoms carbon (C), total nitrogen (D). Salinity was multiplied by -1 and data were normalized in order to highlight the relation with the wave-like fluctuations characteristic of surface salinity' trend.

# 3.3.2    Influence of precipitation on surface salinity

The analyses of the long-term surface salinity variations revealed an overall decreasing trend during all the seasons (negative slope) and a significant negative trend in spring and summer (Fig. 3.6, Tab. 3). In deeper layers, a significant negative trend was recorded only in spring. Such ongoing decoupling between surface and deep-water salinity was better estimated by calculating seasonal trends of Δsal, which resulted to be significant in all the seasons (Tab. 3). The significant increase of Δsal trends in all the seasons suggested that surface and deep salinity varied over time to a different extent. Indeed, although in winter and autumn neither surface nor deep salinity showed a significant upward or downward tendency, the surface trend's slope was steeper than the deep one, resulting in a gradually increasing decoupling between deep and surface layers over the years, traced by the significant trend recorded for Δsal. Similarly, in summer, a slight increase of salinity in deeper layers concurred with a significant decrease in the surface layers, resulting in a significant upward trend of Δsal. In spring, although salinity was decreasing in both layers, surface salinity showed the greatest decrease, and deviated from an average close to 38 in the first years of the millennium, to less than 37 in 2014.
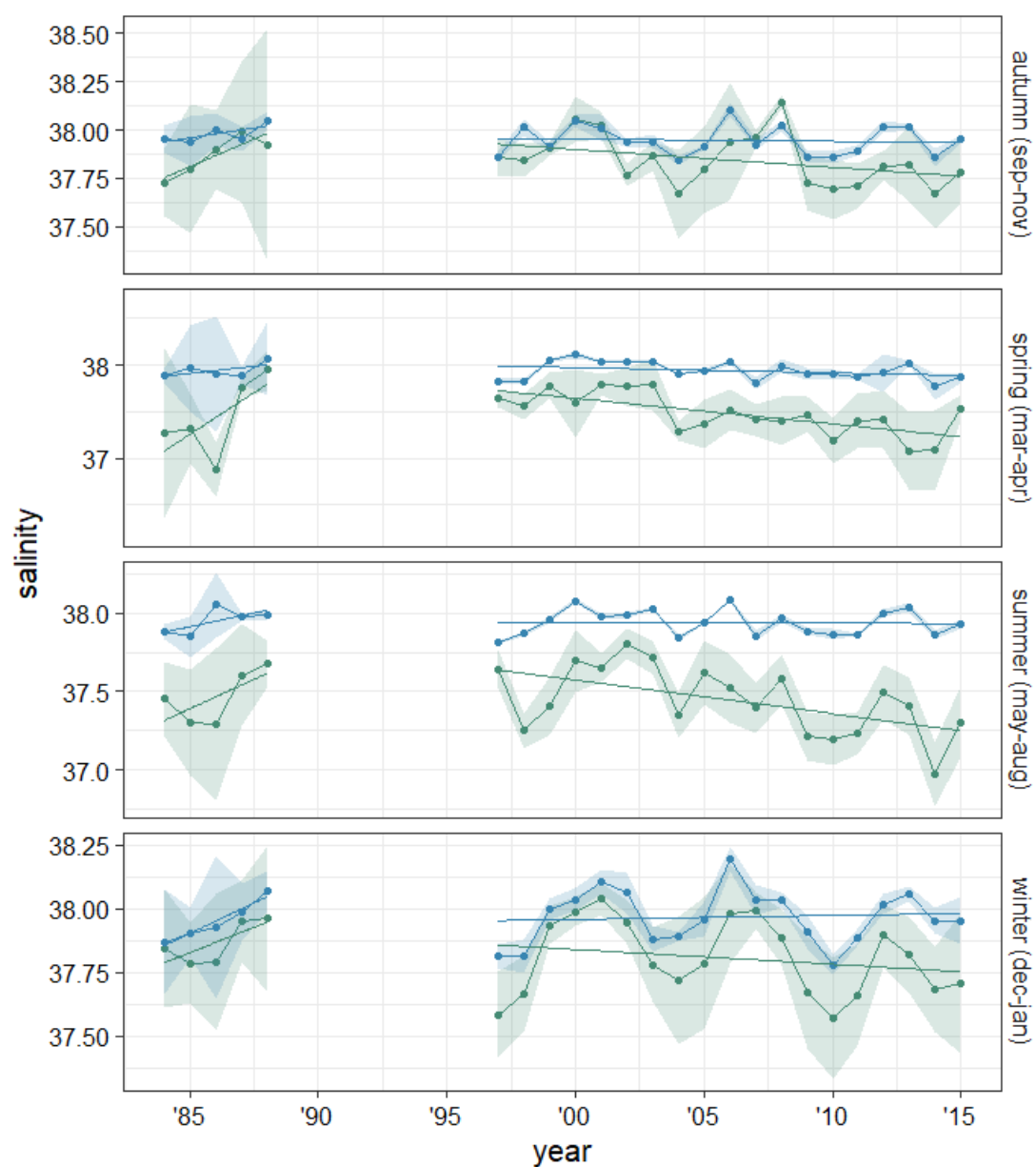
Figure 3.6 Interannual variability of surface (0-5m, in green) and deep salinity (50-70m, in blue). Shaded areas represent 95% confidence intervals.

Table 3 Significance of seasonal trend and slopes of surface (0-5m layer) and deep (5-70 m)-salinity, precipitation and difference between surface and deep salinity (Δsal).

| season | surface salinity slope | surface salinity pvalue | deep salinity slope | deep salinity pvalue | Δsal slope | Δsal pvalue | precipitation slope | precipitation pvalue |
|---|---|---|---|---|---|---|---|---|
| winter | -0.007 | ns | 0.001 | ns | 0.008 | **<0.01** | 3.01 | **<0.01** |
| spring | -0.02 | **0.01** | -0.006 | **0.04** | 0.021 | **0.01** | 0.07 | **0.01** |
| summer | -0.02 | **0.02** | 0.0008 | ns | 0.023 | **<0.01** | 0.03 | **0.03** |
| autumn | -0.01 | ns | -0.0005 | ns | 0.008 | **0.02** | 0.09 | ns |
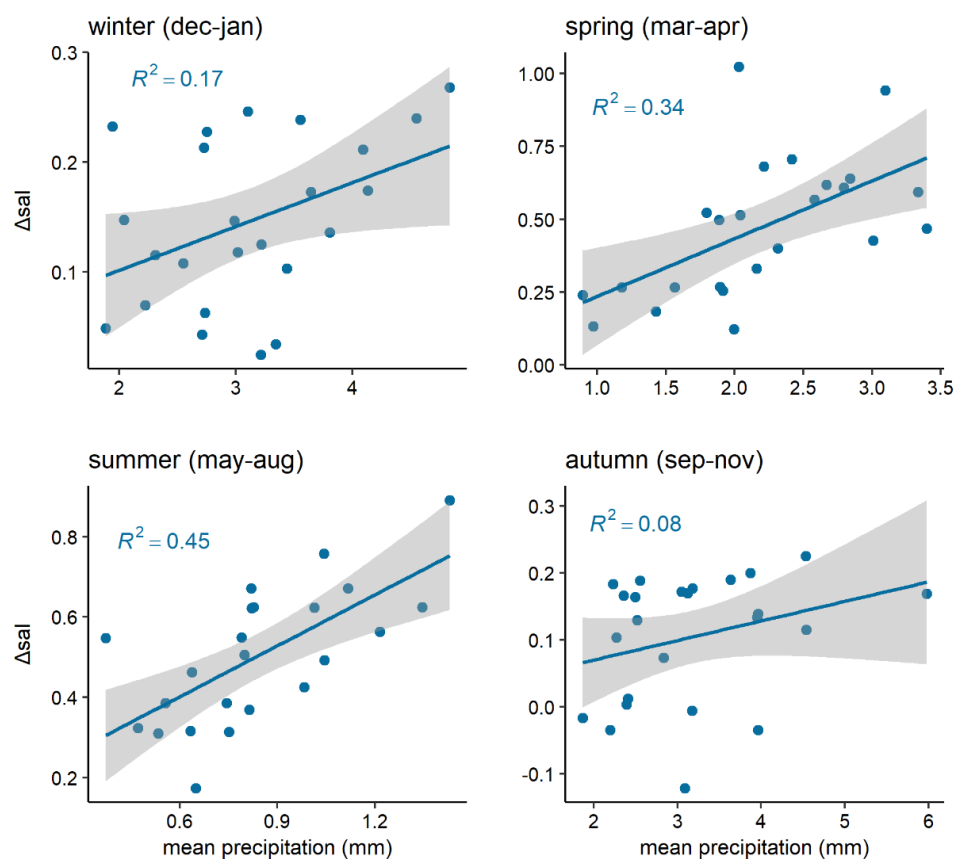


Figure 3.7 Linear regressions relating seasonal cumulative precipitation and difference between surface and deep salinity (Δsal) over the years 1985-2015 at LTER-MC. Dots represent different years.

Results of seasonal linear regressions between mean Δsal and precipitation (Fig. 3.7) revealed that the decoupling between the surface and deep salinity intensified with increasing precipitation especially in spring and summer ($R^2$= 0.34 and $R^2$= 0.45 respectively, pvalue<0.01), and to a lesser extent in winter and autumn ($R^2$= 0.17, pvalue=0.04 and $R^2$= 0.08, pvalue=0.15 respectively).

### 3.3.3    Driver-response curves

Consistent with the interannual relationship found between salinity and precipitation, the driver-response curves (Fig. 3.8) showed surface salinity distributions with positive anomalies at relatively low precipitation values, followed by a marked change in the slope and by negative anomalies at higher precipitation levels in all seasons considered. The response curve of total nitrogen was specular to that of surface salinity, showing a significant change point roughly corresponding to that of surface salinity along the seasonal precipitation gradients (Tab. 4). Specifically, both surface salinity and total nitrogen concentrations shifted, from above average to below average and from below average to above average respectively, when cumulative precipitation exceeded a precipitation threshold that varied with the season considered. In winter, the change point was around 120 mm/month of cumulative precipitation, whereas in autumn and spring a cumulative precipitation value around between 60 and 105 mm/month was enough to shift the system towards a fresher and more nutrient-enriched condition. Accordingly, summer driver-response curves showed higher responsiveness to precipitation compared to other seasons as both salinity and total nitrogen experienced a change in the direction of the curve corresponding to between 9 and 17 mm/month of cumulative precipitation.

While physical and chemical parameters showed a direct response along the precipitation gradient, phytoplankton revealed more complex driver-response curves, slightly different among the groups, whose pattern is indecipherable considering only the Davies' test (Table 4). During winter, at least two change points were present in the distribution of chlorophyll-a, diatoms and dinoflagellates. Indeed, their response curves showed positive anomalies for relatively low precipitation, then, around 70 mm/month of cumulative precipitation, the slope sign reversed showing negative anomalies for intermediate precipitation values. With

cumulative precipitation values higher than 110 mm/month chlorophyll-a, diatoms and dinoflagellates showed again positive anomalies.

In spring, both chlorophyll-a and diatoms showed a characteristic 'v-shaped' response curve that outlined a quite clear association between precipitation and positive phytoplankton anomalies. It is worth noting that, while relatively high precipitation seemed to support, and affect the magnitude of, the spring bloom, additional amounts of precipitation (particularly rainy years or extreme events) depressed phytoplankton growth, especially phytoflagellates and dinoflagellates.

In summer, for very low cumulative precipitation values, both chlorophyll-a and phytoplankton groups followed a clear steep negative slope, particularly evident for phytoflagellates, which roughly matched the negative anomalies of total nitrogen and the positive anomalies of surface salinity. At slightly higher precipitation values (around 9 mm/month), chlorophyll-a and phytoplankton groups showed a steep increase characterized by positive anomalies. Nevertheless, the second part of the curve did not have a well-defined tendency, and the response curves appeared quite scattered over the cumulative precipitation threshold of 9 mm/month. In autumn as in winter, surface salinity and total nitrogen showed one change point along the precipitation gradient, whereas phytoplankton revealed more complex responses characterized by at least two change points. Phytoplankton seemed to be negatively affected by higher precipitation and, except for phytoflagellates, positive anomalies were recorded only at the highest precipitation of the whole time-series.

The nMDS plot revealed that the communities sampled in the different precipitation regimes were quite similar and no clear separation emerged (Fig. 3.9). Only in autumn (Fig. 3.9D), phytoplankton assemblages in high precipitation phases appeared more dispersed compared to low precipitation' ones, without however highlighting a definite clustering. Accordingly, the PERMANOVA tests showed the precipitation effect to be not significant in all the seasons (Tab. 5).
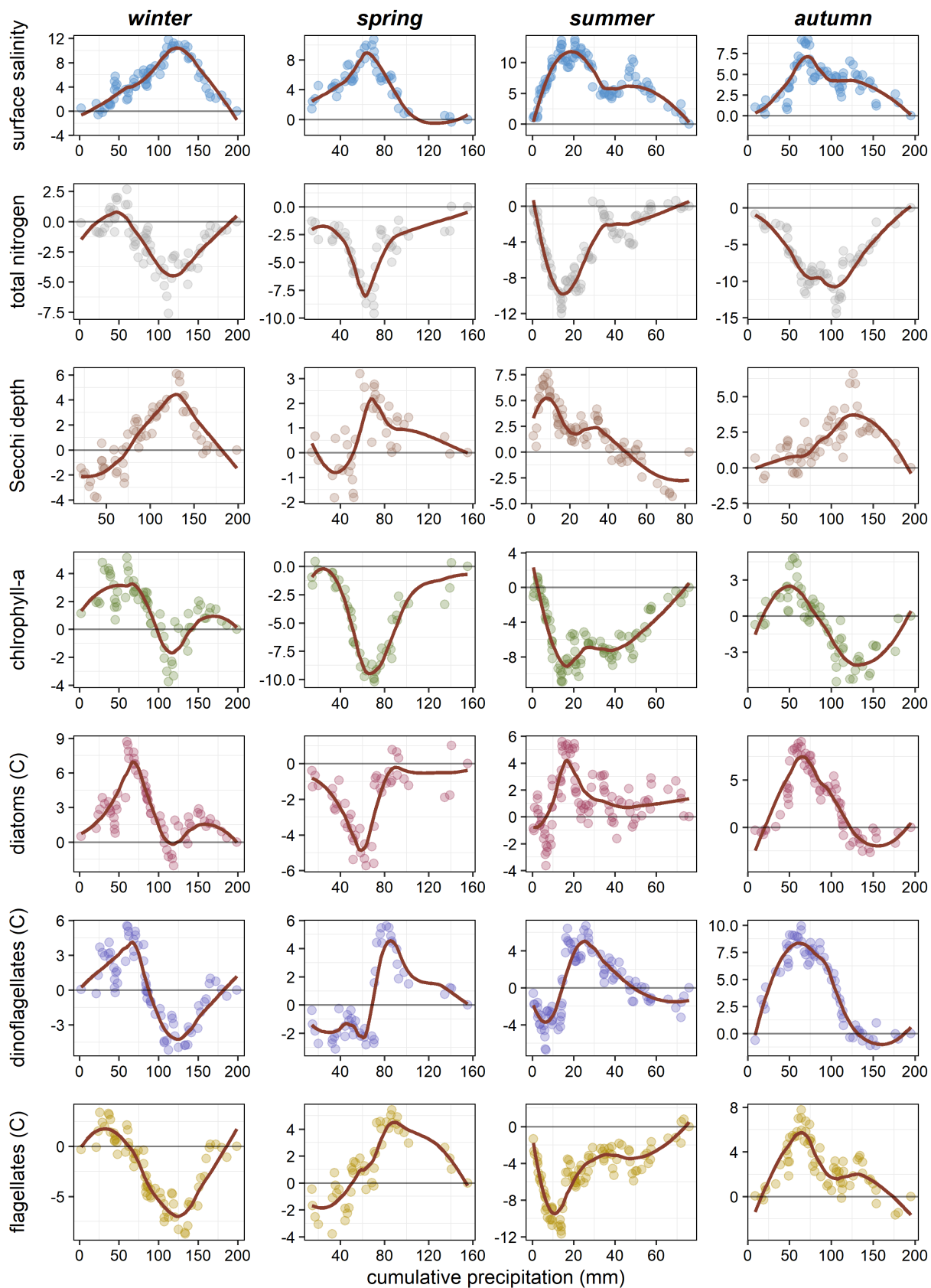
Figure 3.8 Seasonal driver-response curves based on the cumulative sum of normalized physical, chemical and biological parameters along the precipitation gradient (see methods and Fig. 3.1 for explanation).

Table 4 Results of the Davies' test performed on the driver–response curves for each parameter in each season. The precipitation threshold is the value corresponding to a change in the sign of the slope of the response curve along the increasing precipitation gradient.

|        | parameter | precipitation treshold (mm/month) | pvalue |
|--------|-----------|-----------------------------------|--------|
| winter | surface salinity | 119.89 | <0.001 |
|        | secchi depth | 119.89 | <0.001 |
|        | total nitrogen | 119.89 | <0.001 |
|        | chlrophyll *a* | 119.89 | 0.001 |
|        | diatoms | 60.74 | <0.001 |
|        | dinoflagellates | 139.61 | <0.001 |
|        | flagellates | 119.89 | <0.001 |
| spring | surface salinity | 56.78 | <0.001 |
|        | total nitrogen | 56.78 | <0.001 |
|        | secchi depth | 70.74 | <0.001 |
|        | chlrophyll *a* | 70.74 | <0.001 |
|        | diatoms | 56.78 | <0.001 |
|        | dinoflagellates | 84.71 | <0.001 |
|        | flagellates | 98.67 | <0.001 |
| summer | surface salinity | 16.96 | <0.001 |
|        | total nitrogen | 8.90 | <0.001 |
|        | secchi depth | 8.90 | <0.001 |
|        | chlrophyll *a* | 8.90 | <0.001 |
|        | diatoms | 8.90 | <0.001 |
|        | dinoflagellates | 8.90 | <0.001 |
|        | flagellates | 8.90 | <0.001 |
| autumn | surface salinity | 69.68 | <0.001 |
|        | total nitrogen | 105.34 | <0.001 |
|        | secchi depth | 127.58 | 0.006 |
|        | chlrophyll *a* | 141.00 | <0.001 |
|        | diatoms | 51.85 | <0.001 |
|        | dinoflagellates | 51.85 | <0.001 |
|        | flagellates | 51.85 | <0.001 |
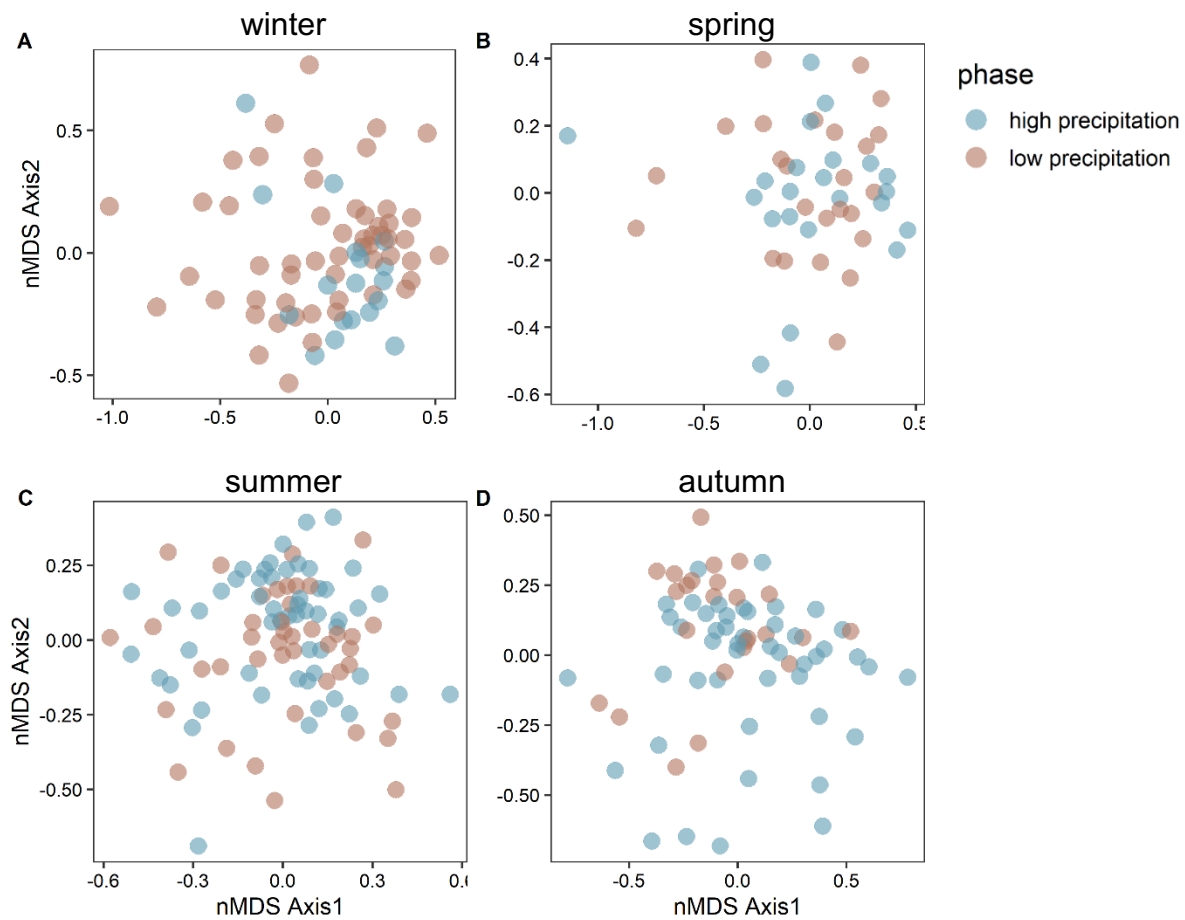
Figure 3.9 Non-metric multidimensional scaling (nMDS) based on Bray-Curtis distances of log-transformed and monthly-averaged phytoplankton abundances according to precipitation phases in different seasons. (A) winter, (B) spring, (C) summer, (D) autumn. Ordination stress was below 0.18 in all representations.

Table 5 Results from PERMANOVA and PERMDISP examining the effect of precipitation regime for each season.

| precipitation regime | Df | PERMANOVA | | PERMDISP | |
| --- | --- | --- | --- | --- | --- |
| | | Pseudo F | P (perm) | F | P |
| winter | 1 | 1.331 | 0.165 | 4.228 | 0.043 |
| spring | 1 | 0.799 | 0.674 | 0.043 | 0.836 |
| summer | 1 | 1.224 | 0.226 | 0.002 | 0.969 |
| autumn | 1 | 1.950 | 0.091 | 2.494 | 0.119 |

# 3.3.4    LTER–MC climatic index

The first axis extracted from the PCA explained 47.3% of the climatic variability at LTER-MC (Fig. 3.10) and it was mostly representative of the temporal variability of anomalies in atmospheric pressure ($R^2$=-0.87), precipitation ($R^2$=0.83), wind speed ($R^2$=0.73) and to a lesser extent relative humidity ($R^2$=0.61),  almost and not representative of air temperature ($R^2$=0.07). The first axis showed a significant correlation with all the climatic indices tested except for the Atlantic multidecadal oscillation index (AMOi) (Tab. 6). In particular, the first axis showed the greatest correlation with the North Atlantic oscillation index (NAOi) and the Mediterranean oscillation index (MOi). Visual inspection of the interannual variability of the first axis and NAOi revealed an evident coupling occurring in the last 10 years of the series (Fig. 3.11), characterized by typical interannual fluctuations of both the indices.
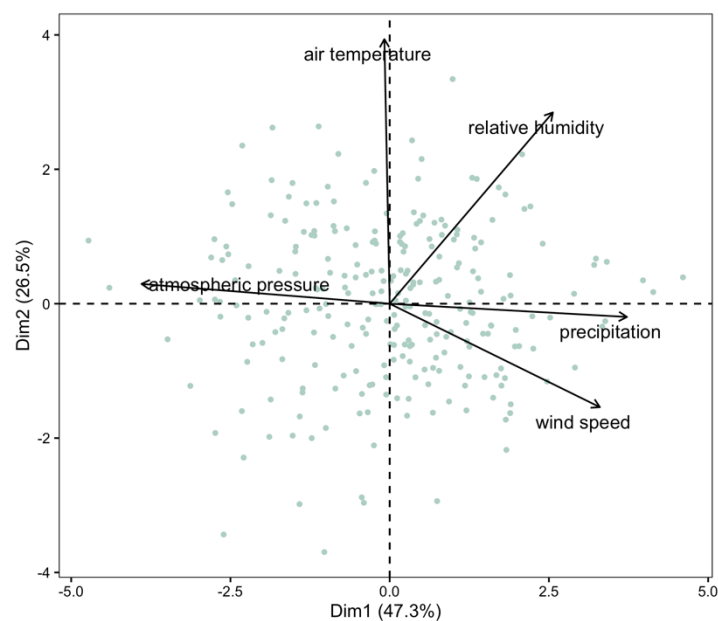


Figure 3.10 PCA of LTER-MC climatic parameters used to extract a proxy of LTER-MC monthly climatic condition.

Table 6 Spearman correlation matrix of the main climatic mid-latitude teleconnection indices and the first principal component (Axis1). Axis1 was extracted from monthly anomalies of atmospheric pressure, wind speed, precipitation and relative humidity.

|  | MOi | WeMOi | AMOi | NAOi | Axis1 |
|---|---|---|---|---|---|
| **MOi** |  | 0.44 | -0.04 | 0.48 | -0.34 |
| **WeMOi** |  |  | -0.21 | 0.11 | 0.31 |
| **AMOi** |  |  |  | -0.26 | 0.14 |
| **NAOi** |  |  |  |  | -0.39 |
| **Axis1** |  |  |  |  |  |
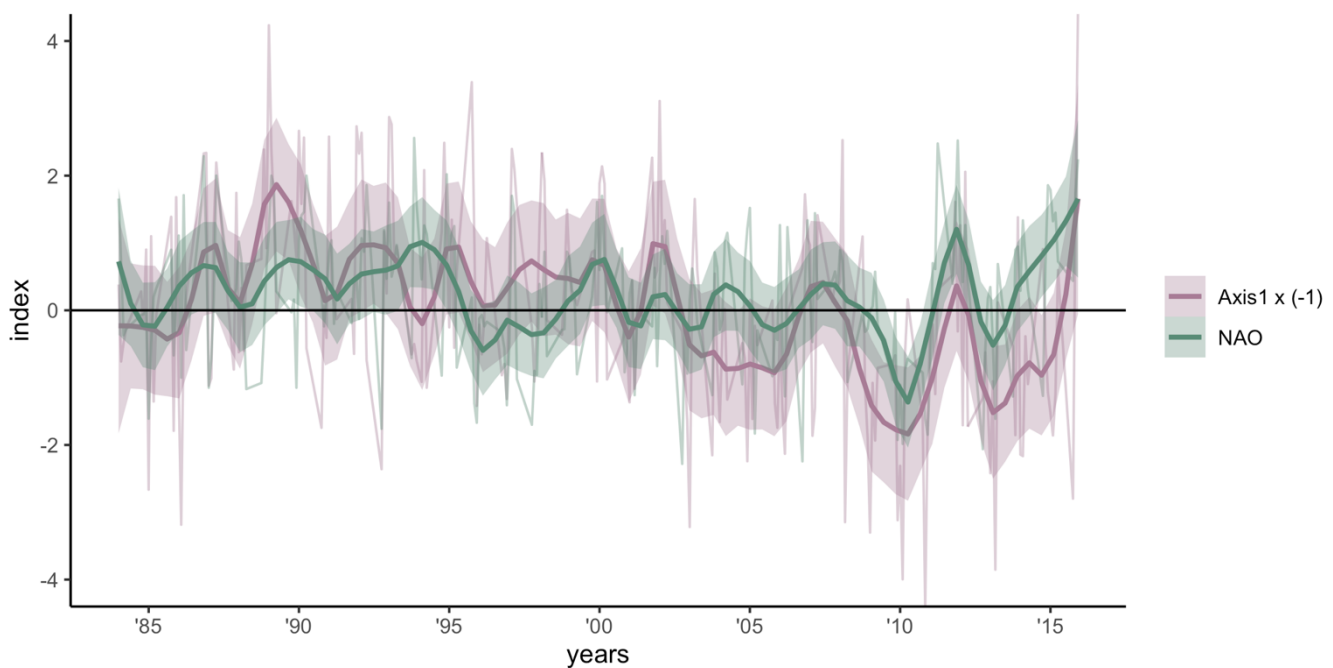


Figure 3.11 Interannual variability of the first principal component (Axis 1) and NAO index described by LOESS curves. Shaded area represents 95% confidence interval.

# 3.4 Discussion

LTER-MC is located in one of the most urbanized coastal areas of Europe. Although the area borders the offshore waters of the open Tyrrhenian sea and interconnects with it, it is strongly influenced by the surrounding lands and in particular by freshwater inputs that introduce new nutrients into the system. The coupling between the surrounding land and the pelagic system is clearly shown by salinity variations both at the seasonal and interannual levels. Previous studies described the pelagic system at LTER-MC as shifting between two main trophic and hydrological phases, defined by different salinity ranges, whereby low salinity was generally associated with nutrient-rich and more productive waters (Carrada et al., 1980; Scotto di Carlo et al., 1985).

The analyses performed on chemical, physical and biological time-series in this study highlighted the main long-term patterns of variability of the LTER-MC system. Although the water surface temperature showed a slight interannual increase especially in late spring and early summer (see also Chapter 2), it did not seem to describe phytoplankton' variability patterns. Rather, this study confirmed the efficacy of salinity variations in describing the state of the coastal pelagic system both in terms of nutrients and biomass, and further extended their relationships with those of the other components of the system on the decadal scale.

The results obtained from long-term studies on different coastal systems have associated interannual variability of salinity with anthropogenic causes, as human modifications of rivers sections (Hayami et al., 2014; Mee, 1992; Petersen al., 2008) or climatic ones, such as changes in circulation patterns or changes in the precipitation regimes (Goberville et al., 2010, Harding et al., 2016; Mozetic et al., 2012; Odebrecht et al., 2010). Here we observed that the climatic signal represented by an increase in precipitation propagated and influenced both the hydrology and the biology of the LTER-MC pelagic system. Although the precipitation regime is expected to decrease in this geographical area in the coming years (IPCC, 2013), we have observed an increase in precipitation in our monitoring and sampling span, whose intensity and variability are part of large-scale climate patterns. Several studies have investigated the connection between fluctuations in south Italy precipitation and Mediterranean and North Atlantic oscillation (Caloiero et al., 2011; De Vita et al., 2012).

However, this is the first time that the forcing of these large-scale atmospheric patterns in this specific area is assessed in the marine context and in particular on phytoplankton.

Larger precipitation amounts involved higher loads of freshwater and nutrients into the system and increased the decoupling between the surface and deep waters as testified by the increase in Δsal. Although in this area the surface layer typically followed a relatively independent dynamic from the rest of the water column (Ribera d'Alcalà et al. 2004; Scotto di Carlo et al., 1985), the increased precipitation intensified the decoupling between the surface and deep layers on the long-term scale. I hypothesized that the intensity and impact on the system varied with the seasons. Indeed, as the hydrological conditions and the availability of resources (as light and nutrients) for the biological community are seasonally variable, I expected a different impact of precipitation on the water chemical and physical properties, and a different degree of sensitivity of the phytoplankton community along the seasonal gradients of precipitation forcing.

From mid-autumn through winter, the water column is fully mixed (Ribera d' Alcalà et al., 2004) and, apart from transient events of stratification, vertical motion is such that the water column is characterized by homogeneous physical, chemical and biological values along with all its depth. Therefore, the volume of water potentially affected by precipitation is higher than in late spring and summer, when the water column is characterized by relative stability and negligible vertical movements. Consistently, we found a clear seasonal progression in the threshold precipitation values associated with a shift of the system towards a fresher, turbid and nutrient-enriched condition (i.e., from 9/mm month in summer to 120 mm/month in winter). The shift occurred in all the seasons following a mechanical dynamic as suggested by the peculiar v-shape of salinity, Secchi depth and nutrients responses along the precipitation gradient. In summary, both the shape of the driver-response curves and the overlay of change points for Secchi depth, surface salinity and total nitrogen are consistent with the runoff-driven dynamics of the system. Once a certain season-dependent precipitation threshold is exceeded, runoff is such that higher amounts of freshwater and new nutrients pour into the basin leading the system to shift from an average condition.

In spite of the mechanical responses showed by physical and chemical variables along the precipitation gradient, phytoplankton responses were heterogeneous. Besides having different seasonal thresholds, they presented different response shapes, mostly non-linear, which suggested the presence of more complex underlying mechanisms. We argue

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that the complexity of phytoplankton driver-response curves was ascribable to the interaction between climate forcing represented by precipitation, and the status of resources limitation (light and nutrients) that phytoplankton experienced in each season, as discussed in the following.

Phytoplankton growth in winter is mostly constrained by the physical mixing of water and by light limitation (Gran, 1931; Sverdrup, 1953). Nevertheless, the occurrence of two particular conditions, at least in coastal areas, can relax these two constraints and favour phytoplankton growth. During prolonged high-pressure conditions, even in the minimum day-length period, light can be such to overcome consumption favouring phytoplankton net growth, a condition observed at LTER-MC (Zingone et al., 2010a) as well as in the northern Adriatic Sea (Totti et al., 2019). Similarly, short interruptions of the shift from autumn to winter characterized by mild and dry high-pressure, the so-called 'St Martin's Summer', were associated with phytoplankton densities higher than the seasonal average (Zingone et al., 1995). A second condition that has been associated with late-autumn and winter blooms is related to the effects of freshwater inputs on the water column stabilizations. Increases in freshwater inputs were observed to limit the vertical displacement towards poorly illuminated depths favouring bloom development (Byun et al., 2005), a condition already observed in winter at LTER-MC (Zingone et al., 2010a). These two conditions could fit with the nonlinear response curves in autumn and winter, in which phytoplankton experienced both positive anomalies for low or no precipitation (high-pressure conditions) and intense precipitation (due to the formation of a stratified surface layer).

As the mixed-layer depth decreases and the hours of light increase, phytoplankton experiences optimal conditions for growth. In my analysis, more precipitations in spring seemed to boost algal biomass growth, as suggested by the shape of the chlorophyll-response curve. Nevertheless, except for a first common positive response, the performance then varied among the different algal groups, with diatoms being the most favoured group. In the literature, there are contrasting results concerning the response of diatoms to precipitation variability. A global study on algal groups response to long-term precipitation variability suggested a negative relationship between diatoms and precipitation (Thompson et al., 2015). Nevertheless, more detailed studies focusing on single time-series reported a positive response of diatoms to increased precipitation in Blanes Bay (NW Mediterranean Sea) (Nunes et al., 2018) and the Adriatic Sea (Totti et al., 2019). In the case of the Gulf of

Naples diatoms, generally representing the bulk of the biomass in spring, may have outcompeted phytoflagellates and dinoflagellates because of their better performance in nutrient-rich conditions (Litchman et al., 2006).

LTER-MC is characterized by dry and hot summers. The water column stratification during summer is considerable and the mixed layer depth is generally less than 5 m (Ribera d' Alcalà et al., 2004). Thus, we expected the phytoplankton community to be particularly responsive to precipitations during this season. Indeed, once a quite low precipitation threshold was exceeded (about 9 mm/month), phytoplankton biomass was positively affected by the runoff effect and shifted from negative to positive anomalies. Interestingly, also in this season, the response was not homogeneous for all groups, whereby small flagellates, which are generally dominant in summer, seemed to further benefit from the impact of precipitation during this period.

An obvious question concerning the response of phytoplankton groups to precipitation fluctuations was whether the community composition also varied between precipitation phases within each season. Although I showed that not all groups benefit from the precipitation impact to the same extent, community composition did not seem to vary during different precipitation phases. This property was consistent with what showed in Chapter 2 and with what was already proposed by Ribera d'Alcalà et al. (2004), namely that taxa occurrence would be regulated by species-specific biological dynamics (i.e. life-cycles, life-strategies), whereas their amplitude (abundance) would be regulated by abiotic forcing.

Although the use of monthly averages provided a consistent framework to investigate long-term processes for nearly 30 years of observations at LTER-MC, further analysis based on finer time scales will be necessary to quantify with more accuracy the response times of the biotic component and abiotic to climatic perturbations in the area.

Overall, my results highlight the important role that the variability of precipitation plays in the functioning of the coastal pelagic system. Unlike open ocean systems where the temperature is the primary influencing factor on the stratification process and nutrient flows, in coastal systems, the influence of precipitation strongly affects stratification dynamics, the supply of new nutrients and the light context by altering water transparency. It is therefore essential in any long-term monitoring activity to take into account the potential of precipitation on the system both in the short-term and in a future projection.

Furthermore, it is to be emphasized that the intensity of the effects of climate change in mid-latitude systems are likely to be season-dependent. The interaction between climate forcing and the life-history of phytoplankton organisms, which in the mid-latitude systems is modulated on the seasons, generates non-linear responses that can only be understood with a deep knowledge of the biology and the life-cycles of phytoplankton organisms.

# 4 Drivers of phenological patterns at LTER-MC, description and hypotheses

## 4.1 Introduction

### 4.1.1    Phenology in the marine environment

Temporal recurrence is a widespread property in the biological realms. A large number of both autotrophic and heterotrophic organisms are strongly tuned to defined temporal windows, and have evolved specific behavioural and strategies to exploit favourable periods in the course of the annual cycle or to achieve synchrony for sexual reproduction (Forrest & Miller-Rushing, 2010). Given the pervasiveness of biological rhythms in nature, phenology is one of the most important indicators of changes in the context of climate variations. A large number of studies on the aquatic systems support the view that climate change, by altering species physiology or their physical and chemical habitats, can impact phenological patterns, with potentially critical effects on the delicate mechanisms of ecological synchronization between different trophic levels (Edwards & Richardson, 2004; Thackeray et al., 2016).

In the marine environment, the annual occurrence of phytoplankton blooms is among the most impressive and extensive phenological manifestations. Annual-recurring phytoplankton blooms are associated with the continuous renewal of energy flows and matter cycles and affect the global functioning of trophic networks and with the continuous renewal of energy flows and matter cycles (Falkowski, 2012; Platt et al., 2003). The temporal variability associated with phytoplankton blooms has been widely studied using time-series observations and, in recent years, advances in satellite data quality have made it possible to create a spatial and temporal mapping of seasonal chlorophyll trends at the global scale. The theoretical foundations behind the recurrence of phytoplankton blooms are attributable to Gran (1931) and were subsequently formalized in quantitative terms by Sverdrup (1953) under the well-known critical depth hypothesis. The Sverdrup model proposes that phytoplankton bloom occurs when the depth of the mixed layer shoals enough to overcome

a certain critical depth, that is, the depth where phytoplankton growth exceeds losses. As both mixed layer depth and critical depth depend on the annual variability of light and radiation, the recurrence of phytoplankton annual blooms reflects the annual regularity of the astronomical cycles (Cloern & Jassby, 2010). However, although the Sverdrup model is successfully adopted in many circumstances, the variability of primary production in terms of timing, magnitude, bloom development and sensitivity to environmental forcing is very wide.

Satellite chlorophyll data have revealed both a component of intrinsic phenological variability dependent on the biogeographical area and a different long-term sensitivity to the impacts of environmental changes. Using global-ocean satellite data, Boyce et al. (2017) highlighted that, even in the open ocean, factors that shape the bloom timing and development can vary depending on the area. For example, in oligotrophic areas, the timing of the bloom is more linked to stratification dynamics, while in more productive and coastal areas it is more sensitive to the light regime. Similarly, the magnitude of long-term trends in bloom timing and seasonal properties of global chlorophyll were found to be regionally-distinct by other global-scale studies (Friedland et al., 2018; Vantrepotte & Melin, 2009).

In coastal areas, the annual biomass cycles are even more variable (Cloern & Jassby, 2008; Winder & Cloern, 2010), whereby changes in phytoplankton phenology appear to be associated with multiple causes. For example, in response to a climatic shift from a warm to cold phase in the eastern Pacific, San Francisco Bay pelagic system experienced an unprecedented autumn bloom (Cloern et al., 2007). A stable increase in water surface temperature was related to an early spring bloom both in Western Scheldt Estuary (Kromkamp & Van Engeland, 2010) and in the Baltic Sea (Smayda, 2004).

Responses to a changing environment vary among taxonomic groups. While dinoflagellates seem to have a significant phenological response to the current temperature increase by anticipating their bloom, diatoms presented more heterogeneous patterns, which are difficult to generalize at the group level (Atkinson et al., 2015; Chivers et al., 2020; Edwards & Richardson, 2004, Widdicombe et al., 2010). In addition to gradual and continuous perturbations such as the increase in surface temperature, climatic fluctuations or short-term events as heat-waves, strong run-off and storms can also induce responses in some taxonomic groups outside their 'typical' period. In summary, we are still looking for a phenological model that includes uniformity of responses or a generalizable pattern. The

same external forcing in different areas (temperature, wind, human interventions just to mention some) influence the phytoplankton community differently (see Zingone et al., 2010b). Yet, despite a certain similarity in the overall composition among coastal phytoplankton communities, e.g., in the dominance of diatoms (mostly *Chaetoceros* spp.), local populations are substantially different, and also diverge in their evolutionary history (Olli et al., 2015). These conclusions pose important limitations towards one of the ultimate goals of long-term ecological research, that is, the ability to predict biological responses to environmental changes. A necessary step for a better understanding of the phytoplankton phenology is undoubtedly to unbundle the aggregated signal of the most used phenological indices (i.e. total biomass, diatoms and dinoflagellates abundance) in species-specific responses. Species have different roles and functions within the pelagic realm, and many of them are of special interest to human health and economy. Deciphering the intricate pattern of species-specific phenological responses can offer valuable insight into our current and future perception of changing environment. Mentioning Cloern & Dufford (2005):

> *«The underlying processes will not be revealed until we fill in details of the matrix of key species and their repertoires of attributes on which the selective processes of community assembly operate, including intra-species genetic diversity (Zingone & Wyatt, 2005). We know surprisingly little about the life cycles, behavior, energetics, biochemical pathways, resource requirements, susceptibility to pathogens, genetic variability and even growth rates of many key species such as the ubiquitous and ecologically important cryptophytes. [...] Resolution of the species assembly puzzle demands investment in a mode of scientific investigation grounded in autecology, organism interactions and life history that is perceived as unfundable or outmoded (Dale 2001, Smetacek et al. 2002). Until this investment is made, our capacity to understand the mechanisms of phytoplankton species dynamics, their ecological and biogeochemical significance, and their variable patterns between ecosystems will remain stifled»*

# 4.1.2    Drivers of marine phytoplankton species phenology

Recent studies showed that the diversity of eukaryotic phytoplankton is higher than previously thought (De Vargas et al., 2015), and one of the big challenges is to incorporate such tremendous diversity into ecological knowledge. Under this view, the observation and the study of the periodic occurrence of phytoplankton taxa along the temporal environmental gradient assumes a lot of value. Under a bottom-up perspective, phytoplankton successional patterns in temperate zones are argued to be strongly determined by the seasonal nature of the chemical-physical environment, whereby light, temperature, nutrients and turbulence are the factors that have historically been associated with the control of phytoplankton growth (Reynolds, 2006; Smayda, 1980; Sommer 2012). The seasonal interplay of these parameters would cyclically shape the environment creating seasonal predictable conditions resulting in a gradual selection of certain species' niche or, in a broader view, a specific phytoplankton assemblage.

Empirical data collected by Margalef both in the lakes of Pyrenees and in Catalan Coastal waters inspired the first models and theories that integrated the physiological characteristics of phytoplankton species and environmental variability. Margalef noted that phytoplankton populations under certain environmental conditions tended to share a set of common characteristics and adaptive strategies, which provided the basis for the classification of phytoplankton into life-forms. These ideas were elegantly summarized in the well-known Margalef's *mandala* (Margalef, 1978; Margalef et al., 1979), which was developed in the 1970s but is still a source of inspiration for phytoplankton ecologists and keeps being regularly cited (e.g., Sellner et al., 2001). According to Margalef, community composition is shaped along an environmental gradient defined by nutrient concentration and turbulence. This approach provides a succession of species that are strategically adapted and selected along the gradient of decaying turbulence and nutrient levels, which is typical of the annual cycle of these environmental variables. In the *mandala*, where the main taxa are proxies of phytoplankton life-forms, fertile conditions (high turbulence and high nutrients concentration) would favour r-strategist and fast-growing species such as small-sized, colonial diatoms, whereas conditions of low turbulence (stratification) and

nutrient limitation would promote k-strategist species such as slow-growing and mobile forms like dinoflagellates and phytoflagellates. In mid-latitude systems, fertile and limiting environmental conditions typically recur cyclically, therefore there is an implicit temporality in the Margalef model which describes and justifies the recurrence and the periodicity of certain species over time.

A similar scheme was proposed by Grime (1977, 1979) for terrestrial plants and then adopted by Reynolds (1980, 1988) to describe the seasonal succession of freshwater phytoplankton. Reynolds explained phytoplankton community variability by the space created by two hard axes defining the availability of both energy (light) and resource (nutrients). Both energy and resource constraints manifest themselves under multiple driving factors, whose influence and temporal variability are relative to the observed system. Environmental constraints act as a filter on specific traits and adaptations of species, and regulate the persistence of a particular phytoplankton assemblage in a given time. Since sets of environmental parameters, in this context called 'habitat-templates' (Southwood, 1977), are seasonally recurrent, specific phytoplankton assemblages are recurrent too.

Initially developed to explain community assembly, the concept of 'habitat template' was then redefined also at the species level (Reynolds, 1998). The idea was to collect species-specific growth rates measured under controlled environmental conditions in the laboratory to map the performance of the species along the environmental gradient. Initially, Reynolds used 6 axes to represent gradients of habitat factors constituting the habitat-template: mean underwater irradiance, mixed layer depth, water temperature, filtration rate of zooplankton, carbon dioxide concentration and biologically available phosphorus. From growth performance measurements in relation to certain environmental variables, species were classified based on functional traits types, which are currently emerging as a useful practical and conceptual tool to explain and predict the seasonal occurrence of phytoplankton (Litchman & Klausmeier 2008; Litchman et al., 2012), and were successfully tested by Edwards et al. (2013) and Edwards (2016) in the English Channel, and more recently by Wentzky et al. (2020) in the Rappbode Reservoir (Germany). Although functional-traits represent a valuable step forward towards the understanding of phytoplankton successional patterns, there are some limitations that need to be addressed. Indeed, there is a growing literature showing that different phenotypic and genomic traits of cells grown in the laboratory can change over time (see Lakeman et al., 2009), which

implies that inferring natural species responses from laboratory conditions must be done cautiously. Furthermore, there is growing evidence that the diversity of marine protists is underestimated, and this diversity manifests itself in the form of a variety of cryptic species that may have different ecological characteristics while retaining identical morphological traits (e.g., Rynearson et al., 2020).

Although species-resolution time-series are still relatively few, new data and new patterns are emerging, and many steps forward have been made in describing the seasonality of phytoplankton in many areas. The temperature has often been associated with the phenological variability of phytoplankton, as it accelerates metabolic processes such as cell replication (Reynolds, 2006), and physically shapes the pelagic habitat by changing parameters generally related to the occurrence of the bloom. Therefore, many studies have focused on the effect of temperature increase on the timing of phytoplankton species occurrence, in most cases regarding the spring bloom. The general view is that the gradual increase in temperature implies an advancement of the phytoplankton species timing, especially in the case of diatoms. This hypothesis is supported by results obtained in different environments from mesocosm experiments in both freshwater and marine environments (Winder et al., 2012), as well as by Continuous Plankton Recorder (CPR) data from the North Sea (Chivers et al., 2019; Edwards & Richardson, 2004) and high-frequency flow-cytometer data in the Gulf of Maine (Hunter-Cevera et al., 2016). Yet, other studies showed contrasting results, with a fairly complex picture emerging from the attempts to identify the drivers of phytoplankton phenology. Indeed, in the effort to verify a potential trophic mismatch between primary and secondary producers in the English Channel (L4 station), Atkinson et al. (2015) found little evidence of the effect of temperature on phytoplankton timing. Similarly, in Helgoland waters (south-eastern North Sea), following a substantial change in abiotic parameters over 50 years of sampling, and an average increase of temperature of about 1.5 °C no change was found in the timing of phytoplankton biomass bloom (Wiltshire et al. 2008), nor a clear trend in the timing of single taxa (Scharfe & Wiltshire, 2019). Specifically, species occurring in the same seasonal periods showed a variety of different responses to different environmental drivers, and showed a non-homogeneous long-term phenological response with a bloom timing that could either anticipate or delay, or in some cases, showed no temporal variation (Scharfe & Wiltshire, 2019).

These conflicting results may be better interpreted in light of the results obtained from very detailed studies on the phenology of freshwater phytoplankton. Using a 48-year length time-series, Thackeray et al. (2008) showed in great detail how different taxa responded in different ways and extents to the same external drivers' changes. In particular, while the phenological variability of *Asterionella* was found to be associated with a nutrient enrichment process, the variability shown by *Cyclotella* was mostly driven by physical processes resulting in an advance in lake thermal stratification. The Authors suggested that the divergence of the phenological response between the two taxa was attributable to differences in both their physiology and morphology, which gave different feedbacks to changes in the nutrient regime and physical habitat respectively.

The examples above clearly illustrate how an in-depth knowledge of the ecology of individual species is essential for the understanding of phenological dynamics, and how such knowledge should be integrative of several aspects of phytoplankton biology, from physiology to morphology. The question that follows is whether species-specific phenological responses coincide in the space dimension, that is, whether the same species show similar phenological behaviours to the same drivers in different areas. To address this question, Feuchtmayr et al. (2012) investigated the phenological variability of 3 taxa in 4 different lakes for more than 50 years, and found that different environmental drivers influenced each taxon differently, whereas, considering each taxon separately, responses were surprisingly uniform between the different lakes. Interestingly, the temperature seemed to have a secondary effect in influencing the timing of taxa compared to nutrients regimes.

Despite the importance of marine phytoplankton phenology in the context of climate change, there are relatively few marine studies characterized by a degree of detail comparable to that of the research in freshwater environments. Nonetheless, while marine time-series are getting longer and longer, the availability of new data has laid the foundations for significant development in the methodologies used to characterize and trace the temporality associated with phytoplankton species. In fact, most of the studies dealing with phytoplankton species phenology are based on the identification of the annual date corresponding to the centre-of-gravity of the annual distribution. Although this method has proven to be successful in tracking long-term phenological variability, it is purely based on the shape of the statistical distribution of species abundance over time, and it is

uninformative of the 'hidden' biological dynamics underlying each sampling point. To the best of my knowledge, Beliaeff et al. (2001) were the first to conceive analytically the phenology of phytoplankton species as constituted by different phases: sudden growth, highest abundance and decline. They identified and integrated this ecologically-relevant information into a reference temporal unit, a 'phytoplankton event'. Further efforts in this direction have been made in studies based on daily records of over 50 years of sampling on the long-term research program Helgoland Roads (Grüner et al. 2011; Mieruch et al. 2010; Schlüter et al., 2012). Among these, a conceptually interesting approach is the fitness-based approach proposed by Grüner et al. (2011). This approach has its foundations on the concept of the ecological niche (*sensu* Hutchinson 1957), and aims to identify the observations in which the species show their highest fitness, analytically represented by the inflection points lying on species curve abundances.

In summary, despite its ecological importance and the extent of spatial and temporal scale of phenology in marine phytoplankton, we are still far from a sufficiently generalizable model targeted with the dual purpose of understanding current changes and forecasting future ones. However, the leitmotif of the studies described above is that the variety of phenological responses seems to be closely connected to the diversity of phytoplankton organisms and the range of their life cycles. Most of the studies focused on the vegetative phase of phytoplankton, the development of which may depend on triggers associated with the quiescence phase, as in the case of internal clocks in some dinoflagellates (Okamoto & Hastings, 2003; Sweeney, 1984), and photoperiod in diatoms (Eilertsen al., 1995; Eilertsen & Wyatt, 2000). Phenology can be conceived as the integrated manifestation of the life cycle of a species and the influence of the external environment (Forrest & Miller-Rushing, 2010). Besides continuing to collect data at long-term sites, the challenge and the intellectual and methodological efforts in this field in the coming years are to try to unveil the dynamics that lead to the prevalence of one component (life cycle or environment) over the other.

The progress made in recent years in the use of molecular techniques for the resolving of phytoplankton diversity represents a significant booster for the advancement in phenology. A higher taxonomic resolution allows to clean the aggregate signal of species not distinguishable with the classical light microscope, and to reveal more clearly the processes involved in the seasonality (Piredda et al., 2017; Rynearson et al., 2020). Although molecular time-series are currently relatively few and not long enough for long-term dynamics

investigation, first results revealed a widespread periodicity of the components of the eukaryotic phytoplankton (Kim et al., 2014; Lambert et al., 2019; Simon et al., 2015), comparable to that already observed in bacterial communities (see Fuhrman et al., 2015).

## 4.1.3    Aims

This Chapter takes up the most relevant results and conclusions from Chapter 2, and aims at applying new conceptual approaches and providing a further level of detail to investigate the phenology of individual taxa in relation to environmental variability. It is composed of two analytical parts. The aim of the first part is to describe the phenology of LTER-MC phytoplankton at the species level on a selection of ecologically and statistically informative taxa. Specifically, I extracted several phenological descriptors from 80 phytoplankton species and used them to investigate long-term changes and species phenological behaviours over 30 years of weekly sampling in the study area. Concurrently, I propose and describe a new set of methods conceived to extract the most relevant phenological descriptors of phytoplankton species (collected in `Rplanktonanalytic` R package). In the second part of this Chapter, I use the phenological descriptors identified in this study to test the relationship between species phenological behaviour and environmental variability. The hypothesis tested is that the timing of bloom initiation and development of individual species is strongly dependent on annual species-specific temporal windows (temporal niches), which prevail over the species response to environmental variability.

## 4.2 Materials and method

## 4.2.1    Taxa selection

The quantitative phytoplankton species dataset of LTER-MC consists of abundance data of 378 taxa characterized by a different level of taxonomic resolution. Most of them (more than 350) are classified at the species level, while others are classified as genera, aggregated taxa (based on supra-taxonomic groups and cell-size parameters) and other types of characterizations (phytoplankton cysts and spores). The aim of the selection applied in this study is to identify phytoplankton taxa that are well represented in the dataset, i.e., all the taxa that present stable populations in the area whose variability patterns are well identified by the overall sampling effort. I used as a starting point the same subset of taxa selected in Chapter 2 but I further discarded a number of them setting a higher relative frequency threshold, greater than 0.08%, in order to include taxa that had been virtually recorded at least 3 times per year. This choice was motivated by the specific aim of this study which is focused on the analysis of the phenology including the identification of the three main phenological phases that are, the start, the maximum and the end of bloom.

## 4.2.2    Characterization of taxa phenology

Generally, phytoplankton species follow a more or less regular unimodal or bimodal occurrence pattern during a solar year (see Chapter 2). It can be assumed that in the ascendant parts of the growth curve (Fig. 4.1) a given species has a net growth rate because there are all the conditions (biotic and abiotic) matching the realized niche of that species, whereas in the descendant part of the curve those conditions are no longer present. Therefore, even though at the beginning of the descendant curve the species is still present with high-density values, it is unlikely that the species' net growth rate has positive values, while high abundances are likely to be a 'residual' of the positive growth phase. According to these concepts, many Authors (Beliaeff et al., 2001; Grüner et al., 2011; Mieruch et al., 2010; Rolinski et al., 2007) successfully developed new methodologies to trace phytoplankton species phenology and their interannual variability.
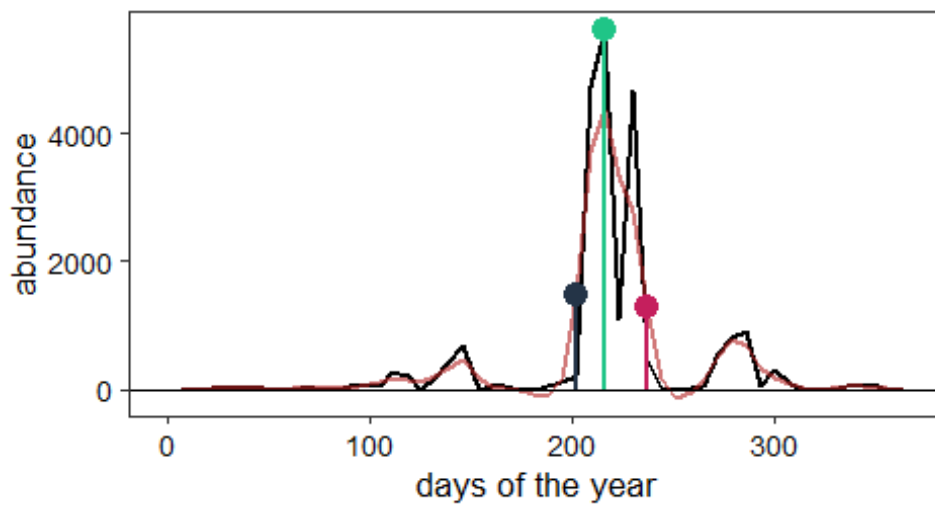
Figure 4.1 Illustration of the phenological phases of *Leptocylindrus danicus* in 2009. The black point is the date of bloom Start; the green point is the maximum abundance; the red point is the end of the bloom.

## 4.2.3    The 'Rplanktonanalytic' package

In collaboration with my external supervisor (Laurent Dubroca), I developed a set of functions useful for the identification of temporal observations associated with different phases of species phenology. The core of these functions is based on the calculation of species growth-rates by estimating the first derivative of the abundance in each sampling point. Furthermore, the package contains the R code useful to compute the timing of the seasonal peak according to the method proposed by Edwards and Richardson (2004), and a set of functions useful to manipulate phytoplankton time-series data in the R-software environment (R Core Team, 2013). All the functions' codes are available on GitHub at https://github.com/ldbk/Rplanktonanalytic.

According to the literature and concepts exposed in section 4.2.2., the annual temporal points having the highest first derivative (highest growth rate in that year) are supposed to reflect the optimal abiotic and biotic conditions experienced by a certain species, and are labelled as corresponding to the start of the bloom. Given the irregularity of several phytoplankton time-series (gaps in the series or heterogeneous sampling frequencies), we developed a set of useful function arguments in order to tune and customize the performance of bloom-time identification tools. In the developed system, besides the possibility to regulate the smoothing parameter (*s_param* argument), it is possible to set a quantile threshold calculated based on the non-zero abundance distribution of a certain species in order to consider as starting-bloom points only those dates when a certain abundance (*ab_treshold* argument) was exceeded. It is also possible to set the minimum number of informative samples to be considered to run the analysis, calculated as the minimum number of samples for each year in which a species showed a non-zero abundance (*obs_year* argument). A very useful argument of the system here developed is *control*, which adds more flexibility to the identification of the start of the bloom. Specifically, this argument takes the maximum and the other highest growth rate values of a year and compare them. If a point has a growth rate very close to the maximum calculated growth rate (MAXgr) and occurs earlier than MAXgr in the year, it will be considered as the probable starting point of the bloom. Specifically, a point is considered very close to MAXgr when its first derivative exceeds the following value: $\mathbf{MAXgr} - (\mathbf{MAXgr} \times \mathbf{control})$

I used abundance data of *Paulinella ovalis* in 2010 to provide a visual example of the functioning of *control* argument (Fig. 4.2). In addition to the bloom-start point, the set of functions returned the dates corresponding to the maximum of the year (as the maximum abundance value after the bloom-start point) and those at the end of the bloom. The latter was identified as the point after the 'Max' that is followed by a prolonged phase (7% of annual observations) of relatively low abundances (abundance values less than the 33$^{rd}$ percentile of the distribution of non-zero abundance of the species in that year). After the visual inspection of several annual cycles of different taxa, I found that the following tuning well-described species' phenology phases: *s_param*=0.4, *control*=0.45, *ab_treshold*=0.25.
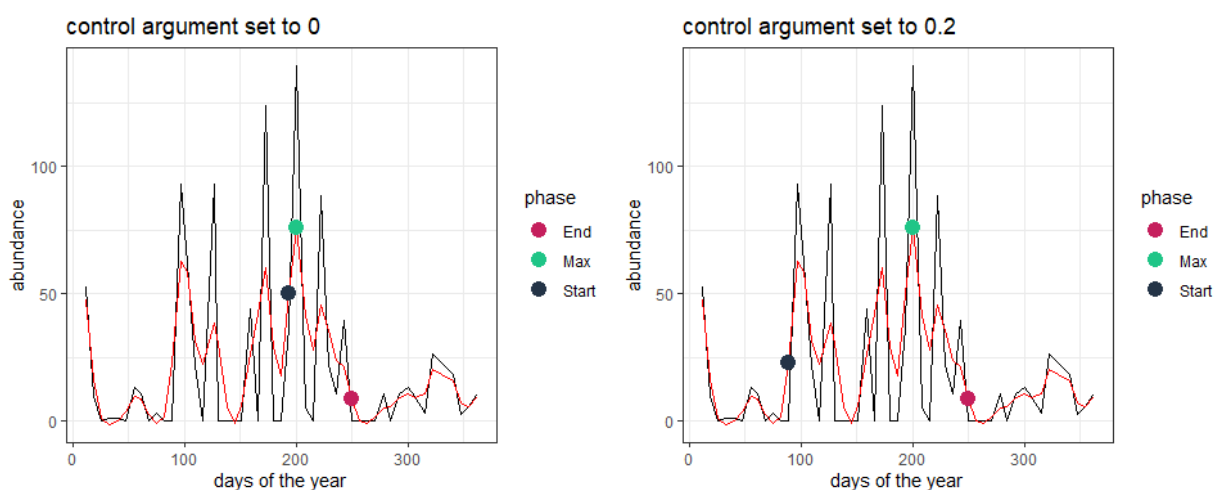


Figure 4.2 Sensitivity of the detection of the start of the bloom of *Paulinella ovalis* (black points) using different control parameters.

The parameters of the functions developed for the identification of phenological phases provide a useful support for the users to customize the analysis according to their objectives. Most of the methods developed and used for the identification of phenological phases in the marine environment are based on aggregate measurements, generally chlorophyll and, from a methodological point of view, on indices based on abundance/biomass' thresholds or on integrative properties such as the centre-of-gravity method (Ji et al., 2010).

Nevertheless, the mechanisms underlying species-specific phenological responses can be different from biomass responses, and the use of the centre-of-gravity can prove to be ineffective and not sensitive when the goal is to characterize the environmental parameters

associated with well-defined phenological phases as those of maximum growth-rate and decline. Also, time series of individual species are generally characterized by quite different properties from those of chlorophyll, indeed, they generally appear as strongly right-skewed signals, quite noisy, and characterized by scattered data points. Approaches based on the rate of change as those proposed by Rolinski et al. (2007) and Mieruch et al. (2010) are certainly among the most flexible and probably the most suitable methods for characterizing phenology at the level of individual species however, an effort to integrate the methodology of the above-mentioned approaches into an analytical unit such as an R package has not yet been made.

I used the methods described above as a baseline to extract a set of informative phenological attributes for each selected species in the LTER-MC dataset. Specifically, I extracted 8 attributes (by *get_pheno* function) related to both phenological and ecological properties of the species (Tab. 7). Then, I performed a PCA on the matrix of species phenological (inter_start_var, mean_duration, duration_trend, start_trend) and ecological properties (mean_abundance, magnitude, rel_freq) to characterize the main ecological behaviours and the main prominent patterns in species phenological variability at LTER-MC. Finally, in order to identify species showing similar phenological behaviour and long-term variability, a cluster analysis was performed using a complete linkage on Euclidean distances based on PCA results.

Table 7 Phenological and ecological attributes of the species blooms returned by the **get_pheno** function.

| attribute code | significance | details |
| --- | --- | --- |
| mean_month | mean month of bloom start | mode of months associated to bloom start |
| mean_abundance | mean abundance | average of species abundance |
| mean_max | mean max abundance | average of species abundance at yearly maxima |
| rel_freq | relative frequency | relative frequency of species in the dataset (%) |
| magnitude | bloom magnitude | average bloom magnitude (first derivative) at the start |
| inter_start_var | bloom-start interannual variability | standard deviation of annual bloom-start days |
| inter_max_var | bloom-max interannual variability | standard deviation of annual bloom-max days |
| mean_duration | average bloom duration | average of annual bloom duration (days) |
| duration_trend | bloom duration interannual trend | Sen's slope of bloom duration |
| start_trend | bloom-start interannual trend | Sen's slope of bloom-start days |
| max_trend | bloom-max interannual trend | Sen's slope of bloom-max days |
| pval_duration | significance of bloom duration trend | p-value of the trend |
| pval_start | significance of bloom-start trend | p-value of the trend |
| pval_max | significance of bloom-max trend | p-value of the trend |

# 4.2.4    Long-term    phenological    changes    and    environmental variability

Based on the results of Chapter 2 and 3, where different environmental parameters showed seasonal trends, I tested the hypothesis of a dependence between species phenological shifts and seasons of the year. Specifically, I performed an ANOVA using phenological indicators as a response variable and seasons as independent and predictor groups.

I performed a random forest classification model (Breiman, 2001) to assess the importance of environmental parameters in predicting species bloom-start points. Random forest is a machine learning method used in a wide variety of applications, from image recognition to finance, health and banking sectors (Boulesteix et al., 2012; Akar et al., 2012; Liu et al., 2015). A random forest model is based on the combined use (ensemble method) of multiple decision trees which, given a set of predictors (features), have the aim to classify the value of a response variable (target). A decision tree has a tree structure where at each node a test is performed on a feature, each branch representing the outcome of the test, and each terminal node (leaf) being associated with the value of the response variable. The tree is therefore trained by the division of the dataset into subsets obtained by the tests performed on the features. This recursive process is completed when each leaf of each subset has a value coinciding with the target variable or when further subdivisions do not add value to the prediction. Random forest models use the predictive power of multiple and diverse decision trees (here the term random forest). Besides performing the prediction, random forest models offer a quantification of the importance of a certain feature by comparing the difference in the prediction accuracy obtained by using or not that feature in the model. Since each decision tree in a random forest model is different from the other, the importance that a feature has in predicting the target value is quantified on different subsets and in relation to different combinations of features, which makes the result quite robust (Breiman, 2001).

In this study, I performed a random forest model set on 500 trees, and I used surface nutrient concentrations ($NO_3$, $NO_2$, $NH_4$, $SiO_4$, $PO_4$), temperature, salinity, radiance (MJ/ $m^2$), day length and Julian day as predictors (the features) of the beginning of the bloom of each species. Furthermore, I tested the statistical significance of the importance of each feature using 5000 permutations. This procedure consists of reshuffling the values of a feature n times in the model (5000 permutations in this work) and calculating the increase in prediction error. The test is based on the assumption that, if a feature is important, reshuffling its values will have the effect of increasing the prediction error because the model relied on that feature for the prediction. Conversely, if a feature is not important, the model 'ignores' that feature for prediction and its value reshuffling will not affect the prediction error.

In order to test the difference in the habitat experienced by the species during the beginning and the end of the bloom, I performed a univariate PERMANOVA (Anderson, 2001; Anderson, 2014) for each of the predictors described above. I used Euclidean distances for each of the environmental parameters and considered the start and the end of the bloom as a conditional factor. The analysis was performed for each species under the theoretical assumption that environmental parameters showing a relatively greater difference between the beginning and end of the bloom would be more important in describing the dynamics of the bloom of a species than those with negligible differences. Furthermore, in order to look for common patterns of environmental variation between the start and end of the bloom, I clustered LTER-MC species according to the annual median difference of environmental parameters experienced at the start and end of the bloom. The clustering analysis was performed on Euclidean distances using the Ward method and the number of optimal clusters was estimated using the gap statistic method (Tibshirani et al., 2001)

# 4.3 Results

## 4.3.1    Temporal    succession    and    species phenological properties

Using a relative frequency threshold higher than 0.08% and the judgement of expert taxonomists (Adriana Zingone and Diana Sarno) 80 taxa were selected from a total of 378 (Tab. 8). Most of the analysed taxa started their bloom from late-winter through early summer, but blooms of a good number of them occurred from mid-summer through late-autumn (Fig. 4.3). On the whole, the bloom-start dates of the selected species cover the entire year, with quite regular occurrences in defined time windows which provide a complete picture of the species succession during a typical year at LTER-MC (Fig. 4.4).
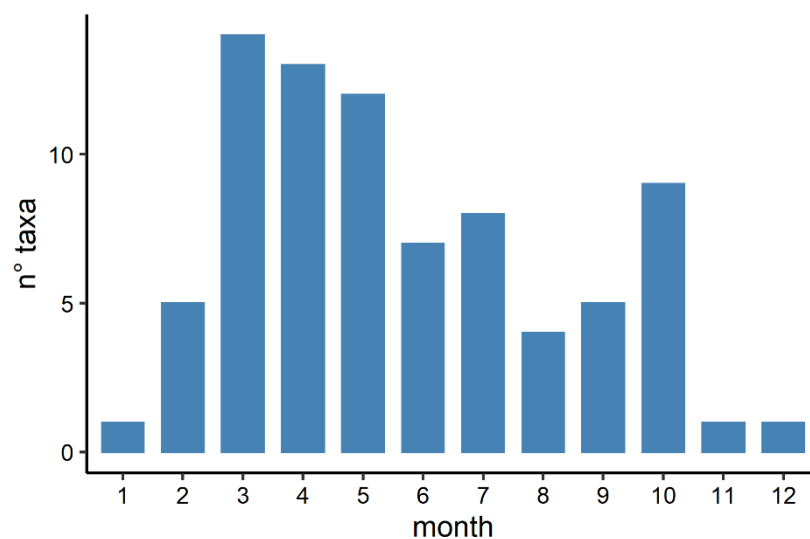


Figure 4.3 Barplot showing the month (mode) of taxa bloom start.

Table 8 Phenological and ecological attributes returned by the get_pheno function for each species. Empty spaces in the last three columns indicated no-significance (pvalue>0.05) of the trend. Colours define clusters according to species' similar ecological and phenological behaviour (see Figs 4.5 – 4.6).

| species | rel_freq | mean_abundance | mean_max | magnitude | inter_start_var | inter_max_var | mean_duration | duration_trend | start_trend | max_trend | cluster |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Emiliania huxleyi | 81.83 | 110.29 | 1163.97 | 45.79 | 66.35 | 48.96 | 127.36 | | | | 2 |
| Chaetoceros tenuissimus | 80.41 | 869.82 | 12793.45 | 286.99 | 53.27 | 59.40 | 135.50 | | | | 4 |
| Leptocylindrus danicus | 75.65 | 513.68 | 6008.09 | 162.06 | 48.10 | 46.97 | 125.71 | | 3.35 | 3.94 | 4 |
| Cylindrotheca closterium | 73.91 | 149.27 | 3118.83 | 61.13 | 51.61 | 47.93 | 109.04 | | | | 2 |
| Ollicola vangoorii | 72.94 | 50.73 | 519.17 | 10.58 | 31.43 | 56.65 | 185.38 | | | | 2 |
| Leucocryptos marina | 64.45 | 27.19 | 294.32 | 6.85 | 33.12 | 34.02 | 134.83 | 7.78 | | | 2 |
| Chaetoceros socialis | 60.62 | 395.91 | 6019.12 | 134.43 | 77.62 | 72.13 | 64.08 | | | | 2 |
| Pseudoscourfieldia marina | 58.17 | 42.14 | 446.46 | 11.70 | 35.75 | 35.75 | 129.26 | | | | 2 |
| Paulinella ovalis | 53.92 | 16.60 | 173.73 | 3.90 | 43.61 | 57.61 | 72.55 | | | | 2 |
| Pseudo-nitzschia galaxiae | 52.09 | 198.79 | 3642.85 | 78.78 | 48.64 | 52.02 | 94.67 | | | | 2 |
| Skeletonema pseudocostatum | 50.73 | 753.15 | 13347.11 | 376.82 | 36.79 | 37.00 | 106.08 | | | | 4 |
| Skeletonema menzelii | 46.28 | 196.24 | 3744.71 | 108.01 | 64.46 | 60.96 | 64.59 | | | | 2 |
| Cerataulina pelagica | 43.16 | 48.88 | 982.04 | 23.33 | 29.36 | 22.81 | 100.29 | | | | 2 |
| Pseudo-nitzschia pseudodelicatissima | 37.61 | 40.67 | 491.85 | 12.78 | 51.81 | 72.21 | 85.29 | | | | 2 |
| Leptocylindrus convexus | 37.17 | 52.49 | 565.36 | 13.42 | 39.69 | 69.62 | 120.00 | | | | 2 |
| Bacteriastrum parallelum | 32.94 | 157.53 | 3898.50 | 96.80 | 57.93 | 54.38 | 66.40 | | | | 2 |
| Dactyliosolen fragilissimus | 30.39 | 13.71 | 192.40 | 4.55 | 67.60 | 64.96 | 68.96 | | | | 1 |
| Pseudo-nitzschia galaxiae "small morphotype" | 28.38 | 13.20 | 198.97 | 4.77 | 58.34 | 55.91 | 65.85 | | | | 1 |
| Calciopappus caudatus | 27.32 | 10.34 | 148.94 | 3.32 | 29.99 | 31.52 | 71.71 | | | -1.89 | 1 |
| Dinobryon faculiferum | 26.38 | 11.83 | 149.40 | 3.50 | 36.74 | 38.73 | 77.08 | -2.00 | | | 1 |
| Chaetoceros "curvi-curvi" | 26.10 | 50.92 | 787.45 | 20.04 | 46.77 | 69.15 | 104.55 | | | | 2 |
| Chaetoceros simplex | 25.83 | 41.18 | 683.41 | 15.10 | 29.73 | 35.69 | 79.65 | 3.50 | -3.31 | | 2 |
| Chaetoceros curvisetus | 25.19 | 21.87 | 358.47 | 9.02 | 90.31 | 90.82 | 61.05 | | | | 3 |
| Minidiscus comicus | 24.85 | 109.99 | 2458.23 | 54.57 | 74.62 | 79.30 | 49.67 | | -8.22 | -8.00 | 3 |
| Meringosphaera mediterranea | 24.79 | 3.26 | 53.45 | 1.33 | 48.69 | 62.48 | 78.95 | | | | 1 |
| Thalassionema nitzschioides | 24.47 | 12.32 | 254.45 | 5.73 | 59.15 | 55.74 | 52.86 | | 3.89 | | 1 |
| Syracosphaera pulchra | 23.64 | 4.99 | 96.68 | 2.31 | 60.54 | 66.89 | 65.48 | | | | 1 |
| Chaetoceros minimus | 22.94 | 30.07 | 589.54 | 12.99 | 112.80 | 108.22 | 54.93 | | | | 3 |
| Pseudo-nitzschia multistriata | 21.52 | 11.41 | 208.15 | 5.10 | 52.58 | 41.53 | 60.79 | | | | 1 |
| Dactyliosolen blavyanus | 21.14 | 6.72 | 134.00 | 3.53 | 55.86 | 45.52 | 67.44 | | | | 1 |
| Oxytoxum variabile | 18.83 | 1.65 | 31.53 | 0.61 | 50.98 | 59.24 | 79.43 | | | | 1 |
| Syracosphaera molischii | 18.59 | 1.97 | 40.28 | 0.96 | 56.24 | 65.45 | 52.93 | | | | 1 |
| Chaetoceros single sp.1 | 17.99 | 225.58 | 6252.34 | 241.53 | 41.43 | 43.11 | 48.83 | | | | 2 |
| Tenuicylindrus belgicus | 17.75 | 23.01 | 583.67 | 13.11 | 18.30 | 22.36 | 56.05 | | -1.25 | | 1 |
| Proboscia alata | 17.70 | 2.98 | 55.32 | 1.03 | 46.76 | 44.75 | 53.83 | | | | 1 |
| Minutocellus polymorphus | 17.65 | 106.32 | 2959.42 | 89.97 | 37.53 | 39.77 | 51.33 | | | | 1 |
| Chaetoceros affinis | 17.56 | 13.35 | 255.53 | 5.25 | 67.18 | 68.19 | 45.69 | | | | 3 |
| Pseudo-nitzschia fraudulenta/subfraudulenta | 16.93 | 7.46 | 120.37 | 2.84 | 43.09 | 46.53 | 48.60 | | | | 1 |
| Thalassiosira rotula | 16.89 | 9.26 | 218.49 | 4.39 | 44.59 | 75.75 | 60.25 | | | | 1 |
| Chaetoceros throndsenii | 16.49 | 94.21 | 2492.48 | 55.19 | 18.33 | 16.69 | 40.00 | | | | 1 |
| Umbilicosphaera sibogae | 16.49 | 0.19 | 2.00 | 0.05 | 21.03 | 32.72 | 69.00 | | | | 1 |
| Calciosolenia brasiliensis | 15.94 | 1.80 | 33.53 | 0.84 | 43.77 | 54.28 | 65.83 | | | | 1 |
| Asterionellopsis glacialis | 15.68 | 12.37 | 266.72 | 6.58 | 42.28 | 31.75 | 46.71 | | | | 1 |
| Rhabdosphaera clavigera | 15.65 | 1.38 | 22.79 | 0.60 | 61.48 | 71.63 | 69.27 | | | | 1 |
| Thalassionema bacillare/frauenfeldii | 14.93 | 3.01 | 49.45 | 1.28 | 70.32 | 69.32 | 38.25 | | | | 3 |
| Prorocentrum triestinum | 14.82 | 7.50 | 155.67 | 3.35 | 23.26 | 34.69 | 54.67 | | | | 1 |
| Acanthoica quattrospina | 14.77 | 0.87 | 19.67 | 0.43 | 90.78 | 89.80 | 61.00 | | | | 3 |
| Ophiaster spp. | 14.69 | 0.64 | 11.49 | 0.25 | 73.84 | 68.18 | 66.94 | | | | 3 |
| Chaetoceros contortus | 14.18 | 31.40 | 716.09 | 18.61 | 14.44 | 16.93 | 45.17 | | | | 1 |
| Chaetoceros costatus | 14.05 | 10.99 | 178.50 | 4.27 | 67.87 | 64.52 | 86.00 | | | | 1 |
| Chaetoceros protuberans | 13.86 | 6.79 | 144.09 | 2.74 | 53.61 | 86.10 | 67.00 | | | | 1 |
| Dactyliosolen phuketensis | 13.77 | 3.75 | 101.43 | 2.25 | 52.87 | 60.86 | 47.67 | | | | 1 |
| Dictyocha fibula | 13.55 | 0.38 | 7.36 | 0.15 | 62.20 | 50.77 | 76.76 | | | | 1 |
| Eutreptiella spp. | 13.33 | 14.09 | 513.98 | 5.69 | 53.30 | 61.63 | 46.44 | | | | 1 |
| Lauderia annulata | 13.33 | 2.62 | 55.80 | 1.27 | 63.02 | 83.25 | 64.80 | | | | 1 |
| Apedinella radians | 13.25 | 1.81 | 47.69 | 1.21 | 79.28 | 78.05 | 50.08 | | | | 3 |
| Chaetoceros diversus | 13.23 | 53.20 | 1736.36 | 43.03 | 20.37 | 16.30 | 38.25 | | | | 1 |
| Lessardia elongata | 13.21 | 2.59 | 52.41 | 1.07 | 44.87 | 42.39 | 45.28 | | | -5.43 | 1 |
| Bacteriastrum furcatum | 13.19 | 12.83 | 430.99 | 11.35 | 10.76 | 16.74 | 41.00 | | | | 1 |
| Chaetoceros decipiens | 13.09 | 3.71 | 81.88 | 1.75 | 103.31 | 102.74 | 26.89 | | | | 3 |
| Guinardia striata | 12.96 | 2.84 | 65.65 | 1.53 | 28.77 | 31.33 | 43.65 | | | | 1 |
| Chaetoceros wighamii | 12.42 | 54.97 | 1345.78 | 34.12 | 53.27 | 55.27 | 31.86 | | 19.25 | 2.00 | 1 |
| Diplostauron cf. elegans | 12.29 | 4.77 | 110.39 | 2.42 | 70.11 | 61.75 | 57.64 | | | | 3 |
| Thalassiosira mediterranea | 12.20 | 12.34 | 319.56 | 8.42 | 53.37 | 47.80 | 46.46 | | | | 1 |
| Chaetoceros diadema | 12.08 | 14.98 | 426.69 | 10.83 | 68.36 | 70.46 | 31.53 | | | | 3 |
| Dinobryon coalescens | 11.68 | 13.53 | 482.79 | 9.23 | 23.91 | 28.83 | 48.75 | | | | 1 |
| Coronosphaera mediterranea | 11.56 | 0.31 | 4.43 | 0.13 | 38.23 | 114.08 | 102.00 | | | | 1 |
| Lithodesmium cf. variabile | 11.54 | 4.10 | 122.97 | 2.05 | 56.44 | 53.15 | 30.60 | | | | 1 |
| Chaetoceros pseudocurvisetus | 11.50 | 5.14 | 124.54 | 2.82 | 102.02 | 112.17 | 36.00 | | | | 3 |
| Sphaerocalyptra quadridentata | 11.38 | 2.71 | 60.09 | 1.52 | 24.12 | 29.33 | 38.57 | | | | 1 |
| Heterocapsa niei | 11.32 | 3.32 | 76.67 | 1.86 | 28.51 | 28.47 | 31.45 | | | | 1 |
| Eucampia zodiacus f. cylindricornis | 11.08 | 2.04 | 52.71 | 1.48 | 56.00 | 67.65 | 39.71 | | | | 1 |
| Solenicola setigera | 10.95 | 4.77 | 112.62 | 2.72 | 56.85 | 56.75 | 37.86 | 7.50 | | | 2 |
| Chaetoceros peruvianus | 10.76 | 0.76 | 19.36 | 0.46 | 85.64 | 55.23 | 22.89 | | | | 3 |
| Leptocylindrus mediterraneus | 10.66 | 0.62 | 18.19 | 0.37 | 14.81 | 27.00 | 46.83 | | | | 1 |
| Skeletonema tropicum | 10.51 | 7.17 | 184.48 | 5.34 | 24.12 | 20.38 | 38.43 | | | | 1 |
| Chaetoceros anastomosans | 10.34 | 6.46 | 164.85 | 4.57 | 69.60 | 84.87 | 58.89 | | | | 3 |
| Protoperidinium bipes | 9.94 | 1.24 | 22.33 | 0.54 | 76.87 | 78.23 | 40.29 | | | | 3 |
| Lioloma spp. | 9.54 | 0.74 | 14.70 | 0.42 | 53.38 | 60.63 | 35.20 | -9.42 | | | 3 |
| Algirosphaera robusta | 8.81 | 0.18 | 3.61 | 0.10 | 55.33 | 44.18 | 47.25 | | | | 1 |
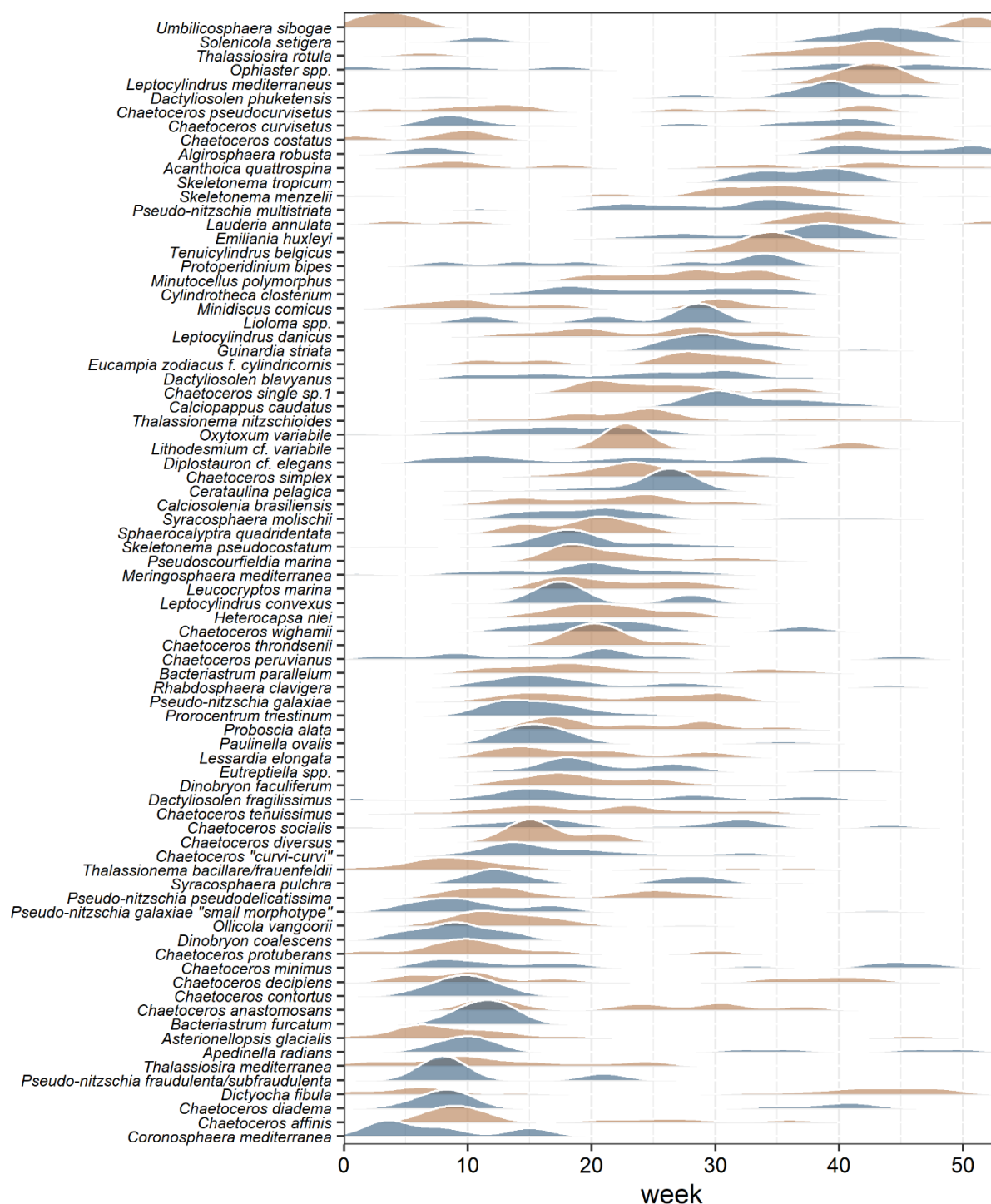
Figure 4.4 Kernel density distribution of bloom-start weeks of each phytoplankton taxa at LTER-MC.

According to the phenological and ecological attributes considered, the PCA explained 58% of total species phenological variability (Fig. 4.5). The first axis was the most representative (40.5% of explained variation) and was mostly correlated with the ecological properties of the species such as their relative frequency (rel_freq), bloom magnitude (magnitude) and average abundance (mean_abundance), as well as with some phenological properties such as the average duration (mean_duration). The second axis (17.4% of explained variation), on the other hand, is representative of the long-term phenological variability and is correlated with both the interannual variability of the start of the bloom (int_start_var), and the trends of the start of the bloom (start_trend) and bloom-duration (duration_trend). According to the angles formed by the variables in the PCA representation, species mean abundance (mean_abundance) and their propensity for rapid growth (magnitude) were highly correlated, and most abundant species were associated with higher relative frequency and longer bloom duration (duration). These attributes were very little or not at all related to the ones characterizing long-term phenological variability.

Cluster analysis of the phenological multidimensional space identified 4 clusters, with groups 1, 2 and 3 including most of the species and group 4 only three species. Cluster 1 and 3 were accounted together for a large number of species (51) despite a low dispersion in the multi-dimensional space. These clusters identified temporally regular species as *Skeletonema tropicum* and *Tenuicylindrus belgicus* (cluster 1), characterized by relatively low abundances and short blooms, and with negligible long-term variability (Tab. 8, Fig. 4.6), whereas cluster 3 defined species which showed high variability in both the bloom-start timing and annual maxima as *Chaetoceros decipiens*, , *C. pseudocurvisetus* and *C. minimus*. Cluster 2 was characterized by a quite wide multidimensional dispersion and included species among the most abundant, frequent, and characterised by long-lasting blooms such as *Chaetoceros socialis*, *Emiliania huxleyi* and *Ollicola vangoori* (Tab. 8). Cluster 4 identified the species with the highest abundance and bloom-magnitude of the LTER-MC pelagic system, i.e., *Leptocylindrus danicus*, *Chaetoceros tenuissimus* and *Skeletonema pseudocostatum* (Tab. 8). The variability in long-term phenological descriptors was identified in all clusters. Indeed, some species of cluster 2 (e.g., *Leucocryptos marina* and *Chaetoceros simplex*) showed a long-term phenological variability in the form of a significant and positive trend in bloom duration, while *Thalassionema nitzschioides* (cluster 1) and *Leptocylindrus danicus* (cluster

4) showed a long-term delayed trend in bloom timing (Tab. 8). Figure 4.7 summarises the most emblematic species for each cluster and their interannual phenological phases.
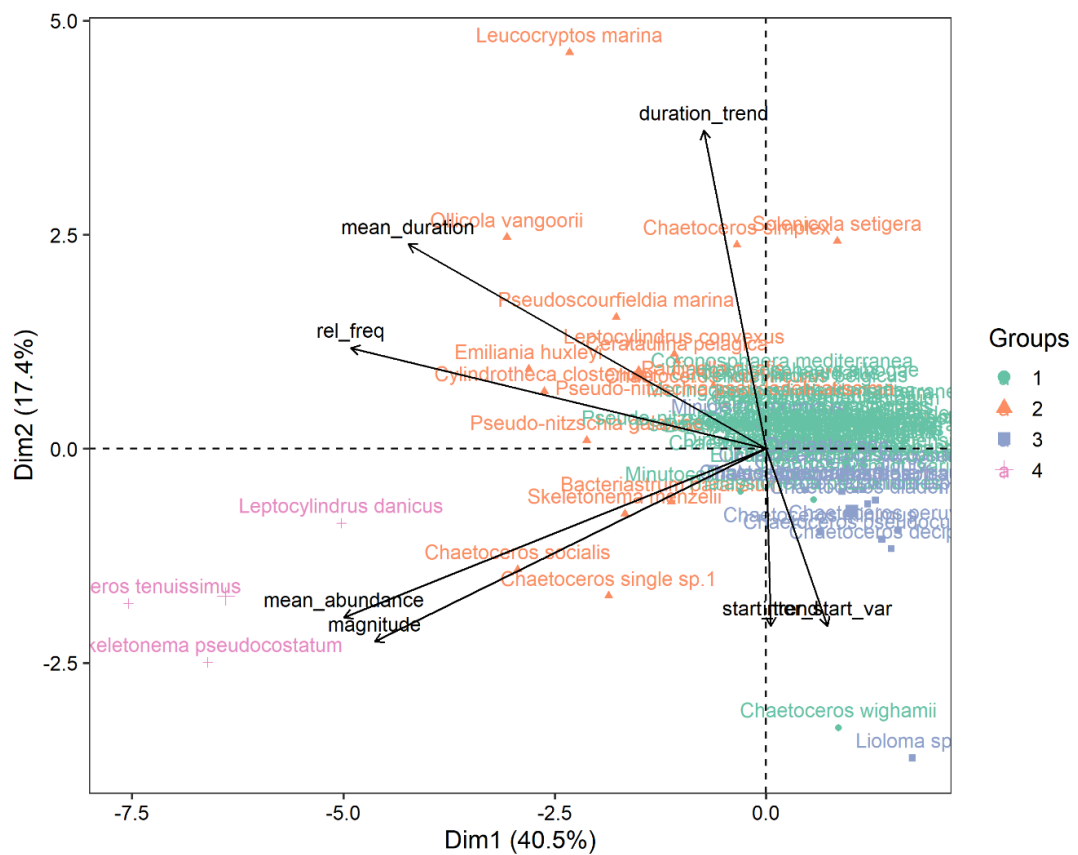


Figure 4.5 Principal component analysis of species abundance, relative frequency, and phenological attributes (see Tabs. 7 and 8).
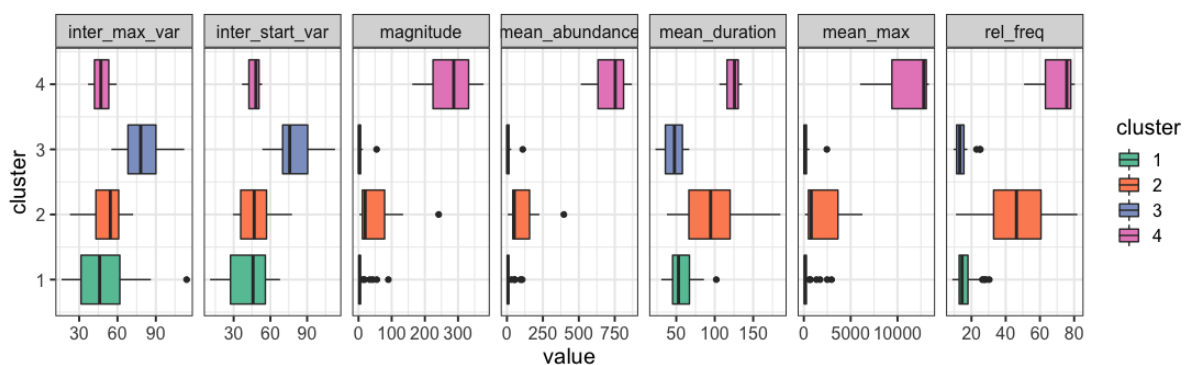


Figure 4.6 Distribution of ecological and phenological attributes' properties according to the clusters defined in the PCA (Fig. 1.5).
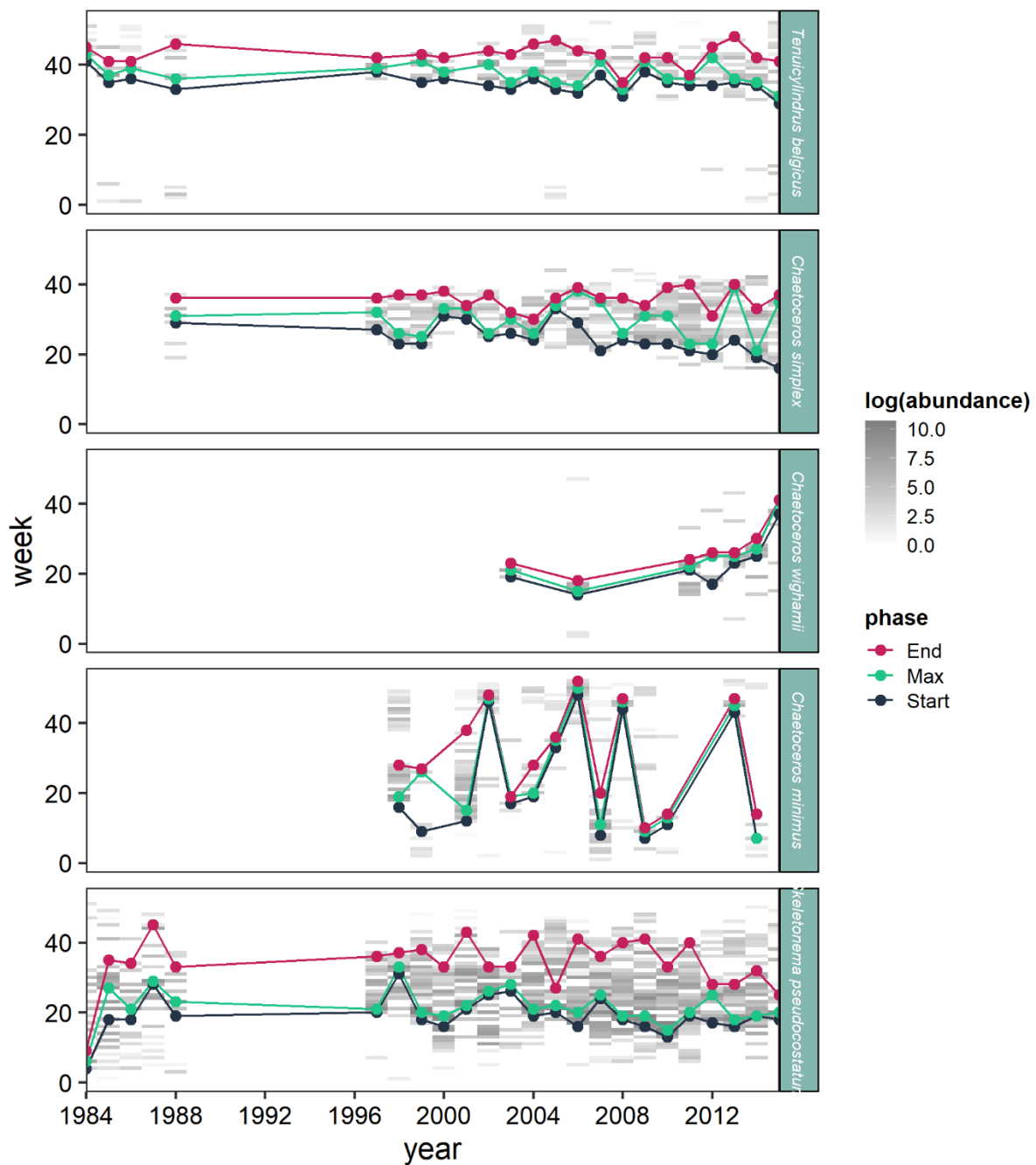
Figure 4.7 Emblematic species of the phenological variability at LTER-MC, from the top to the bottom: *Tenuicylindrus belgicus*, a species with a regular bloom timing; *Chaetoceros simplex* and *Chaetoceros wighamii*, examples of long-term trends in bloom duration and timing respectively; *Chaetoceros minimus*, a long-term timing-variable species; *Skeletonema pseudocostatum*, a very abundant species characterized by high bloom magnitudes and duration, as well as consistent timing over the years.

# 4.3.2    Long-term changes and environmental variability

According to PCA results, relatively few species showed long-term variability in phenological characteristics. A significant trend was found in the bloom timing of only six species, of which *Chaetoceros simplex*, *Minidiscus comicus* and *Tenuicylindrus belgicus*, showed an average advance of -3.31, -8.22 and -1.25 days per year, while *Chaetoceros wighamii*, *Leptocylindrus danicus* and *Thalassionema nitzschioides* showed an average delay in the timing of 19.25, 3.35 and 3.88 days, respectively. *Chaetoceros simplex* showed a significant positive trend also in the duration of the bloom with an average increase of 3.5 days each year, and also *Leucocryptos marina* and *Solenicola setigera* showed an average extension of their bloom of 7.77 and 7.5 days per year respectively. By contrast, *Dinobryon faculiferum* and *Lioloma* spp. showed an average reduction in bloom duration of -2 and -9.04 days per year respectively. Results from ANOVA performed to investigate the seasonal-dependence of long-term phenological variability indicated no significant seasonal effects in any of the phenological descriptors used (Tab. 9).

Table 9 ANOVA results of the seasonal effect on the phenological attributes of phytoplankton taxa.

| attribute | Sum.Sq | Mean.Sq | F.value | pvalue |
|---|---|---|---|---|
| *bloom-start interannual variability* | *3081.18* | *1027.06* | *2.41* | *0.07* |
| *bloom-start interannual trend* | *12.11* | *4.04* | *0.65* | *0.58* |
| *bloom-max interannual trend* | *7.78* | *2.59* | *0.39* | *0.76* |
| *bloom duration interannual trend* | *12.37* | *4.12* | *1.53* | *0.21* |

Considering the complexity of the dataset, the results of the random forest models performed on each species were characterized by an overall good accuracy. The models correctly classified more than 60% of the observations for each species, and it reached an accuracy of around 90% for very regular species such as the cases of *Cerataulina pelagica*, *Paulinella ovalis* and *Bacteriastrum furcatum* (Fig. 4.8). However, the models did not have the same performance when considering only the observations associated with the start of the blooms. Although models correctly classified more than 50% of the beginning of the bloom in most of the species, others were below 50% and some did not exceed 30%. Less regular species were the ones associated with model misclassification, including several less abundant species belonging to the genus *Chaetoceros* such as *C. pseudocurvisetus*, *C. decipiens* and *C. anastomosans*, but also some of the most frequent and abundant ones such as *C. tenuissimus* and *C. socialis*.

The models indicated that the photoperiod was the main factor associated with a correct classification of the observations followed by temperature and radiance, while salinity, nutrients and zooplanktonic biomass would seem to have a minor role in classifying correctly the beginning of the bloom of each species (Fig. 4.8). The significance test conducted on each model with 5000 permutations on each predictor identified that 50 out of 80 species considered had a statistically significant association with predictors. For most of the species examined, the photoperiod, radiance and temperature were significant in improving the performance of the model (Fig. 4.9). Similarly, the Julian day was significant for many species (22) although its removal from the models resulted in a minor overall error relative to the other significant predictors. Instead, nutrients, as well as salinity and zooplankton biomass had a significant effect on the model performance for very few species.
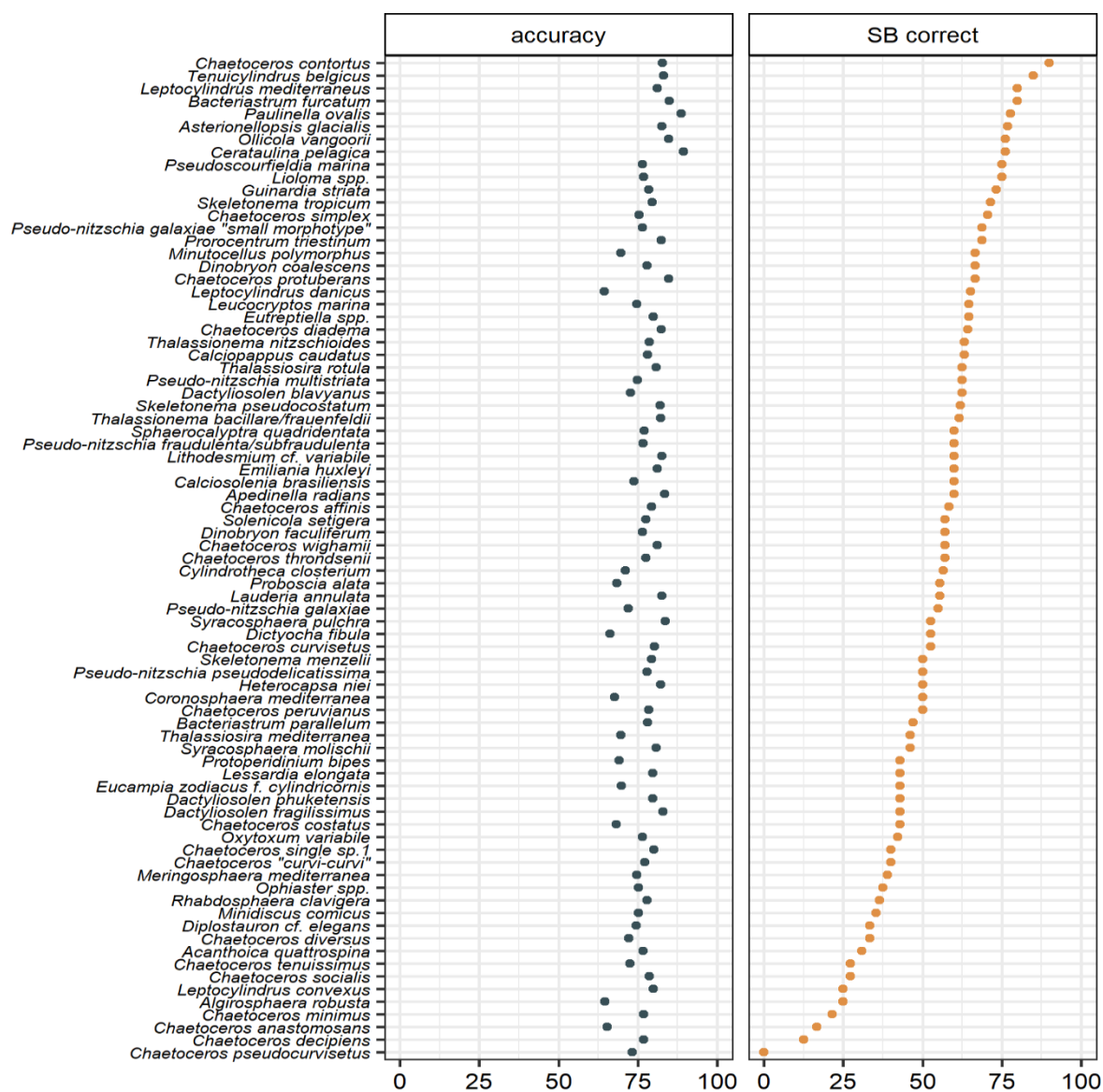
Figure 4.8 Summary of random forest model performance for each species. Accuracy indicates the proportion of the samples that were correctly classified for each species. SB correct indicates the proportion of 'start bloom' samples correctly classified for each species.
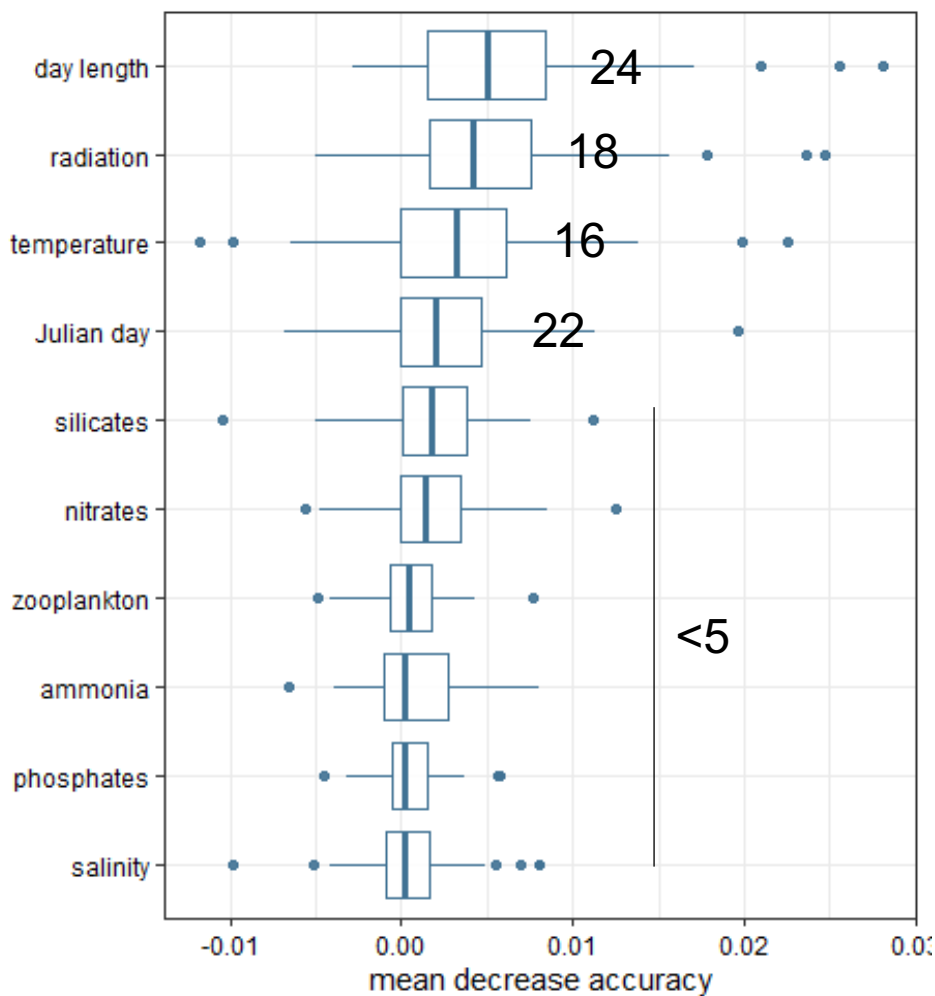
Figure 4.9 Mean decrease accuracy of features used in the random forest model. Numbers indicated the number of species for which the corresponding feature resulted significant in the overall performance of the model.

The results of PERMANOVA (Fig. 4.10) performed to test a significant variation in the distribution of predictors between the beginning and end of the bloom revealed that the nutrients and biomass of zooplankton did not seem to define the phenological phases of LTER-MC species. Conversely, photoperiod, radiation and temperature were the parameters that most characterized the difference between the environment experienced by each species between the beginning and the end of the bloom.

The cluster analysis performed on parameters difference between the start and end of the bloom identified 3 main clusters characterized by common patterns of parameters variation. Cluster 1 included most of the species (Fig. 4.11) and was mainly composed of spring and

early summer species such as many representatives of the genus *Chaetoceros* including *C. socialis* and *C. tenuissimus*, and other representative species of the LTER pelagic system such as *Skeletonema pseudocostatum* and *Paulinella ovalis*. Except for temperature which seemed to be slightly warmer at the end of the bloom than at the beginning, the species belonging to cluster 1 did not experience a notable difference in the environment between the beginning and the end of the bloom (Fig. 4.12). Groups 2 and 3, on the other hand, experienced a mirrored trend in the change of environmental conditions between the beginning and end of bloom phases. Cluster 2 species occurring in late summer and autumn (e.g., *Emiliania huxleyi*, *Leptocylindrus danicus*, *Cylindrotheca closterium* and *Cerataulina pelagica*) at the end of the bloom experienced an environment characterized by temperature, photoperiod and radiance lower than in the beginning phase while nutrients followed an opposite trend. Conversely, cluster 3 was composed of typically winter and early-spring species (e.g., *Coronosphaera mediterranea*, *Apedinella radians*, *Asterionellopsis glacialis* and *Thalassiosira mediterranea*), for which the bloom demise occurred when the temperature and the light levels were higher compared to the bloom start phase.
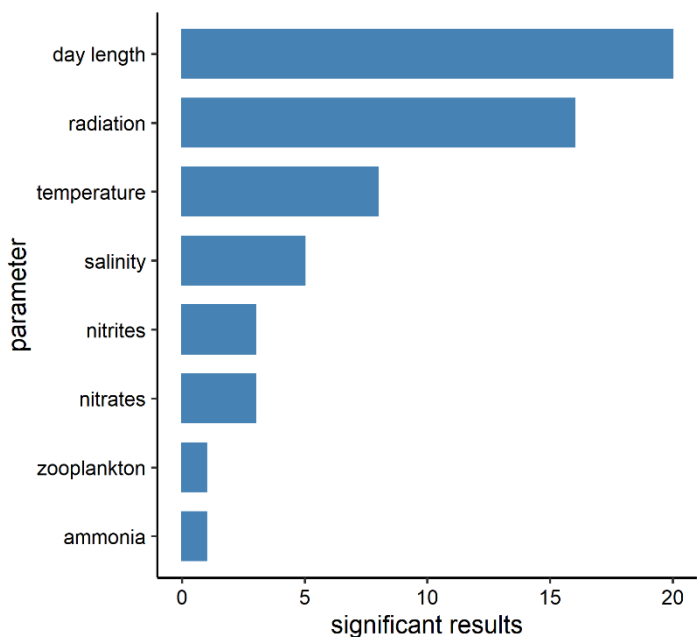
Figure 4.10 Number of species that experienced a significant difference in the values of each parameter between the start and end of bloom.
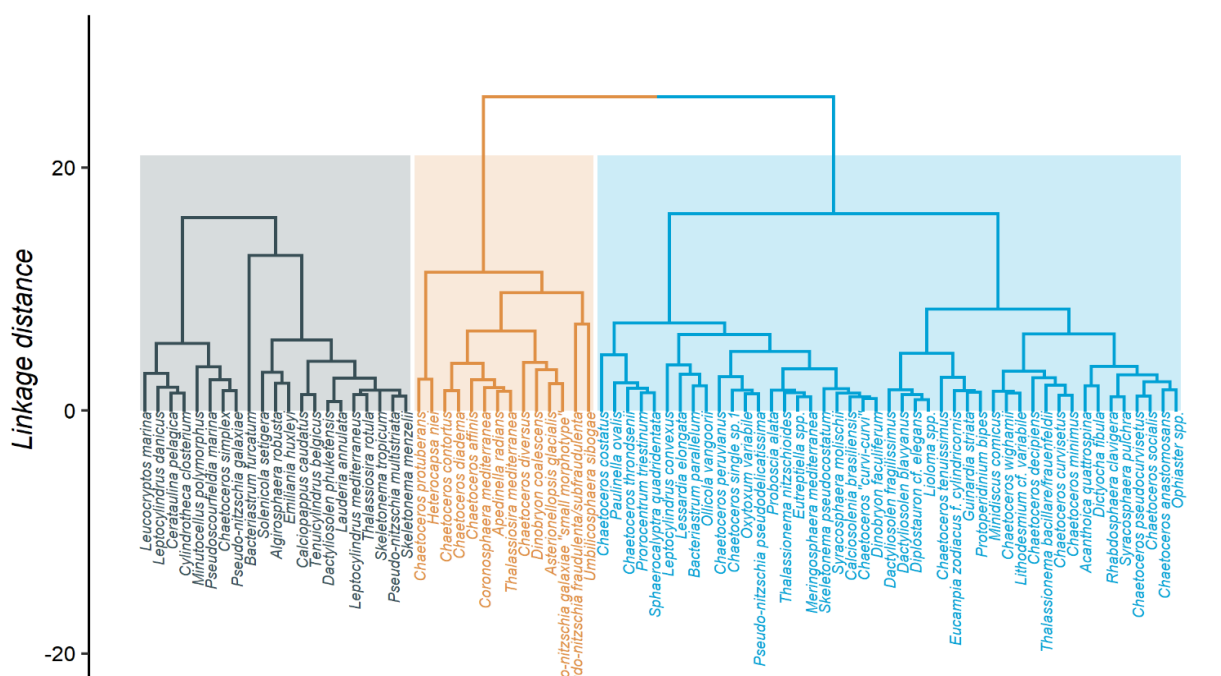


Figure 4.11 Cluster analysis based on median differences of features between the start and the end of the bloom. The dendrogram gather together the species experiencing common patterns of parameter variation between the start and end of the bloom.
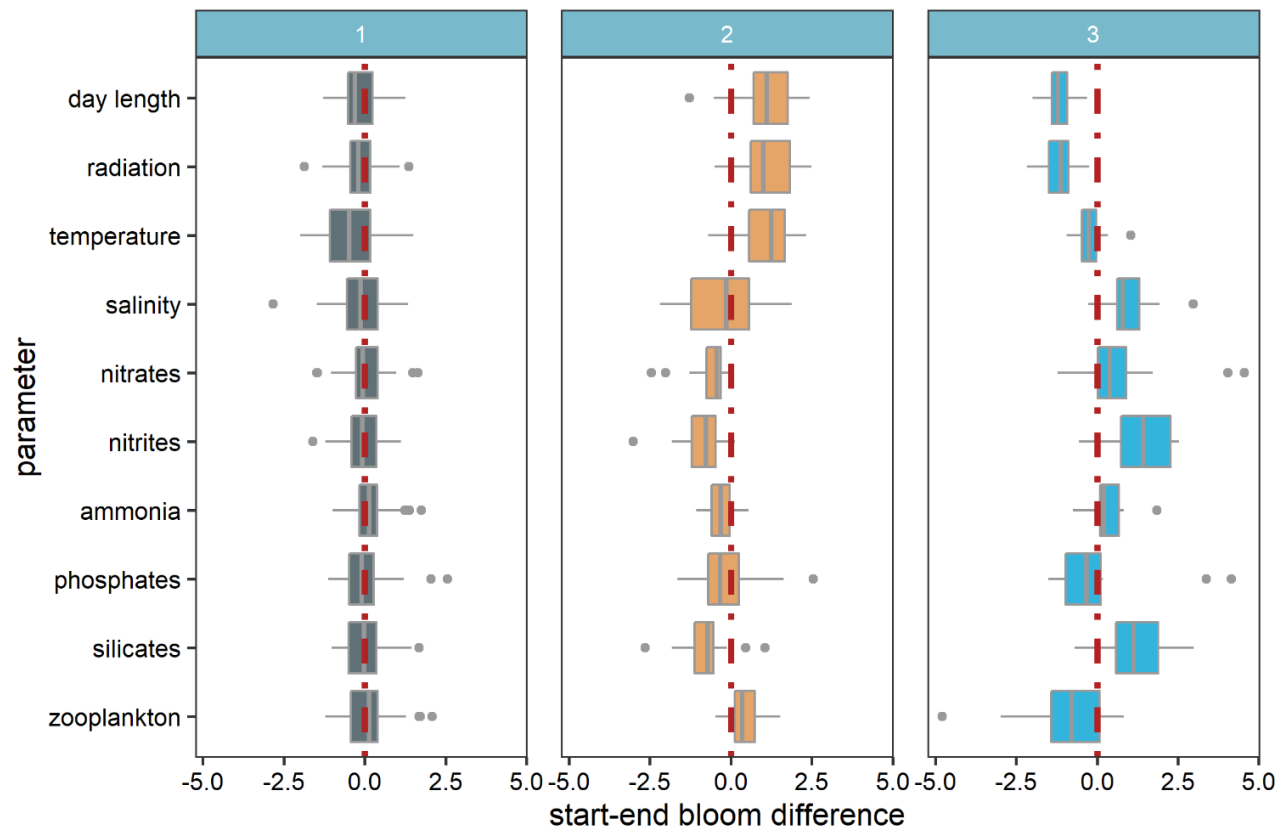
Figure 4.12 Median difference in the values of physical, chemical and biological variables experienced by phytoplankton species at LTER–MC during the start and the end of the bloom. Box numbers and boxplots colours refer to the groups identified by cluster analysis (Fig. 4.11).

# 4.4 Discussion

The phenological properties of the major marine autotrophs are considered important descriptors of the dynamics of the pelagic system and reliable sentinels of any changes taking place in the marine environment. Also, the effort towards the understanding of phytoplankton phenology contributes to the knowledge of the behaviours and adaptations of the individual species which, especially for microbes and in the marine environment, is still poorly understood. Intuitively, time-series examined for these purposes require an appropriate sampling length and frequency to produce reliable results on the dynamics of planktonic organisms (Adrian et al., 2012). Moreover, an appropriate taxonomic resolution is crucial to unbundle the phenological signals generated by aggregated measures as chlorophyll and functional groups, and therefore recognize the factors actually involved in phenology. However, time-series on marine phytoplankton are relatively few, and those with a species-level resolution are even fewer (McQuatters-Gollop et al., 2017).

In this study, based on weekly abundances of 80 phytoplankton species over 30 years, most of the species share a robust temporal regularity associated with the beginning of their bloom. A comparable species-specific regularity was reported for 15 species from Helgoland Roads station (North Sea), which showed a quite stable occurrence in well-defined temporal windows on the interannual scale (Scharfe & Wiltshire. 2019). Some of those species slightly anticipated or delayed their bloom within such temporal windows over nearly 50 years in a similar way as already observed at other North Sea sites, such as the L4 station in the Western English Channel (Atkinson et al., 2015). Conversely, the results in this study indicated that long-term phenological variability is not a characteristic of the LTER-MC phytoplankton community, as only 6 out of 80 species considered showed a significant change in the timing of the bloom.

Our methodology proved valid and effective in characterizing the phenological phases of the 80 species selected. From a theoretical point of view, our approach followed the wave of growth-rate based methods such as those proposed by Beliaeff et al., (2001), Rolinski et al. (2007), Mieruch et al. (2010), which are certainly among the most flexible and probably the most suitable kind of methods for characterizing phenology at the level of individual species. Unlike methods based on predefined thresholds, the use of estimated growth rates is less sensitive to phenological variations that are potentially attributable to factors external to

those of the growth of the species. Indeed, especially in coastal systems, biomass can be subject to prominent changes according to the hydrological regime and water residence times, and a methodology based on thresholds would be unreliable.

Compared to the use of the Weibull functions proposed by Rolinski et al. (2007), and using Scharfe & Wiltshire (2019), our approach assumes no particular statistical distribution, which is a great advantage given the "noisy" shape of individual time series. From a practical point of view, our method provides a set of parameters useful to make the identification of the phases more flexible and to customize the use based on the properties of the time series (such as the sampling frequency and the occurrence of the species). At the moment, a potential limitation of the method proposed lies in handling multimodal patterns, as the algorithm is set up to characterize only one phenological event (meant as the start, maximum and decline) on an annual basis.

Phytoplankton growth rates are strongly influenced by temperature. Accordingly, observations from both mesocosms experiments (Winder et al., 2012) and the natural environment (Chivers et al., 2020; Edwards & Richardson, 2004) reported a relationship between phenological variability and temperature fluctuations. In the wake of the findings of the famous paper of Edwards and Richardson (2004), many studies focused on changes in surface temperature as the most probable cause of phenological variations, including the ones mentioned above for L4 and Helgoland sites. However, the results are conflicting when the relationships between temperature and timing are investigated at the species rather than at the chlorophyll or functional groups' levels. Indeed, while some species (mainly dinoflagellates) seem to anticipate the timing of their blooms following an increase in temperature in the long term, in other groups some species responded anticipating and others delayed the timing, highlighting a much less clear pattern (Ji et al., 2010). Similarly, while it is true that at LTER-MC there was a significant positive trend in surface temperature in summer (see Chapter 2) and *Chaetoceros simplex*, a typical summer species, experienced a significant advance in the timing of the bloom, it is also true that other summer species (including other *Chaetoceros* species) did not show any phenological trends. These results support the idea of a strong specificity in the association between temperature changes and the timing of phytoplankton bloom. Therefore, according to LTER-MC data and results of my analyses, any possible association between timing and temperature changes should be

addressed at the species level, because of non-homogeneous responses between species belonging to the same taxonomic group observed in this study.

According to Reynolds (1984), meteorological fluctuations or particular weather conditions can influence the phytoplankton community by changing successional patterns. Indeed, in the Bahía Blanca Estuary (Argentina), particularly dry years have been associated with a temporal reorganization of the community (Guinder et al., 2010). Similarly, large-scale atmospheric and hydrographic circulation patterns in the North Atlantic seemed to determine variations in the seasonality of *Skeletonema costatum* 'complex' in Narragansett Bay (Borkman & Smayda, 2010). In freshwater environments, changes in species periodicity resulted from fluctuations in nutrient regimes, especially following changes in phosphorus levels (Anneville et al., 2018; Carey et al., 2016). In the present study, neither significant signs of change in the phytoplankton community composition as a whole (see Chapter 2) nor the temporal patterns of single species emerged which could have been attributable to climatic fluctuations and/or changes in the nutrient regimes. Seasonal-dependent climatic fluctuations were observed at LTER-MC (Chapter 3), with the system experiencing significant negative trends for phosphorous levels and salinity. Furthermore, temporal phytoplankton patterns have remained almost unchanged over more than 20 years suggesting that the phytoplankton phenology at LTER-MC is governed by mechanisms other than environmental forcing, to which they likely superimpose determining the temporal regularity that is observable.

A previous study on the whole plankton system at LTER-MC hypothesized, that while the amplitude of phytoplankton peaks was modulated by abiotic forcing, the regular periodicity of the peaks noticed for several species was attributable to biological rhythms linked to their life-cycles (Ribera d'Alcalà et al., 2004). The same hypothesis was made by observing a strong species recurrency in the North Adriatic (Aubry et al., 2012), an even more variable area than the Gulf of Naples. Nevertheless, no specific analyses were presented in support of this hypothesis. In this study, the analysis of the abundance patterns performed on each species has allowed me to identify the potentially favourable and unfavourable abiotic conditions for the development of the bloom, and provide a basis for a better comprehension of the widespread regularity among a wide number of phytoplankton species at LTER-MC. The results largely agree in identifying the light regime as the most important dimension describing the phase of maximum fitness of the species (in terms of

growth) and therefore in the development of their bloom. In fact, except for the light regime (photoperiod and radiance) and to a lesser extent of the temperature, the general environmental context experienced by the species during their maximum growth phase varies from year to year, with heterogeneous salinity and nutrient levels over the years (see also Chapter 2 and 3). These observations, together with the interesting result that there were no significant differences between the nutrient parameters, zooplankton biomass and salinity between the beginning and the end of the bloom, support the idea that these variables were likely to be marginal in regulating species-specific bloom timing, at least considering the ranges of variability observed in this study. In the light of my assumption and interpretation of the beginning and the end of the bloom as informative time-points of species fitness, and under a perspective of abiotic forcing as the main driver of the temporal variability of the species, those two phenological phases should have been characterized by quite divergent environmental conditions. However, it is worth noting that other factors such as parasitism and species-specific ecological interactions, not considered in this study, can influence the species seasonality (Sommer et al., 2012). Also, the aggregate zooplankton biomass I used in the analyses hardly represents the complexity of the trophic dynamics between phytoplankton and primary consumers.

The fact that nutrients do not appear to affect species timing has already been discussed in Chapter 2, where the community and species' regular occurrence were compared to the environmental variability. The discussion of their minor role remains unchanged in the light of the results presented in this Chapter, where the growth phase of individual species was specifically addressed. Regarding the temperature, the discussion is more complex. Although the temperature has an undoubted role in influencing the physiology of microalgae, accelerating their metabolism and division rates, it is not clear to what extent it influences their occurrence timing. In analysing the optimal temperature ranges of 138 taxa in Narragansett Bay over 22 years, Karentz & Smayda (1984) reported a significant interannual temperature variability associated with both the annual maxima and the timing of the species (as the first detectable annual occurrence of the species), and concluded that other factors could play a more important role or at least concur in this process. Accordingly, If the species timing were driven only by the temperature, it would have been plausible to find a more marked difference in temperature between the bloom start and end for most species, which I have not found. In the Thau Lagoon (French Mediterranean coast) an

increase in temperature triggered a bloom of the whole community, not just of some species (Trombetta et al., 2019). This circumstance does not seem to be adaptable to our data, indeed it is in sharp contrast to my results where many species have their maximum growth phase coinciding with a period of rapid cooling of the water column. My results, also in the light of those of Chapter 2, rather support the idea that it is the light regime and in particular the photoperiod to determine the timing, while the temperature and the hydrological regime may affect the amplitude of the peak, as hypothesized by Ribera d'Alcalà et al. (2004). Results obtained from an experiment with mesocosms conducted in the Kiel Fjord (Western Baltic Sea) (Sommer & Lengfellner, 2008) would also seem to support this hypothesis.

The question arising at this point is whether the photoperiod should be interpreted in terms of quantity of light, and therefore of a radiation gradient to which the species are differently adapted, thus generating their succession, or it rather acts as a seasonal signal triggering a physiological reaction. The fact that the Julian day, together with the photoperiod, contributed significantly to the performance of the random forest model of a large number of species, leads me to support the second hypothesis. The photoperiod and Julian day are the only numerically constant and stationary predictors among those used in my analysis, and the fact that they have such an impact on the classification model suggests an implicit temporality that superimposes to the environmental conditions, including radiation, which is subject to continuous weather condition variability.

I have already discussed the potential role of photoperiod as a driver of species succession in Chapter 2. In this regard, and based on the results of the present Chapter, in the following paragraphs I present further arguments in support of the hypothesis of light as the main factor involved in the timing of species.

Studies focused on sexual reproduction of different phytoplankton species, and in particular of diatoms, showed how synchrony is linked to the success of sexual reproduction. In particular, there is evidence of a density-dependent mechanism that triggers sexual reproduction in the diatom *Pseudo-nitzschia multistriata* (see Montresor et al., 2016). Indeed, high cells concentration would increase the probability of contact and perception of chemical cues, and would trigger the formation of gametes aimed at sexual reproduction. The cell cycle of all photoautotrophic organisms is finely regulated by light, and it is widely documented that the progression of the cell cycle in microalgae requires a light-dark

periodicity (circadian rhythm) that regulates the progress of mitotic division as well as physiological adaptations (Bišová & Zachleder, 2014; Chisholm et al., 1981; Mori et al., 2000). The evolutionary advantage of a circadian rhythm would be to tune the physiology of the cell to predictable changes in the environment, thus providing the optimal condition for a cell to perform its life cycle (Suzuki & Johnson; 2001).

Although to the best of my knowledge a biorhythm with a period greater than 24h (circadian rhythm) has not been described for microalgae, it would be reasonable to hypothesize a circannual mechanism in which the photoperiod would act as a synchronization signal between local populations, also in the light of recent studies describing a complexity of molecular and photoreceptor mechanisms involved in the biological processes of microalgae, unknown or underestimated until recently (Depauw et al., 2012; Jaubert et al. 2017). Besides having a crucial role in the vegetative phase, it has been shown that photoperiod influences the sexual phase as well. Several studies have demonstrated that light has a fundamental role in the sexualisation of cells (see Montresor et al., 2016), while others reported how the light regime associated with the greatest reproductive success of different species measured in the laboratory corresponded to the photoperiod at which these species occurred in the natural environment (Hiltz et al., 2000; Mouget et al., 2009).

Given the significant environmental variability that phytoplankton experience at LTER-MC, photoperiod could represent a constant signal, not subject to short-term perturbations, useful to synchronize internal biological processes and imprinted in the evolutionary history of the population of this area.

In summary, despite the significant environmental and hydrological variability of the coastal area where LTER-MC is located, both individual species and the community showed robust patterns of temporal regularity. The results of this Chapter and Chapter 2 indicate that the light regime (and in particular the photoperiod) is the main factor involved in the timing and succession of species. This does not mean that other parameters considered (especially the temperature) are not involved in these processes but that, at least in the area studied, they are partly masked by species internal processes that seemed to be strongly associated with the light regime.

# 5 Comparing phytoplankton niches from global coastal seas

## 5.1 Introduction

The ongoing environmental changes represent a crucial challenge for the scientific community and as never before there is an urgent need for appropriate tools useful to assess the direction and intensity of the changes taking place. One of the most interesting themes is to assess the adaptive potential of organisms and, the availability of historical observations represents an essential component to achieve this purpose is the availability of historical observations.  Indeed, in order to understand organisms' adaptive potential and dynamics versus environmental changes it is necessary to have a reference state, a 'time zero' representing the basis on which to discuss the present and make projections on the future.

Ocean models and observations predict that the ongoing ocean warming will result in a significant change of phytoplankton structure and composition (Daufresne et al., 2009; Winder & Sommer, 2012), and an overall reduction of phytoplankton primary productivity and abundance (Boyce et al., 2010; Bopp et al., 2000; D'Alelio et al., 2020.). In coastal systems, the complex of environmental changes linked to the SST warming trend is likely to exacerbate already known issues as eutrophication, regime shifts, and the occurrence of invasive allochthonous species (see Cloern et al., 2016). As phytoplankton is a fundamental component for ecosystem functioning, the understanding of the extension of their adaptive potential is of great importance to better understand the effects of human-induced changes in marine habitats.

In this context, the concept of the ecological niche of a species has regained popularity in the last years in the scientific community as a potential tool useful for the interpretation of biological dynamics in a rapidly changing ecosystem (Holt, 2009; Soberón, 2007). Grinnell (1917) was the first to introduce the term 'niche' to summarise the complex relationships between species performance and resource availability. However, it was Hutchinson in 1957 who proposed a quantitative argument to the topic.  The niche of a species *sensu* Hutchinson (1957) is depicted as an 'n-dimension hypervolume that defines the space of the resources

in which a species can persist indefinitely. Later, the term 'indefinitely' was better formulated in terms of a growth-rate parameter (Hutchinson, 1957; Maguire, 1973), and defined the niche of a species as the n-dimension hyper-volume where a species achieves a positive net growth rate. Hutchison made a distinction between the 'fundamental niche', that is the full range of environmental conditions that a species can use without any other limiting factor and the 'realized niche'. The distinction has its foundation considering that natural biological communities experience an incredible set of interactions. Competition, mutualism or parasitism (Pearman et al., 2008) reduce the shape of the fundamental niche in the realized niche, that is the actual space of the resources exploited by a species, an expression of the dynamics that characterize a species in its environment and its relations with it.

In recent years, by taking advantage of the increasing number of open-ocean and coastal observations, phytoplankton niche models are being extensively adopted to predict species responses to changing climate and assess species' extinction rates. These species distribution models (SDMs) are based on observed correlations between species and environmental variables, and generally assume that species have a fixed environmental niche, a condition referred to as 'niche conservatism', a term initially introduced by Harvey and Pagel (1991) indicating the tendency of a species or a group of congeneric species to retain their niches over time. Overall, in view of predicted warming of the oceans, SDMs studies predict a general biogeographical shift in species' thermal niches, followed by a decline in phytoplankton diversity (Flombaum et al., 2013; Thomas et al., 2012).

Nevertheless, there are several limitations in niche models that need to be further addressed. Indeed, the ecological concept of niche is strictly related to the concept of species, which in phytoplankton ecology is very labile due to the large genetic variability among populations and strains that are considered to belong to the same species. Even if morphologically identical, individuals (morpho-species) of the same population can express a range of physiological and biochemical behaviours widely divergent among them. Braarud (1951) was the first to discuss the existence of different 'races' in phytoplankton species, and his intuitions have been deepened and confirmed in subsequent years leading to re-evaluate the species concept in microalgae and its implications in phytoplankton ecology (Gallagher, 1982; Wood & Leatham, 1992). Moreover, apart from genetic variation across the range of a taxon, which may result in adaptive evolutionary processes, phytoplankton

can show phenotypic, species-specific responses to environmental forcing without changes in genotype (see Collins et al., 2014), a condition defined as 'plasticity'. Finally, most SDMs studies on phytoplankton rely on presence-absence observations, underestimating the temporal and seasonal dynamics of a certain species in a given area (see Chapter 4).

In recent years, the analysis of coastal phytoplankton time-series has contributed to characterize and describe the role and the ecology of different species in their respective environments. Comparative studies on different coastal sites reported a preponderant role of the sampling area in defining the structure and composition of phytoplankton communities (Olli et al., 2015; Spatharis et al., 2019). Previous global analyses at the level of individual species, aimed at characterizing their patterns of occurrence in relation to environmental parameters in different areas, did not find a consistent association between species-specific blooms and global environmental ranges (Carstensen et al., 2015).

The effort of such study is addressed to a better understanding of the adaptive/conservative dynamics of phytoplankton, and advance a further contribution also in the context of phytoplankton potential plasticity and prediction concerning environmental changes. I used a unique set of 10 worldwide-distributed coastal phytoplankton time-series in order to test whether regionally different morpho-species share the same ecological niche, and to clarify whether they conserve a common dynamic/organization during comparable environmental conditions from different regions.

## 5.2 Materials and methods

## 5.2.1    Data

The dataset used in this study includes 9 time series of phytoplankton-species abundances distributed in different biogeographical regions and characterized by a taxonomic resolution at the species level (Fig. 5.1, Tab.10). In Europe, two time-series are located in the Mediterranean Sea namely, *station C1* located in the Gulf of Trieste (Adriatic Sea, http://nettuno.ogs.trieste.it/ilter/GoTTs/en_c1_lter.html) and *LTER-MC* located in the Gulf of Naples (Tyrrhenian Sea, http://szn.macisteweb.com/), whereas the other two, *L4* (Western Channel Observatory, https://www.westernchannelobservatory.org.uk/) and *At So* (REPHY, https://wwz.ifremer.fr/lerpc/Activites-et-Missions/Surveillance/REPHY) stations, are located in the western and eastern areas of the English Channel respectively. Two time-series are located along the Atlantic coasts of North America: *Station II*, in Narragansett Bay, Rhode Island (https://web.uri.edu/gso/research/plankton/), and station *WE4.2*, in the polyhaline zone of the Chesapeake Bay Estuary (Chesapeake Bay Program, https://www.chesapeakebay.net/). A time-series in San Francisco Bay (United States Geological Survey, https://www.usgs.gov/mission-areas/water-resources/science/water-quality-san-francisco-bay-research-and-monitoring?qt-science_center_objects=0#qt-science_center_objects) consists of data from multiple stations located on the Pacific U.S.A. coasts. Finally, I used data from two time-series from stations located in tropical areas of the southern hemisphere, *CARIACO* (*CARIACO* Ocean Time-Series Program, http://www.imars.usf.edu/cariaco) on the continental shelf of Venezuela, and *Yongala* station in Northern East Australia, belonging to the Australian Integrated Marine Observing System (IMOS, https://imos.org.au/facilities/shipsofopportunity/auscontinuousplanktonrecorder).

The selection of phytoplankton species was made according to their presence shared among multiple time series and their frequency in each series. Initially, I have included all the species shared by at least 4 time-series: subsequently, I have selected only those species that showed a relative frequency of at least 20% in each time series. The use of such a high-frequency threshold is justified by the aim of identifying only those species that were

statically representative of each time series, and which were assumed to represent populations characterized by a stable presence in each area.

Environmental data used to define species niche included physical parameters (surface temperature and salinity, day length) and surface concentrations of inorganic nutrients (nitrates, silicates and phosphates). Since time-series data showed different date formats as well as different measurement units for both biological and environmental data, a quite extensive data pre-processing with cleaning and normalization was performed before the analyses. All the time-series were therefore converted into common formats, and measurements units were homogenized in cells ml$^{-1}$ for phytoplankton abundance, μM for inorganic nutrients concentration and °C for temperature. Moreover, species nomenclature was updated according to the *World Register of Marine Species* (WoRMS, http://www.marinespecies.org/).
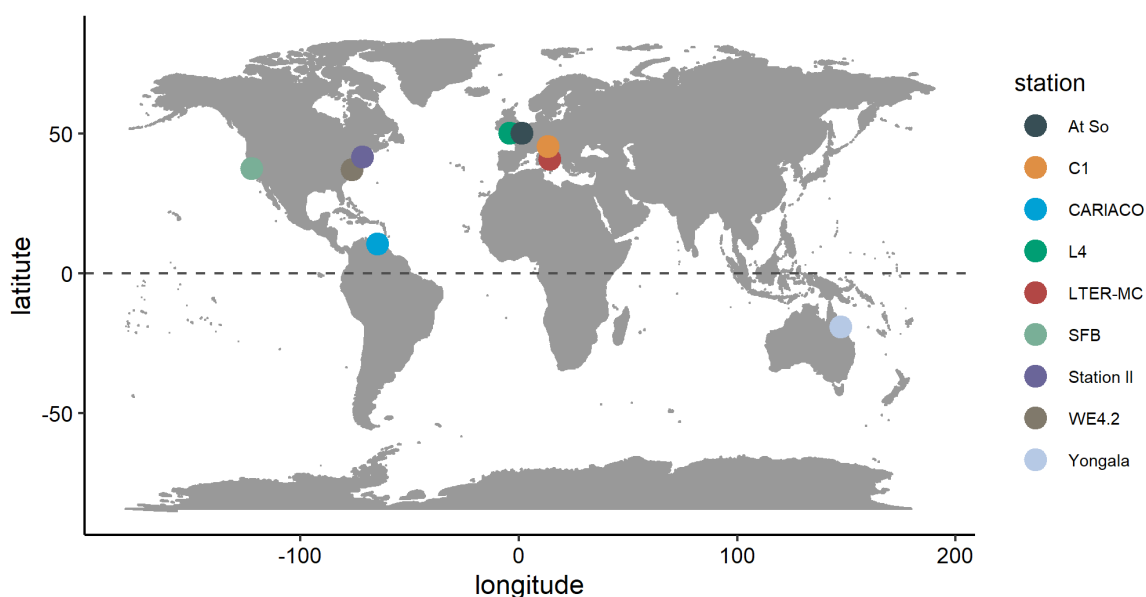


Figure 5.1 Location of the sites of the phytoplankton time–series analysed in this study.

Table 10 Geographical region, station name and temporal properties of the time-series used in this study.

| Region | Station | Latitude | Longitude | Frequency | Monitored period | Length (years) |
|---|---|---|---|---|---|---|
| Chesapeake Bay (USA) | WE4.2 | 37.11 | -76.29 | monthly | 1985-2018 | 33 |
| Cariaco basin (Venezuela) | CARIACO | 10.50 | -64.67 | monthly | 1995-2017 | 22 |
| San Francisco Bay (USA) | SFB | 37.50 | -122.10 | monthly | 1992-2011 | 19 |
| Gulf of Naples (Italy) | LTER-MC | 40.82 | 14.25 | weekly | 1984-2015 | 31 |
| Narragansett Bay (USA) | Station II | 41.57 | -71.39 | weekly | 1959-2018 | 59 |
| Western English Channel (England) | L4 | 50.15 | -4.13 | weekly | 1992-2015 | 23 |
| Gulf of Trieste (Italy) | C1 | 45.42 | 13.42 | monthly | 1986-2010 | 24 |
| Eastern English Channel (France) | At so | 50.20 | 1.47 | fortnightly | 1990-2018 | 28 |
| NE Australia (Australia) | Yongala | -19.19 | 147.37 | monthly | 2009-2019 | 10 |

## 5.2.2    Construction of species realized-niche

Annual maxima for each species in each location were used as representative of high-fitness conditions. To make the analysis more robust and accurate and to exclude potentially undersampled or unrepresentative years from the analysis, only annual maximum values greater than the third quartile of the entire positive abundance distribution of a species in each location were taken into consideration. Therefore, this process returned a new dataset collecting species' annual events of maximum abundance.  As argued in Chapter 4, maximum recorded annual abundance may not always associate with the high-fitness conditions of a species, making an approach based on the properties of species' abundance curves conceptually more correct. Yet, in this case, the frequency of the time series data was not homogeneous nor adequate to obtain reliable abundance curves. Moreover, at the scale of comparison in this study (global), the annual maximum values can be considered suitable for the purposes of this study. Therefore, the new dataset was visually explored to compare the physical-chemical ranges associated with species' high-fitness, and to verify whether the same species shared the same physical-chemical ranges across different biogeographical areas.

Then, in order to verify whether individual species exploited the same environmental gradient in different geographical regions, I followed a geometric-like approach specifically designed to characterize the realized niche (*sensu* Hutchinson, 1957) of different organisms, the Within Outlying Mean Index (WitOMI, Karasiewicz et al., 2017). The WitOMI is an extension of The Outlying Mean Index (OMI) (Doledec, 2000), an analysis implemented for the construction of the Huchinsonian niche (Hutchinson, 1957). To this aim, the method is based on ordination techniques aimed to represent the multidimensional environmental space exploited by different taxa into a new bi-dimensional space that maximizes the distance between the mean habitat conditions used by the taxonomic unit and the mean habitat condition of the entire studied area. This process allows to highlight and quantify the position (the centre of gravity) and the tolerance (dispersion of points) of the taxonomic units along the environmental gradient. With respect to the OMI, which is designed to investigate species distribution on a single environmental space (i.e. a single area of investigation), the WitOMI, has the further feature of admitting two or more areas of investigation characterized by common species and environment tables.

Initially, a PCA including physical and chemical data from all sites was made to create a common 'environmental map' representing the whole environmental space. Before this process, environmental tables from each location were scaled to analyse the environmental dynamics relatively to each location. Species abundances were squared-root-transformed in order to down weight the influence of most abundant species in the construction of the multidimensional space. Following the same logic, nutrient concentrations were log-transformed. Based on the PCA scores, ordination axes were rotated to maximise the mean marginality of each species, that is, the mean squared distance between the centroid of the space used by each species (environmental gradient exploited by the species) and the centroid of the cloud of available points in the ecological space (average environmental condition of each area). From the new axes generated from the rotation of PCA, it is possible to quantify 3 attributes related to the species realized niches: the niche position, corresponding to the centre of gravity of species maximum annual abundances in the multivariate space, the niche breadth (species tolerance), given by the dispersion of these points, and the marginality (species specialization), given by the distance of the species-niche distance from the origin of the axes.

In addition to these attributes, I have calculated the geometric area occupied by each species in each location in the environmental space to test the overlap between the environmental conditions experienced by the different species. Specifically, I have calculated the percentage of niche overlapping between all possible species pairwise. Values of percentage area shared among all possible species pairwise were used to test the hypothesis that the same morpho-species in different locations shared, on average, a significantly higher area than that shared with random subsamples of other morpho-species.

Before these analyses, I made a check on the quality of the information available for each time series. Given that the target is to extract and compare the niches of phytoplankton species in a common environmental gradient, it was necessary that species were well represented in the space of environmental variables, and that the data used covered homogeneously all the seasonal variability to avoid any bias caused by the under-representation of the time-series of certain periods of the year. Moreover, the creation of the multidimensional environmental space requires non-missing observations for each parameter, i.e., samples that include both environmental and biological records without missing values (hereafter complete samples). Therefore, in order to have a picture of the number of complete samples for each location, I have calculated the number of observations that included non-missing biological (species counts) and environmental data (physical and nutrients data) for each month in each time series. Since time series had different lengths, I took the last 10 years for each location to avoid an over-representation in the multidimensional space of species and environmental parameters of the series characterized by the highest number of observations.

# 5.3 Results

## 5.3.1    Habitat variability

The general environmental context experienced by phytoplankton populations at each long-term observation site was quite heterogeneous (Fig 5.2). Except for the two tropical sites (*CARIACO* and *Yongala*), all other time series showed a clear seasonality despite different absolute values. *Station II* in Narragansett Bay and *WE4.2* in the Chesapeake Bay showed the highest amplitude of the annual temperature cycle, being characterized by very cold winters (temperature often below 0 at *Station II* in winter) while summer temperatures reached relatively high values (up to 27 °C at WE4.2). At *L4* and *At So* reported a lower difference between winter and summer temperatures was reported, with an average difference of around 6 °C. In the Mediterranean Sea, average winter temperature values at *LTER-MC* and *C1* were around 15 °C and 10 °C respectively, whereas they reached almost the same summer maxima, with surface waters around 25 °C.  Day length showed a clear difference between stations located in tropical and temperate regions. While in all temperate stations there was a difference in the hours of light between winter and summer of at least 4 hours, *CARIACO* and *Yongala* did not exceed two hours. The stations located in San Francisco Bay (*SFB*) and Chesapeake Bay (WE4.2) had a typically estuarine character, with significantly lower salinity than the other stations and higher nutrient concentrations (especially in the Chesapeake Bay), whereas both the Mediterranean and tropical stations showed an opposite condition, characterized by higher salinity and lower nutrient concentrations. In particular, *Yongala* station was characterized by the lowest concentrations of inorganic nutrients among all the time series. Is it worth noting the divergence of the environmental context between *L4* and *At So* which, although located in the same biogeographical region (English Channel) showed very different ranges in nutrients and salinity.  *At So* is located near the mouth of the Somme River and was characterized by fresher and nutrient-rich waters compared to *L4* which was more influenced by saltier and nutrient-poor offshore waters, being located relatively far from the coast.
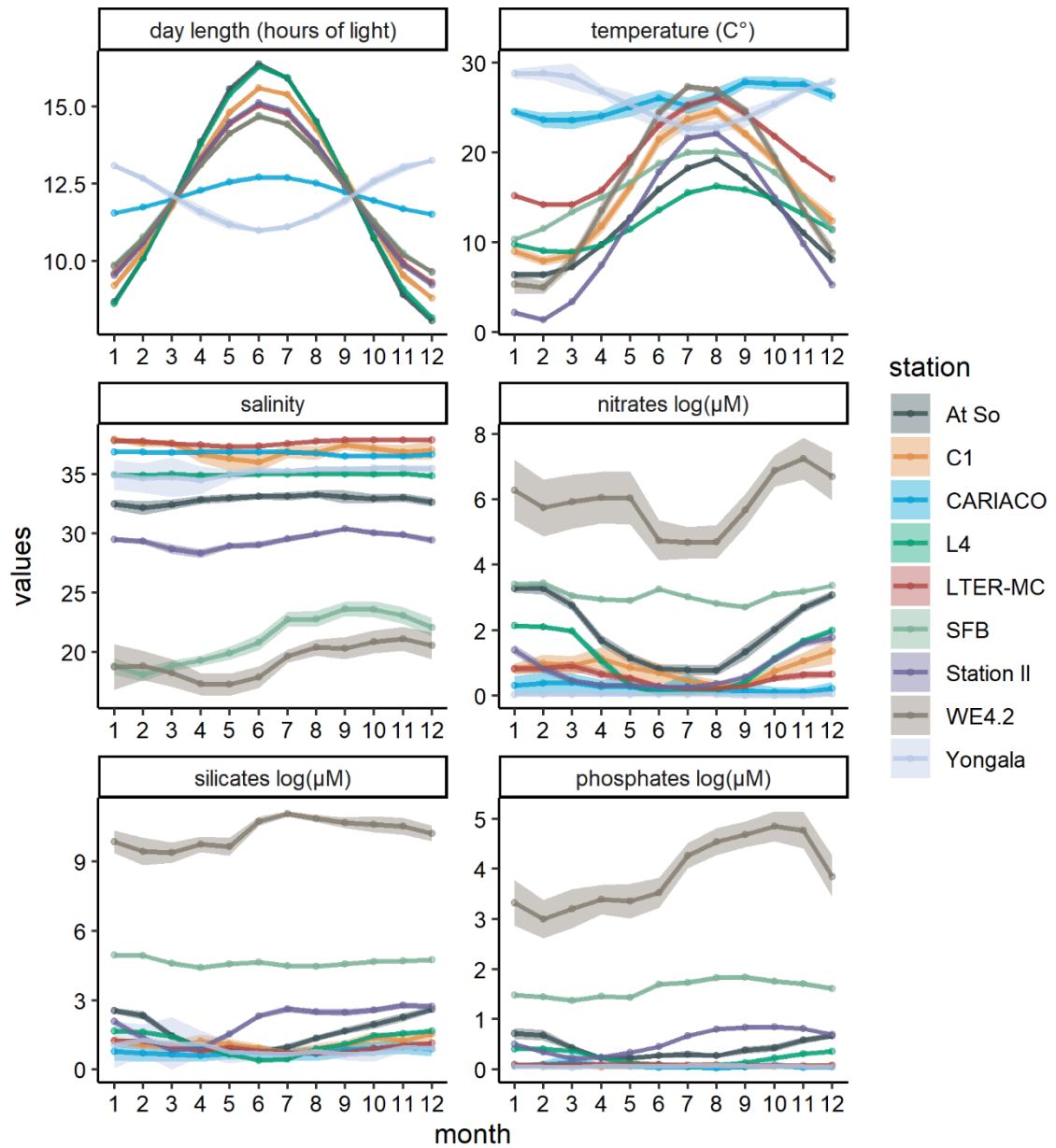
Figure 5.2 Average monthly variability of physical and chemical parameters in each time-series. Shaded areas represent 95% confidence interval.

## 5.3.2     Species selection and distribution

According to the criteria described in the methods above, I have selected 10 species (Table 11). Some of them as *Cerataulina pelagica*, *Asterionellopsis glacialis*, *Dactyliosen fragilissimus*, *Cylindrotheca closterium* and *Thalassionema nitzschioides* were common to all the time-series but were characterized by very different ranges of abundance and relative frequency (Tab. 8). The stations located in the tropics tended to show lower average species abundances, whereas *WE4.2* and *LTER-MC* stations were characterized by higher average abundances compared to other locations. This was also evident from the monthly species climatology (Fig. 5.3) in which in both *WE4.2* and *LTER-MC* many species showed their maximum values across all series despite a different annual cycle. In addition to heterogeneous ranges of abundance and frequency among the different time-series selected, species often showed a distinctive average annual cycle from region to region, as can be seen from their monthly distribution (Fig. 5.3). *Asterionellopsis glacialis* showed a predominant winter distribution for most of the series except for the *At So* station, where it occurred in midsummer. Similarly, *Prorocentrum micans* showed different distributions among the series. It was typically autumnal in the Gulf of Trieste (station *C1*), whereas it occurred in summer at *At So* and all year round in station *WE4.2* in the Chesapeake Bay. *Thalassionema nitzschioides* also a very different annual distribution from region to region, characterized by high densities during summer at *WE4.2* while it occurred predominantly in winter or early at *At So*, *SFB* and *CARIACO*. Other species, on the other hand, seemed to show more regular patterns associated with the annual occurrence such as *Cylindrotheca closterium* and *Rhizosolenia delicatula*. The former appeared to be characteristic of the late summer / autumnal period in several stations, while the latter reached the highest densities in spring in many locations.

Table 11 List of the species selected and their relative frequency, mean abundance and standard deviation for each station.

| species | Yongala | | | LTER-MC | | | L4 | | | WE4.2 | | | CARIACO | | | SFB | | | Station II | | | At So | | | C1 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | freq. | mean | sd | freq. | mean | sd | freq. | mean | sd | freq. | mean | sd | freq. | mean | sd | freq. | mean | sd | freq. | mean | sd | freq. | mean | sd | freq. | mean | sd |
| A. glacialis | 3.77 | 0.02 | 0.16 | 12.48 | 9.62 | 51.44 | 0.73 | 0.01 | 0.11 | 58.47 | 84.57 | 355.8 | 7.69 | 1.6 | 15.94 | 9.23 | 1.3 | 15.92 | 23.54 | 106.1 | 636.5 | 55.6 | 170 | 1247 | 8.53 | 6.66 | 62.1 |
| C. closterium | 94.3 | 0.77 | 1.27 | 72.53 | 134.5 | 1062 | 84.4 | 1.53 | 4.22 | 77.49 | 69.94 | 298.2 | 26.7 | 0.46 | 2.21 | 40.33 | 17.14 | 102.26 | 30.68 | 11.56 | 273.4 | 8.4 | 0.37 | 1.83 | 39.3 | 1.26 | 5.16 |
| C. pelagica | 14.2 | 0.04 | 0.14 | 43.93 | 45.16 | 295.1 | 43.7 | 1.86 | 12.8 | 65.43 | 266.6 | 1472 | 19.46 | 0.41 | 2.76 | 1.43 | 0.46 | 8.54 | 16.64 | 42.19 | 617.2 | 7.63 | 0.53 | 6.45 | 61.1 | 15.3 | 58.9 |
| D. fragilissimus | 27.4 | 0.28 | 1.29 | 28.25 | 12.16 | 52.53 | 23.4 | 1.78 | 20.3 | 75.41 | 208.3 | 923.7 | 51.13 | 3.24 | 20.95 | 2.64 | 0.06 | 0.49 | 16.43 | 53.1 | 613.5 | 12.5 | 1.72 | 11.8 | 17.4 | 1.41 | 13.8 |
| L. danicus | 56.6 | 1.44 | 5.23 | 75.56 | 516.3 | 1460 | 40.4 | 16 | 72 | 32.25 | 30.4 | 267.2 | 36.65 | 6.93 | 30.59 | 1.54 | 0.4 | 7.51 | 21.61 | 35.85 | 303.1 | 18.2 | 31.6 | 277 | 49.2 | 14.3 | 68 |
| P. micans | - | - | - | - | - | - | 36.8 | 0.41 | 3.14 | 88.86 | 16.99 | 44.07 | 33.03 | 0.02 | 0.05 | 9.34 | 0.34 | 3.33 | 4.73 | 0.27 | 2.22 | 36.4 | 1.32 | 19.7 | 45.1 | 2.06 | 7.55 |
| P. seriata | - | - | - | - | - | - | 37.4 | 2.65 | 20.3 | 46.87 | 27.62 | 127 | 32.58 | 4.31 | 25.79 | 3.19 | 2.06 | 35.97 | 5.71 | 2.24 | 19.7 | 21.6 | 7.3 | 47.8 | 22.5 | 13 | 44.8 |
| R. setigera | 74.5 | 0.58 | 2.54 | 4.85 | 0.54 | 4.58 | 37.2 | 0.59 | 7.35 | 46.87 | 3.51 | 27.27 | 31.22 | 0.91 | 5.08 | 18.02 | 1.31 | 8.76 | 22.59 | 3.22 | 19.91 | 39.5 | 2.03 | 13.4 | - | - | - |
| R. delicatula | 5.66 | 0.01 | 0.07 | - | - | - | 72.2 | 5.8 | 23.3 | 39.44 | 38.5 | 162 | 42.99 | 1.6 | 8.84 | 12.09 | 0.34 | 2.77 | 29.77 | 46.64 | 309.8 | 70 | 32 | 109 | 8.87 | 0.69 | 2.94 |
| T. nitzschioides | 5.66 | 0.04 | 0.25 | 22.1 | 10.66 | 50.54 | 45.5 | 1.59 | 18.2 | 86.77 | 83.32 | 299.3 | 33.48 | 2.39 | 23.85 | 15.27 | 4.1 | 39.58 | 24.52 | 13.35 | 102.7 | 55.1 | 8.46 | 29.5 | 19.5 | 1.36 | 12.4 |

Figure 5.3 Average monthly variability of the ten selected species in each time-series.

### 5.3.3     Species physical–chemical range

Visual inspection of the distribution of the annual maxima of each species revealed that overall, species experienced quite different physical-chemical ranges (Fig. 5.4). Indeed, the spectrum of temperatures during the annual maxima of *Thalassionema nitzschioides* ranged from values below 10 °C at *At So* to values greater than 30 °C at *Yongala*. Between these two extremes, the optimal temperature range of *Thalassionema nitzschioides* was on average 14 °C at *SFB*, *Station II* and *L4* and greater than 20 °C for the other time series. Similarly, *Rhizosolenia setigera* and *Dactyliosen fragilissimus* showed optimal temperatures over a gradient of temperature of 15 °C without showing any clear range of shared values. Other species such as *Asterionellopsis glacialis* and *Rhizosolenia delicatula* showed slightly overlapped optimal temperature values and, excluding tropical stations, seemed to generally prefer colder temperatures at all sites.

However, while the optimal temperature conditions of *Asterionellopsis glacialis* and *Rhizosolenia delicatula* between the different stations seemed to overlap, the same cannot be said for the lighting regime. While in both the Gulf of Trieste (*C1*) and in the English Channel (*At So*) *Asterionellopsis glacialis* showed an optimal temperature of around 13 °C, its optimal light regime varied from about 10 to 15 hours of light at *C1* and *At So* respectively. In addition, the species showed very different optimal temperature ranges between the Gulf of Naples and *CARIACO* Basin (about 8 °C of difference), it experienced the same optimal light conditions. As observed for temperature ranges, several species appeared to occur in a wide gradient of light conditions. Indeed, *Leptocylindrus danicus* and *Cylindrotheca closterium* showed their maximums at 9 hours to 16 hours of light at *C1* and *At So* respectively, while other species seemed to cluster around a narrower spectrum of light hours such as *Rhizosolenia delicatula* and *Dactyliosen fragilissimus*.

The distribution of the maximum annual abundances along salinity and nutrient gradients revealed a marked separation in the environment experienced by the species in San Francisco Bay (*SFB*) and Chesapeake Bay (WE4.2) from the rest of the stations considered. In both *SFB* and *Station II* all species occurred in fresher and nutrient-rich conditions than in the rest of the sites, which showed their optimal conditions at much lower nutrient concentrations instead, sometimes very close to zero as in the case of *Yongala* and *CARIACO*.
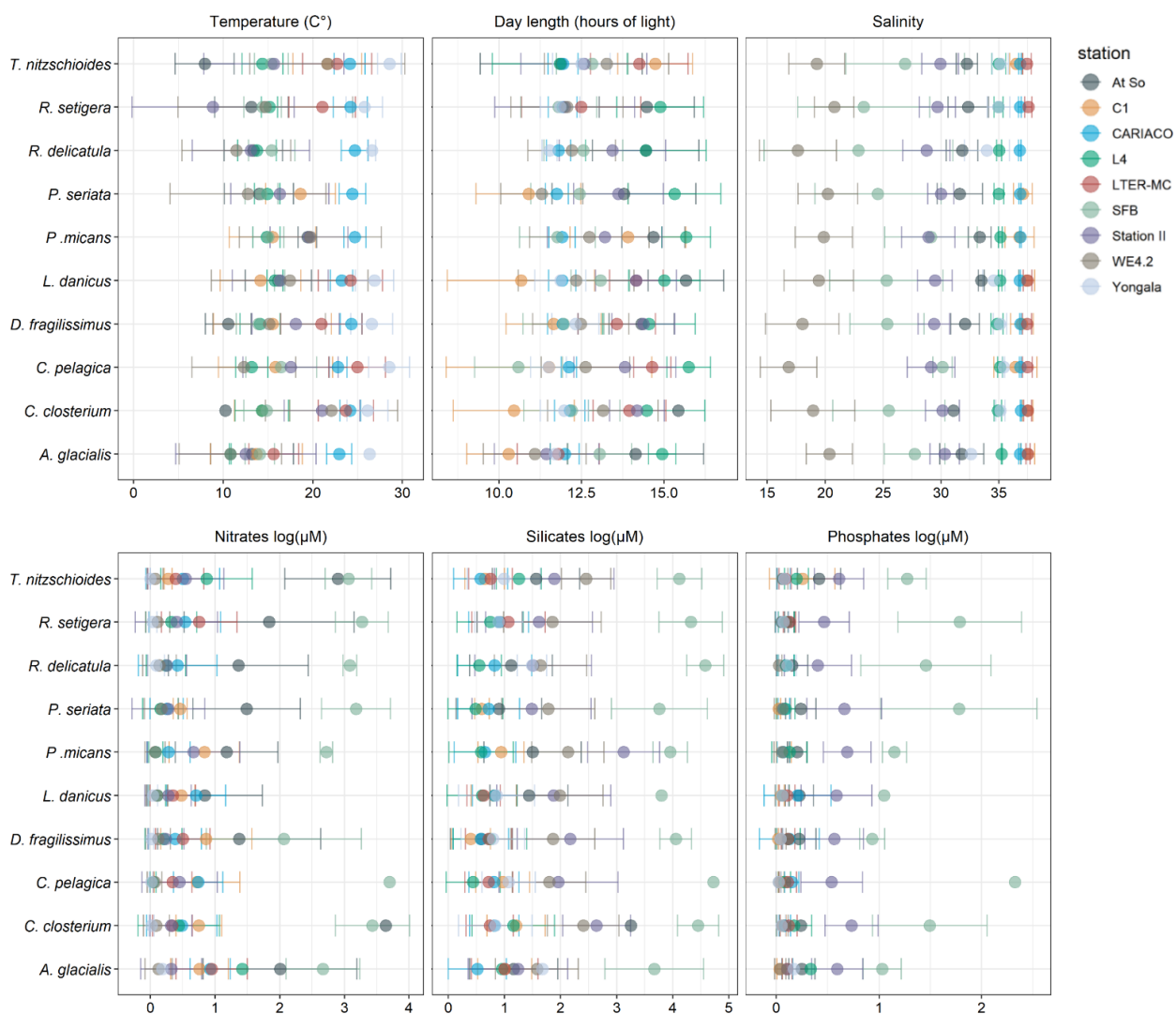
Figure 5.4 Distribution of species annual maxima along the gradients of environmental parameters relative to each location.

## 5.3.4    Realized niche

The amount of complete monthly observations obtained by combining species and environmental matrices are displayed in Fig. 5.5. Although autumn and winter were slightly under-sampled, even seasonally unbalanced time series were generally characterized by a large number of observations which made such unbalance negligible for the purpose of the analyses. Yet, *C1* and *Yongala*, which had few total complete observations compared to other stations, showed homogeneity of observations along the seasons. One notable exception is that of *SFB* in San Francisco Bay, where summer, autumn and winter were significantly under-represented compared to the spring period, a potentially biasing factor for the interpretation of the results of the subsequent analyses.
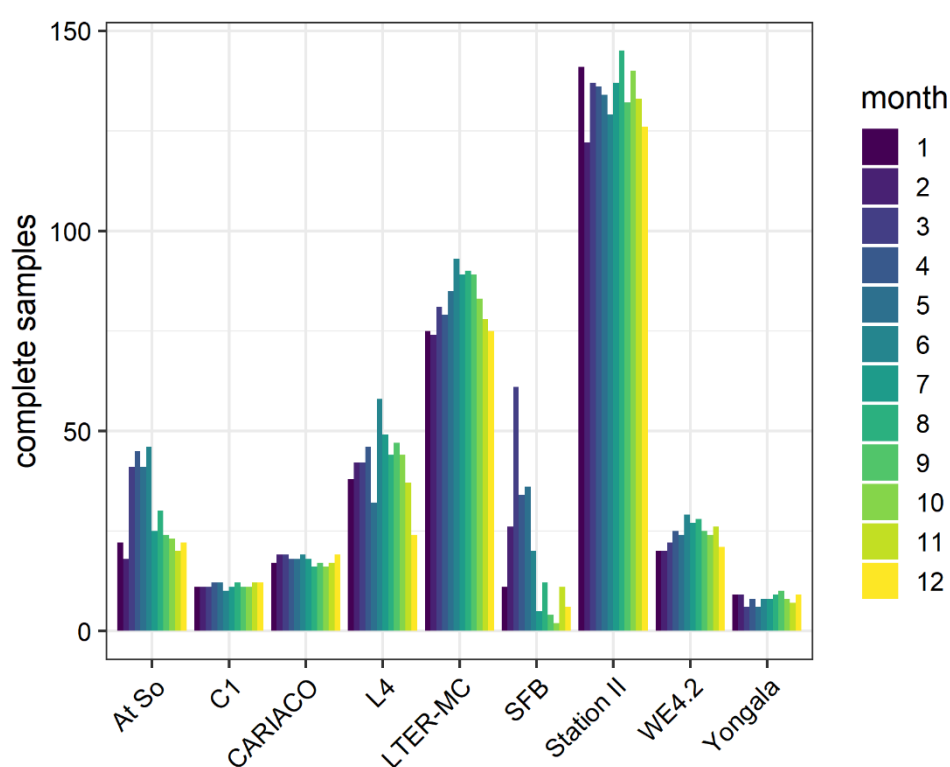


Figure 5.5 Number of observations, in each time-series, characterized by non-missing values in both biological (species counts) and environmental data (physical and nutrients data) grouped by month.

The multidimensional environmental space generated by the rotation of environmental axes described 86.12% of the separation of species marginality (Fig. 5.6), that is the distance of species niches (weighted centres) from the average environmental condition common to all the stations. The first OMI axis explained most of the variability in species niche positions (52.44%) and it was most representative of the temperature and in a minor part of silicates and phosphates gradients. The second axis represented the gradient defined by light conditions (day length) and nitrates and described the 33.66% of species' niche variability.

The position of the weighted centres (niche positions) calculated for each species using WitOMI revealed that the species considered in the study exploited a wide range of environmental conditions and that the same species in different locations occurred in a wide range of physical and chemical contexts (Fig. 5.7). For instance, *Asterionellopsis glacialis* shared a preference for cold and nitrate-rich waters in most of the stations considered, except for *At So* that occurred in relatively warmer temperatures. *Leptocylindrus danicus* exploited a relatively cold environment compared to the *Leptocylindrus danicus* collected in the English Channel (*L4* and *At So*) and in the Gulf of Naples, and it seemed to be quite tolerable to a wide range of nitrates conditions as it occurred in conditions of high nitrates relative to *CARIACO* station, and to lowest relative nitrates concentrations at *WE4.2* in the Chesapeake Bay.

Other species as *Cylindrotheca closterium* and *Pseudo-nitzschia seriata* seemed to be advantaged at high temperatures relative to each location although they differ for the general condition of nutrients experienced when they reached the maximum abundances. Similarly, *Cylindrotheca closterium*, *Prorocentrum micans* and *Rhizosolenia delicatula* were experienced a wide range of nitrates, silicates and phosphates conditions, whereas they seemed to be more constrained by day length and temperature. Indeed, *Prorocentrum micans and Rhizosolenia delicatula* were quite emblematic in this sense. *Prorocentrum micans* collected in different locations seemed to be advantaged in longer day lengths, whereas *Rhizosolenia delicatula* preferred lower hours of light.

In contrast to the regularities described above for some species in different locations, other species as *Thalassionema nitzschioides* and *Cerataulina pelagica* showed very different habitat preferences. *Cerataulina pelagica* collected in the English Channel showed the maximum abundances in relatively cold temperatures compared, for example, to the Gulf of Naples or to Narragansett Bay, where it reached the highest densities in summer when

the highest surface water temperatures were usually recorded. Similarly, the group of *Thalassionema nitzschioides* occupied all environmental conditions described by the multidimensional space, from relatively high temperatures and day length conditions at *C1* and *LTER-MC* to the opposite conditions at *CARIACO* and *At So*.
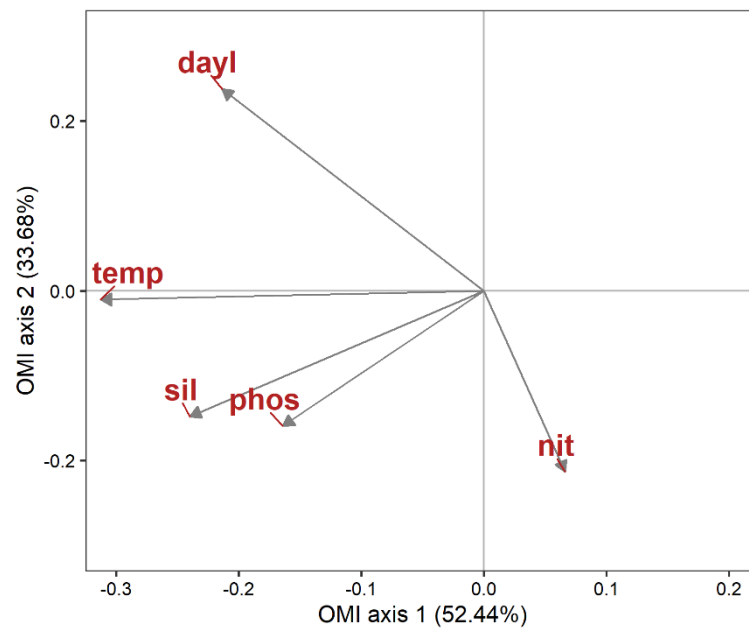


Figure 5.6 Canonical weights of the environmental variables depicting the multidimensional environmental space. The length of each arrow represents the relative importance of each variable in separating species niche positions.
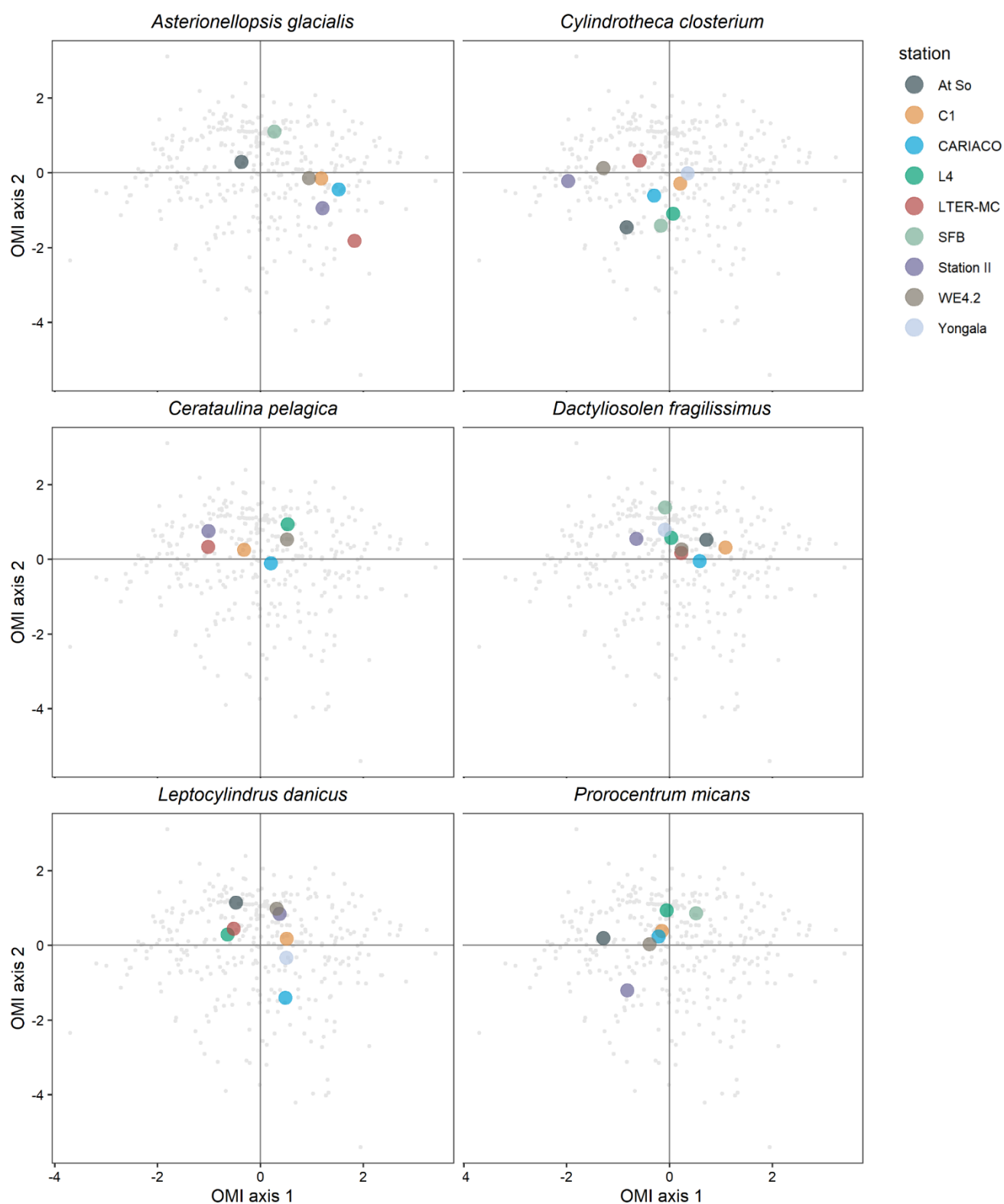
Figure 5.7 Weighted positions of the species in the multidimensional environmental space (see Fig. 5.6) coloured for each station. Species that had low marginality occupied positions near the origin of the space, whereas those that exploited a more specialized environment occupied positions far from the origin.
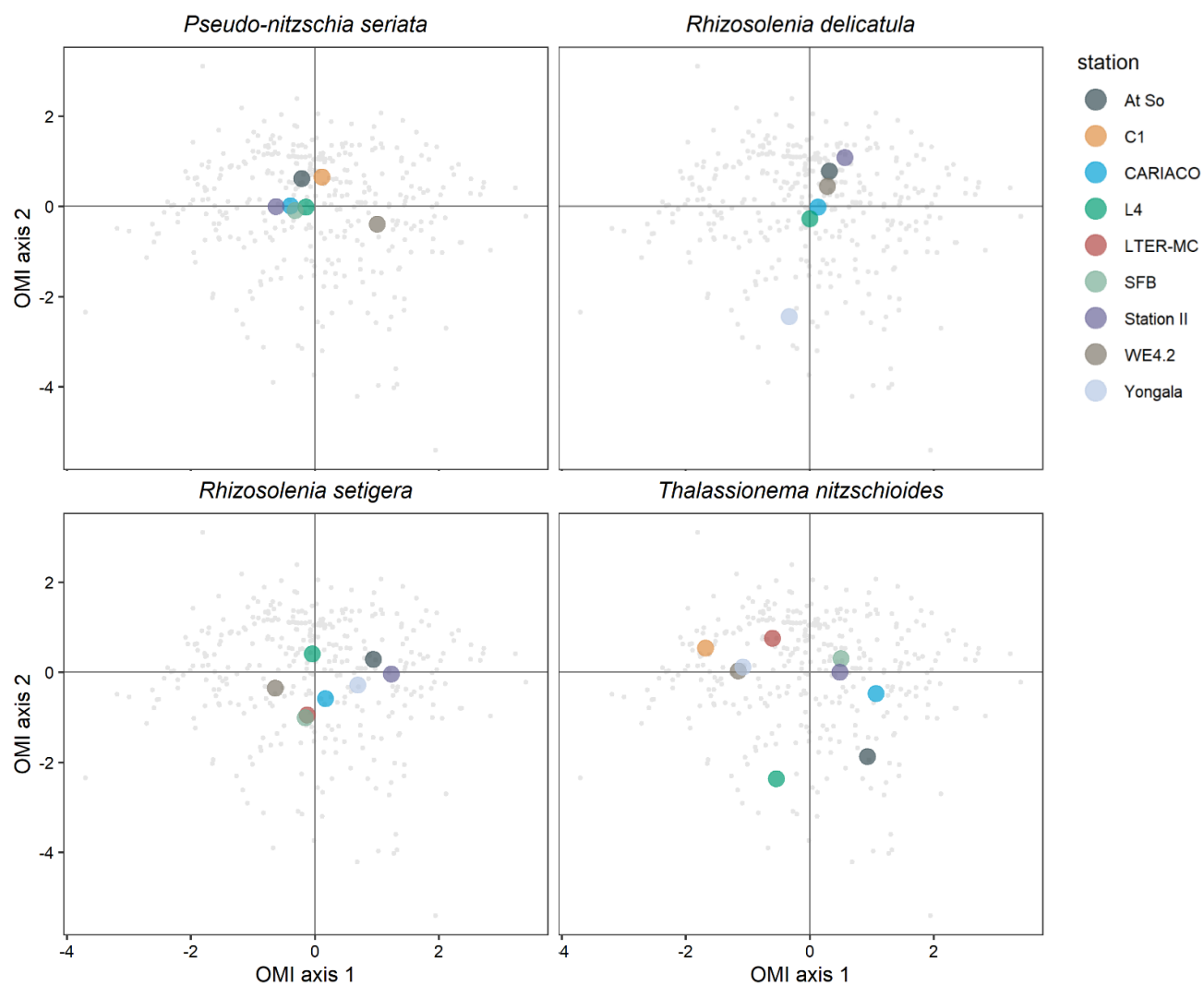
Figure 5.7 Continued.

The quantitative comparison of the areas exploited by the species revealed that the same species tend to share, on average, a higher percentage of the area than that with different species (Fig. 5.8). Nevertheless, the results of ANOVA indicated that, statistically, this was true only 4 species (Tab. 12).

Table 12 Significance of the ANOVA test performed comparing the proportion of shared area among same species with respect to different species. Significant species (in bold) shared a proportion of area in the multidimensional space significantly higher than that shared with random subsamples of different species

| species | Sum Sq. | Mean Sq. | F value | pvalue |
|---|---|---|---|---|
| Asterionellopsis glacialis | 528.41 | 528.41 | 1.14 | 0.2893 |
| Cerataulina pelagica | 28.50 | 28.50 | 0.09 | 0.7608 |
| **Cylindrotheca closterium** | 3242.68 | 3242.68 | 12.80 | 0.0006 |
| **Dactyliosolen fragilissimus** | 1603.57 | 1603.57 | 6.32 | 0.0130 |
| Leptocylindrus danicus | 335.30 | 335.30 | 0.98 | 0.3240 |
| Prorocentrum micans | 634.47 | 634.47 | 1.46 | 0.2317 |
| **Pseudo-nitzschia seriata** | 1184.86 | 1184.86 | 3.97 | 0.0497 |
| **Rhizosolenia delicatula** | 4459.80 | 4459.80 | 17.53 | 0.0002 |
| Rhizosolenia setigera | 115.29 | 115.29 | 0.34 | 0.5616 |
| Thalassionema nitzschioides | 14.19 | 14.19 | 0.05 | 0.8325 |
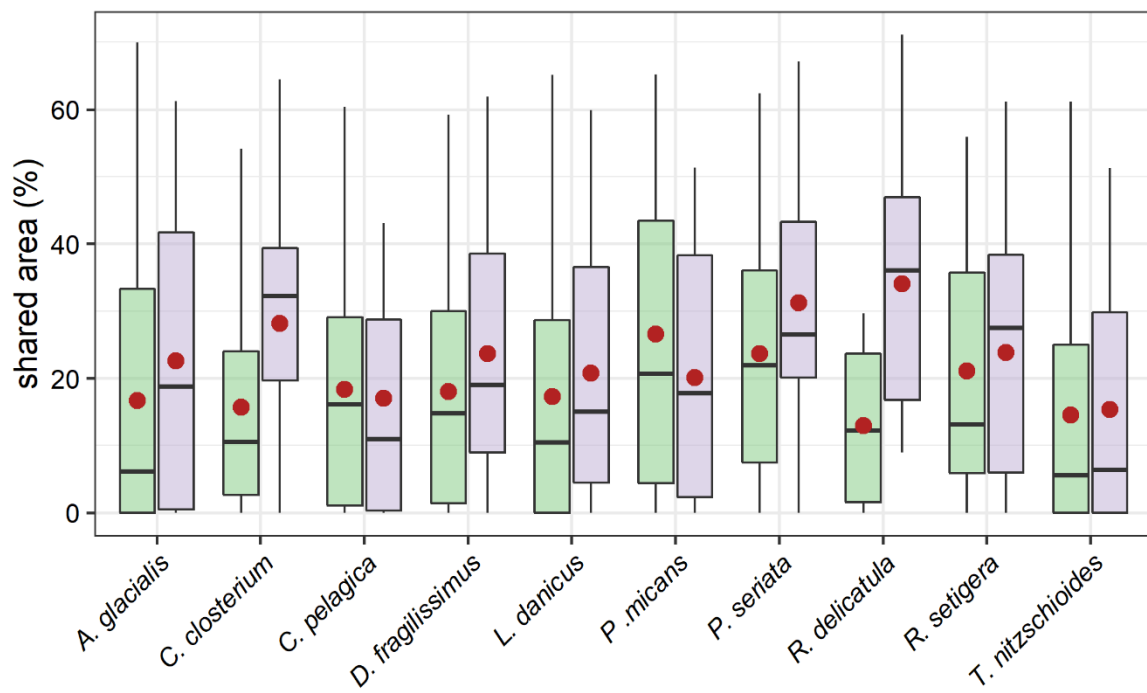
Figure 5.8 Proportion of shared area in the multidimensional environmental space. Green boxplot refers to the shared area of same species with respect to different species, whereas green area is the shared area common to the same species in different locations.

# 5.4 Discussion

The identification of phytoplankton species' niche is a quite complex task given the uncertainty associated with the correct identification of phytoplankton species and the scarcity of field observations. Many efforts aimed to characterize species best performance (in terms of growth rate) were made under controlled conditions although it is not clear whether the resulting information can be successfully applied in the natural environment. Indeed, biological interactions are likely to play an important role in how a species interacts with the environment, and also because in natural habitats species experience changes of multiple environmental drivers (Brennan et al., 2017). Even when field data are available, they rarely include species counts and extensive seasonal coverage which, as highlighted in previous chapters, provide crucial information on the temporal variability associated with species-specific biological processes.

In this study, by integrating multiple time series of phytoplankton abundance, I have exploited temporally regular observations from different biogeographical regions to compare the ecological niche of widespread coastal species. The location selected included very heterogeneous environments and provided an ideal set of observations useful to test the degree of adaptability of individual species. I am aware that niche models set up in this study lack at least of one important niche dimension that is the grazing pressure. Since the coastal areas analysed in this study are very different from each other, the consumer communities may be divergent in terms of diversity, but even if they are not, it has been shown how pelagic trophic networks structure and reorganize considerably even in the same system (D 'Alelio et al., 2015). These conditions could result in different trophic relationships between the consumer communities relative to each area and the phytoplankton species analysed considered here, invalidating partially the realized ecological niches modelled in this study.

The first point on which to discuss is the fitness of a species in a given environment is represented by its occurrence and abundance. Under this perspective, it could be argued that since *Leptocylindrus danicus* showed the highest abundance and proportion of occurrence at *LTER-MC* (Gulf of Naples) it is more adapted to the Gulf of Naples than to other environments. Nevertheless, given the heterogeneity of the hydrological and hydrographic features of the regions considered in this study as well as the differences in

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sampling procedures among the time series, a discussion based on the parameters of occurrence and abundance would be limiting and not adequate to cover the complexity of the topic. Hence, the reconstruction of the realized niches based on selected samples from species temporal variability relative to each area is likely to be more appropriate to address the ecological characteristics of the species ecology.

Phytoplankton species showed a substantial difference in the range of environmental parameters during their annual maxima at different sites, and these results supported the idea of niche divergence rather than niche conservatism. In comparing habitat preferences of several bloom-forming species from 86 coastal sites (estuaries and lagoons within the 35°-65° latitudinal range), Carstensen et al. (2015) reported a certain convergence among sites in the thermal niche of *Cerataulina pelagica* and *Dactyliosen fragilissimus* which, over the range considered, preferred intermediate and high temperatures respectively. However, in my study that takes into account data from a wider latitudinal range, including tropical (*Yongala*, *CARIACO*) and Mediterranean sites (*LTER-MC*, *C1*), both *C. pelagica* and *D. fragilissimus* appeared to tolerate a wider range of temperatures than that reported by Carstensen et al. (2015).

Several hypotheses can explain the divergences in the range of phytoplankton biogeographical niches. First, many of the widespread phytoplankton species have undergone deep taxonomic investigations that have revealed a much higher diversity than that appreciated by gross morphological characters in light microscopy (LM). Such studies have led to an increase in the number of species in some genera, which are not always identifiable in LM. This is the case of the widespread taxa *Skeletonema costatum* (Sarno et al., 2005), *Pseudo-nitzschia delicatissma* (Amato et al., 2007), *Leptocylindrus danicus* (Nanjappa et al., 2013), and *Asterionellopsis glacialis* (Kaczmarska et al., 2014), which have all proven to consist of several distinct species. The taxonomic changes related to these taxa have not always been applied in species identification, and some species names that are present in the time-series checklists are not updated. A particular case in our global time series analysis is that of *Pseudo-nitzschia seriata*, a name that has been often used to lump several different species of this needle-like, chain-forming diatom genus, sharing the characteristic of relatively wide valves but otherwise not distinguishable in LM.  In addition to interspecific differences, intraspecific genetic variation as a result of adaptive evolutionary processes may be reflected by ecological differences. Both interspecific and

intraspecific variability in the genetic pool of phytoplankton species, widely observed even among populations in the same area (e.g., Amato et al., 2007), whereby different strains or cryptic species can have different niches. The molecular analyses performed on the population of *Thalassionema* spp. in Narragansett Bay also revealed a previously underestimated diversity characterized by a complex of cryptic species showing different thermal niches (Rynearson et al., 2020). Something similar is likely to concern also the taxa considered in this study, and which could explain the occurrence of some species along with a wide range of physical-chemical conditions in their respective area. Indeed, the environmental variability in some regions almost reaches a connotation of environmental disturbance on which measure niches' plasticity. The *WE4.2*, *At So* and *Station II* stations are located in coastal areas characterized by a considerable variability of salinity and nutrients to which the species are well adapted. Similarly, *Rhizosolenia setigera* and *Pseudo-nitzschia seriata* showed their maximums along a temperature range of about 19 °C at Narragansett Bay and Cheesapeake Bay respectively.

Therefore, bringing these observations at the biogeographical level, it is quite reasonable to hypothesize that the niche divergence shown by the same species in different environments would be associated with genetic, and thus evolutionary processes. Experimental manipulations revealed that several phytoplankton species showed high adaptive responses to many environmental stressors (see Collins et al., 2014 and Reusch & Boyd, 2013). Lohbeck et al. (2012) found strong evidence of evolutionary adaptation to increasing $CO_2$ in several strains of *Emiliania huxleyi* after approximately 1 year (~500 generations), while Jin and Agustí (2018) reported pieces of evidence of evolutionary adaptations of four diatoms to a stable increase of temperature. Although these studies considered a single environmental driver, similar evolutionary adaptation patterns were also found in mesocosm experiments (Schaum et al., 2017), and a recent study reported that evolutionary adaptation is stronger when primary producers experience conditions more similar to natural habitats, characterized by the persistence of multiple environment drivers (Brennan et al., 2017). Consistent with the experiments described above, studies conducted on time series have reported a gradual modification of the niche of different phytoplankton species to environmental changes (Ajani et al., 2018; Chivers et al., 2017; Irwin et al., 2015a), supporting the hypothesis that phytoplankton populations adapt quite rapidly to

environmental changes although the range of changes (temperature) observed in the field was much smaller than those analysed under controlled experimental conditions.

To the best of my knowledge, there are no other studies specifically targeted to construct and compare the niche of phytoplankton species in different environments based on time-series data, therefore I will refer to some biogeographic studies in the following discussion, despite the differences in methods used and areas of investigation covered.

Results from analyses performed on phytoplankton presence-absence data on the North Atlantic Ocean (Irwin et al., 2012) and the open ocean at a global scale (Brun et al., 2015b), showed that mixed layer depth, temperature and irradiance were the most important factors characterizing phytoplankton species' niche. Although in this study I did not take into account the mixed layer depth, I found that the majority of species showed a great dispersion along the nutrient gradient, and that temperature, together with irradiance (indexed by day length) had the most important contribution in discriminating species' niches. However, although both Irwin et al. (2012) and Brun et al. (2015b) reported a certain degree of niche overlapping among many taxa, they recognized quite clearly a separation between the environment exploited by different phytoplankton functional groups (i.e., diatoms, dinoflagellates, coccolithophores). My data did not cover diversity at the scale of functional groups except for the dinoflagellates *P. micans* all other species were diatoms. Nevertheless, the set of diatoms considered in my study was characterized by very different sizes and shapes, a diversity of morphological traits which are generally associated with the selective process exerted by the environment (Reynolds, 2006; see Naselli-Flores et al., 2007). My results did not show any relation between morphology and environment. Indeed, many *Thalassionema nitzschioides* and *Pseudo-nitzschia seriata* showed a preference for relatively high-light conditions despite their characteristic elongated shapes, which would represent adaptations to light-limited environments (Charalampous et al., 2018). I have also observed an almost random distribution of different-sized species in the environmental gradient, although the role of size in influencing many functional traits and physiological processes is widely recognised (Litchman & Klausmeier; 2008).

However, it is important to emphasize that there is a substantial difference between the open-ocean environment investigated by Brun and Irwin, and the coastal environment analysed in this study. Coastal phytoplankton populations are likely characterized by a lower dispersal compared to those populating the open ocean, and it is conceivable that they

undergo a strong characterization by the environmental and trophic peculiarities of the specific coastal area (Carstensen et al., 2015).

Consistent with this hypothesis, Olli et al. (2015) measured the evolutionary dissimilarity among phytoplankton communities collected in different coastal areas and reported a clear geographical characterization. Similarly, a recent study conducted on both molecular and morphological phytoplankton data highlighted the predominant role of the spatial factor on environmental variability in structuring the composition of the communities (Spatharis et al., 2019). Furthermore, recent experiments aimed at characterizing the adaptive potential of phytoplankton revealed a higher rate of molecular evolution in fluctuating environments (Schaum et al., 2016; 2018) that are typical of coastal systems, which provides further evidence in support of the hypothesis of an evolutionary process associated to region-specific peculiarities of the environment, resulting in the niche divergence of phytoplankton species observed in this study.

# 6 General conclusions and future perspectives

The objective of my thesis was to shed light on the main processes driving phytoplankton variability at different temporal scales. To this aim, I used an extensive set of data obtained from temporal environmental and biological observations spanning more than 25 years and considered levels of organization encompassing species, functional groups and the whole community. The results of this thesis provided new and interesting insights into the variability patterns of primary producers at the seasonal and interannual scale in relation to the environment in a pelagic Mediterranean coastal system, at the same time contributing to a better understanding of several issues related to phytoplankton ecology.

The first part of the thesis provided a detailed description of the biological and abiotic context of the study area 16 years after the first comprehensive description of the planktonic system (Ribera d'Alcalà et al., 2004). That study, as well as other efforts (Zingone et al., 2003; Zingone & Sarno, 2001), noticed the annual recurrence of the main phytoplankton species, but no statistical analysis nor any extensive characterization of the temporal patterns of phytoplankton of the Gulf of Naples had ever been attempted. As argued in the Introduction, and in Chapters 2 and 4, the issue of the temporal regularity in marine phytoplankton is of considerable importance given the possible effects on upper trophic levels, and because it provides a baseline to decipher long-term dynamics in marine systems (Ji et al., 2010). Therefore, I planned to investigate the issue of the temporal recurrence of primary producers in detail and assess this feature quantitatively in relation to environmental patterns.

The results of this exercise highlighted a consistent periodical pattern in most of the analysable species (i.e., for which data were adequate). The most striking result of my thesis is indeed the predominant temporal regularity of phytoplankton despite a high-variable environment. Noteworthy, such result is even more important considering that the study area is a coastal system which, apart from being characterized by strong seasonal and interannual fluctuations in the main oceanographic physical and chemical parameters (temperature, salinity and inorganic nutrients), is located in one of the most densely

populated areas in Europe and inevitably subject to perturbations of various nature including organic and inorganic pollution (Tornero & Ribera d'Alcalà, 2014). Although recurrence patterns were already reported in other phytoplankton time series (Aubry et al., 2012; Karentz & Smayda, 1984; Scharfe & Wiltshire, 2019), this is the first time that they are robustly demonstrated, against environmental variability, for the whole community, and over almost 30 years.

In support of my findings are a wide variety of analytical tools, from relatively simple univariate methods such as periodograms to multivariate techniques including STATICO (Thioulouse et al., 2004) and Bray-Curtis pseudocorrelation, to more sophisticated machine-learning approaches as discriminant function analysis and random forest. In particular, it is important to underline the relationships between Chapter 2 and Chapter 4 where quantitative results and conclusions converged despite the application of a different methodological approach on a different data structure. All these methods have provided very consistent results with each other and highlighted two main points: a periodic pattern prevailing in most of the taxa, and a relative uncoupling of taxa occurrences with respect to environmental variability. Noteworthy, these properties have been observed to persist at different scales of investigation, from single phytoplankton taxa to the whole community, and from weekly to pluriannual scale.

The possibility of exploring in detail the relationship between species' turnover and environmental variability is the result of the informative potential and the quality of the time series under study. In particular, the high taxonomic resolution and the weekly frequency are crucial to cover topics that are generally poorly explored in the current phytoplankton time series (McQuatters-Gollop et al., 2017). Chapter 4 is quite emblematic from this point of view. Indeed, the identification of species' annual highest-fitness temporal points offers a significant advance in the understanding of the dynamics of phytoplankton species to environmental variability. Although a similar approach has been used in analysing the patterns of variability for some species at Helgoland (Scharfe & Wiltshire, 2019), it has never been used for an entire community, and it has never been integrated with more sophisticated techniques aimed at evaluating the hierarchical role of individual environmental parameters. It must be stressed that the approach that has been developed in Chapter 4 is not to be meant as limited to this thesis. It can be used and further developed based on the functions of the R package '`Rplanktonanalytic`'. Moving from a theoretical

approach to a reproducible and shareable analytical framework confers additional value to this thesis. The R package developed in this study provides a homogeneous tool for the analysis and comparison of phytoplankton phenology, a research field so far little explored and tackled with different methods. Moreover, besides phytoplankton, the identification of phenological phases is relevant to various components of ecological systems, to which the tools contained in the package can be easily adapted, and further developed for specific applications.

The results showing the role of the day length in driving occurrence patterns of individual species (Chapter 4) and, more broadly, of the entire community (Chapter 2) were quite new, considering similar studies on phytoplankton time-series based on optical identification methods. Interestingly, my results are similar to those obtained from HTS-based time-series analyses (e.g. Lambert et al., 2018; Nagarkar et al., 2018; Marquardt et al., 2016; Kim et al., 2014; Gilbert et al., 2012), a relevant convergence for several reasons. First, the predominant periodicity of phytoplankton and its relationship with day length are properties that are not purely local, that is, observed only at LTER-MC in the Gulf of Naples, but also emerge from accurate studies conducted in other coastal areas also characterized by a highly fluctuating environment (e.g. Lambert et al., 2019). Second, the results obtained in this thesis extend the association between photoperiod and phytoplankton periodicity to the microphytoplankton component since long-term studies conducted on molecular data generally focused on smaller components of phytoplankton, mostly nano- and picophytoplankton. Finally, I showed that these relationships persisted on a decadal scale. As for the higher percentage of taxa shown to be non-periodical in molecular-based studies (see Giner et al., 2019) compared to my results, it is worth considering that the level of taxonomic resolution of the two approaches is quite different. Although metabarcoding can detect many more taxa than microscopy, and also help in the identification of cryptic species in many cases, using scarcely variable DNA fragments cause distinct species to be lumped together in many cases, which blurs species-specific phenological patterns.

There is an urgent need to integrate the knowledge of the species variability patterns related to life-cycle peculiarities with long-term observations (see D'Alelio et al., 2010), and create a synergy between experienced researchers from the two fields. This integration would also be of fundamental importance in orienting and distinguishing between the multiple sources of variability that phytoplankton populations show, and help to formulate relevant questions

in the context of long-term observations. Further, my thesis is mostly conceived on a bottom-up approach although I am aware of the relevance of biological interactions to phytoplankton variability patterns. In particular, the comprehension of trophic dynamics in the planktonic realm is still in its infancy and places serious limitations on an exhaustive understanding of the dynamics of phytoplankton in the long-term context (D'Alelio et al., 2015; 2016).

As commented above, detailed taxonomic information and high sampling frequency are rarely available in phytoplankton time series (Zingone et al., 2010). Nonetheless, it has been worth making an effort to explore the hypotheses and conclusions of this thesis on a global scale. To the best of my knowledge, this is the first time that multiannual phytoplankton time series from very different biogeographical coastal regions have been analysed to get relevant insights into individual species performances. By showing that the same species can thrive under environmental conditions varying from site to site, Chapter 5 highlighted a remarkable adaptation potential for phytoplankton, which is probably related to their high genetic variability, and has pointed to the role of the environmental selection in shaping the ecological characteristics of individual species. Besides their scientific value, the results on species niches are also relevant in a forecast and management perspective, as they contribute to the understanding of individual species' behaviour in light of future environmental changes in particularly relevant areas such as coastal areas.

However, some critical issues emerged from this global comparison that should be addressed in the future. Firstly, the data collection and homogenization phase were enormously challenging given the enormous heterogeneity of data quality and formats. As mentioned in the introduction, although efforts are being made to render phytoplankton time-series data compliant to the 'FAIR' concept (Findability, Accessibility, Interoperability, and Reusability, from Wilkinson et al., 2016), there is still much work to be done. As yet, given the heterogeneity of the operators' skills and the methods used for taxa identification, microscopy-based phytoplankton time-series are difficult to compare and misclassification is likely to occur, highlighting the need to adopt common protocols and data quality assessment procedures, as proposed by Zingone et al. (2015).

While Chapters 2 and 4 have shown the resilience of the composition of phytoplankton populations on a seasonal scale, Chapter 3 has revealed similar dynamics also in relation to multiannual environmental changes. Phytoplankton bulk properties as chlorophyll-a and

functional groups' biomass did show marked fluctuations as a result of the influence of atmospheric forcing on the general hydrology of the LTER-MC' system, but no comparable shifts or oscillations were detected in the overall community composition. An interesting result of the study described in Chapter 3 is the evidence of the quite different impact of climate variations on the system depending on the seasons, a property that is neglected when using annually aggregated data, as it occurs in the large majority of decadal time-series analyses. In fact, the alternation of the seasons which is typical of temperate areas has a tremendous impact in shaping both environmental and biological dynamics, each season having its own characteristic sets of conditions to which different organisms adapt differently. My results show the relevance to evaluate the effects of climate change considering these differences among seasons and integrate the results to have a coherent view of the changes taking place (Mat, 2018). Yet, nonlinear responses are likely to occur in the biological component of complex environments as the pelagic realm. A good example is the complex, nonlinear response of phytoplankton groups to precipitations, likely deriving from the interplay of environmental constraints and life strategies, compared to the mechanistic response of the abiotic component. Beyond the interesting scientific aspects, the analysis of 30 years of biological and environmental observations in Chapter 3 highlights the relationships between local and large-scale climate patterns (NAO), and provides a quantitative assessment of the response of coastal salinity and plankton to precipitation fluctuations, which is potentially useful for management purposes. Furthermore, Chapter 3 remarkably stresses the need to consider the different aspects of climate change, rather than infer on a single paradigmatic factor, which is generally seawater temperature, to bring to light and possibly predict future directions of changes in the complex coastal systems. Indeed, other factors such as precipitation and salinity may provide a more direct indication of the changes taking place in coastal systems and in phytoplankton communities associated with them. Finally, the results of Chapter 3 highlight the importance of the continued collection of long-term observations, because environmental changes in the marine environment rarely occur as linear trends, but rather as oscillatory phases or wave-like patterns (Wilkinson et al., 2020). Fluctuations similar to those found for precipitation and salinity at LTER-MC can be identified only by the continuous collection of biological and oceanographic data.

The role of light as a biological signal emerging from this thesis points to complex biological mechanisms regulating phytoplankton organisms, which operate both within and among species. While the role of environmental parameters in determining the structure and composition of the community is widely taken into account, little or no consideration is generally given to these endogenous mechanisms that can have a prominent influence on the observed variability as well (Zingone & Wyatt, 2005). Indeed, the large analytical and intellectual efforts towards highlighting the passive role of phytoplankton versus environmental constraints has rarely been counterbalanced by a comparable effort aimed to explore (or at least consider) the active role that phytoplankton organisms can play in virtue of their biological and life-cycle characteristics. It was very interesting to find the evidence that, in a highly fluctuating habitat as the coastal pelagic environment, the major source of phytoplankton community variability is a periodically constant signal such as day length. In most of the ecological studies on phytoplankton, light is generally conceived as a resource exploited based on species-specific traits (Edwards et al., 2015), similarly to what happens for nutrients. In fact, besides being a direct resource, in a great variety of organisms light plays a crucial role as a signal associated with the activation of certain biological processes (Bradshaw et al., 2010; Nelson et al., 2010; Putterill et al., 2004), a function that has often been overlooked in phytoplankton ecology. Nevertheless, studies focused on the molecular and cellular mechanisms of diverse phytoplankton species have revealed the role of light as a signal in activating important responses related to different phases of the life cycle, including sexual and vegetative reproduction (see Chapter 4 and references therein).

Therefore, these observations, together with the convergence between the results of this thesis and the recent studies carried out on molecular data from different marine systems, suggest that light (day length) can act as a biological signal for diverse taxa and contribute to shaping the complex of variability' patterns that we observe in long-term phytoplankton observations. Although there are encouraging data in this sense, further studies at a global scale will be needed to confirm this theory. More specifically, crucial indications could emerge by comparing the phenological patterns of phytoplankton communities as a whole along the latitudinal gradient.

In conclusion, the results in this thesis highlighted the importance of biology-driven behaviour in phytoplankton at different temporal scales and provided new insights into the understanding of phytoplankton species and community ecology. Although most of this

thesis focuses on the analysis of a single phytoplankton time series, it offers broader hints and hypotheses potentially testable in different contexts. In a historical moment characterized by rapid environmental changes, when most forecasting tools simply link species to changes in environmental gradients, the results of this thesis assume great importance to me, and demand urgent considerations on the general view of the driving forces determining the observed variability in long-term studies focusing on marine phytoplankton.

# References

Adrian, R., Gerten, D., Huber, V., Wagner, C., & Schmidt, S. R. (2012). Windows of change: Temporal scale of analysis is decisive to detect ecosystem responses to climate change. *Marine Biology*, *159*(11), 2533–2542.

Adrian, R., Wilhelm, S., & Gerten, D. (2006). Life-history traits of lake plankton species may govern their phenological response to climate warming. *Global Change Biology*, *12*(4), 652–661.

Ajani, P. A., McGinty, N., Finkel, Z. V., & Irwin, A. J. (2018). Phytoplankton realized niches track changing oceanic conditions at a long-term coastal station off Sydney Australia. *Frontiers in Marine Science*, *5*, 285.

Akar, Ö., & Güngör, O. (2012). Classification of multispectral images using Random Forest algorithm. *Journal of Geodesy and Geoinformation*, *1*(2), 105–112.

Albright, L. J., Yang, C. Z., & Johnson, S. (1993). Sub-lethal concentrations of the harmful diatoms, Chaetoceros concavicornis and C. convolutus, increase mortality rates of penned Pacific salmon. *Aquaculture*, *117*(3–4), 215–225.

Amato, A., Kooistra, W. H., Ghiron, J. H. L., Mann, D. G., Pröschold, T., & Montresor, M. (2007). Reproductive isolation among sympatric cryptic species in marine diatoms. *Protist*, *158*(2), 193–207.

Andersen, J. H., Carstensen, J., Conley, D. J., Dromph, K., Fleming-Lehtinen, V., Gustafsson, B. G., Josefson, A. B., Norkko, A., Villnäs, A., & Murray, C. (2017). Long-term temporal and spatial trends in eutrophication status of the Baltic Sea. *Biological Reviews*, *92*(1), 135–149.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*(1), 32–46.

Anderson, M. J. (2006). Distance-based tests for homogeneity of multivariate dispersions. *Biometrics*, *62*(1), 245–253.

Anderson, M. J. (2014). Permutational multivariate analysis of variance (PERMANOVA). *Wiley Statsref: Statistics Reference Online*, 1–15.

Anderson, M. J., Ellingsen, K. E., & McArdle, B. H. (2006). Multivariate dispersion as a measure of beta diversity. *Ecology Letters*, *9*(6), 683–693.

Andersson, A., Samuelsson, K., Haecky, P., & Albertsson, J. (2006). Changes in the pelagic microbial food web due to artificial eutrophication. *Aquatic Ecology*, *40*(3), 299–313.

Anneville, O., Dur, G., Rimet, F., & Souissi, S. (2018). Plasticity in phytoplankton annual periodicity: An adaptation to long-term environmental changes. *Hydrobiologia*, *824*(1), 121–141.

Atkinson, A., Harmer, R. A., Widdicombe, C. E., McEvoy, A. J., Smyth, T. J., Cummings, D. G., Somerfield, P. J., Maud, J. L., & McConville, K. (2015). Questioning the role of phenology shifts and trophic mismatching in a planktonic food web. *Progress in Oceanography*, *137*, 498–512.

Aubry, F. B., Cossarini, G., Acri, F., Bastianini, M., Bianchi, F., Camatti, E., De Lazzari, A., Pugnetti, A., Solidoro, C., & Socal, G. (2012). Plankton communities in the northern Adriatic Sea: Patterns and changes over the last 30 years. *Estuarine, Coastal and Shelf Science*, *115*, 125–137.

Auladell, A., Sánchez, P., Sánchez, O., Gasol, J. M., & Ferrera, I. (2019). Long-term seasonal and interannual variability of marine aerobic anoxygenic photoheterotrophic bacteria. *The ISME Journal*, *13*(8), 1975–1987. https://doi.org/10.1038/s41396-019-0401-4

Backer, H., Leppänen, J.-M., Brusendorff, A. C., Forsius, K., Stankiewicz, M., Mehtonen, J., Pyhälä, M., Laamanen, M., Paulomäki, H., & Vlasov, N. (2010). HELCOM Baltic Sea Action Plan–A regional programme of measures for the marine environment based on the Ecosystem Approach. *Marine Pollution Bulletin*, *60*(5), 642–649.

Barber, R. T., & Chavez, F. P. (1983). Biological consequences of el nino. *Science*, *222*(4629), 1203–1210.

Barbosa, A. B., Domingues, R. B., & Galvão, H. M. (2010). Environmental Forcing of Phytoplankton in a Mediterranean Estuary (Guadiana Estuary, South-western Iberia): A Decadal Study of Anthropogenic and Climatic Influences. *Estuaries and Coasts*, *33*(2), 324–341. https://doi.org/10.1007/s12237-009-9200-x

Barrera-Alba, J. J., Abreu, P. C., & Tenenbaum, D. R. (2019). Seasonal and inter-annual variability in phytoplankton over a 22-year period in a tropical coastal region in the southwestern Atlantic Ocean. *Continental Shelf Research*, *176*, 51–63. https://doi.org/10.1016/j.csr.2019.02.011

Beaugrand, G. (2002). Reorganization of North Atlantic Marine Copepod Biodiversity and Climate. *Science*, *296*(5573), 1692–1694. https://doi.org/10.1126/science.1071329

Beaugrand, G. (2005). Monitoring pelagic ecosystems using plankton indicators. *ICES Journal of Marine Science*, *62*(3), 333–338. https://doi.org/10.1016/j.icesjms.2005.01.002

Behrenfeld, M. J., O'Malley, R. T., Siegel, D. A., McClain, C. R., Sarmiento, J. L., Feldman, G. C., Milligan, A. J., Falkowski, P. G., Letelier, R. M., & Boss, E. S. (2006). Climate-driven trends in contemporary ocean productivity. *Nature*, *444*(7120), 752–755. https://doi.org/10.1038/nature05317

Beliaeff, B., Gros, P., Belin, C., Raffin, B., Gailhard, I., & Durbec, J.-P. (2001). 'Phytoplankton events' in French coastal waters during 1987–1997. *Oceanologica Acta*, *24*(5), 425–433.

Bethoux, J. P., Gentili, B., Morin, P., Nicolas, E., Pierre, C., & Ruiz-Pino, D. (1999). The Mediterranean Sea: A miniature ocean for climatic and environmental studies and a key for the climatic functioning of the North Atlantic. *Progress in Oceanography*, *44*(1–3), 131–146. https://doi.org/10.1016/S0079-6611(99)00023-3

Bi, J. (2012). A review of statistical methods for determination of relative importance of correlated predictors and identification of drivers of consumer liking. *Journal of Sensory Studies*, *27*(2), 87–101.

Bianchi, C. N., & Morri, C. (2000). Marine biodiversity of the Mediterranean Sea: Situation, problems and prospects for future research. *Marine Pollution Bulletin*, *40*(5), 367–376.

Bidigare, R. R., Chai, F., Landry, M. R., Lukas, R., Hannides, C. C. S., Christensen, S. J., Karl, D. M., Shi, L., & Chao, Y. (2009). Subtropical ocean ecosystem structure changes forced by North Pacific climate variations. *Journal of Plankton Research*, *31*(10), 1131–1139. https://doi.org/10.1093/plankt/fbp064

Bišová, K., & Zachleder, V. (2014). Cell-cycle regulation in green algae dividing by multiple fission. *Journal of Experimental Botany*, *65*(10), 2585–2602.

Blauw, A. N., Los, F. J., Huisman, J., & Peperzak, L. (2010). Nuisance foam events and Phaeocystis globosa blooms in Dutch coastal waters analyzed with fuzzy logic. *Journal of Marine Systems*, *83*(3–4), 115–126.

Boero, F., Kraberg, A. C., Krause, G., & Wiltshire, K. H. (2015). Time is an affliction: Why ecology cannot be as predictive as physics and why it needs time series. *Journal of Sea Research*, *101*, 12–18. https://doi.org/10.1016/j.seares.2014.07.008

Bopp, L., Monfray, P., Aumont, O., Dufresne, J.-L., Le Treut, H., Madec, G., Terray, L., & Orr, J. C. (2001). Potential impact of climate change on marine export production. *Global Biogeochemical Cycles*, *15*(1), 81–99.

Borcard, D., Gillet, F., & Legendre, P. (2018). *Numerical ecology with R*. Springer.

Borkman, D. G., & Smayda, T. J. (2009). Gulf Stream position and winter NAO as drivers of long-term variations in the bloom phenology of the diatom Skeletonema costatum "species-complex" in Narragansett Bay, RI, USA. *Journal of Plankton Research*, *31*(11), 1407–1425.

Boulesteix, A.-L., Janitza, S., Kruppa, J., & König, I. R. (2012). Overview of random forest methodology and practical guidance with emphasis on computational biology and bioinformatics. *Wiley Interdisciplinary Reviews: Data Mining and Knowledge Discovery*, *2*(6), 493–507.

Boyce, D. G., Lewis, M. R., & Worm, B. (2010). Global phytoplankton decline over the past century. *Nature*, *466*(7306), 591–596.

Boyce, D. G., Petrie, B., Frank, K. T., Worm, B., & Leggett, W. C. (2017). Environmental structuring of marine plankton phenology. *Nature Ecology & Evolution*, *1*(10), 1484–1494. https://doi.org/10.1038/s41559-017-0287-3

Boyd, P. W., & Trull, T. W. (2007). Understanding the export of biogenic particles in oceanic waters: Is there consensus? *Progress in Oceanography*, *72*(4), 276–312.

Braarud, T., & Pappas, I. (1951). *Experimental Studies on the dinoflagellate Peridinium triquetrum Ehrb. Lebour*. Dybwad i komm.

Bracco, A., Provenzale, A., & Scheuring, I. (2000). Mesoscale vortices and the paradox of the plankton. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1454), 1795–1800.

Bradshaw, W. E., & Holzapfel, C. M. (2010). Light, time, and the physiology of biotic response to rapid climate change in animals. *Annual Review of Physiology*, *72*, 147–166.

Brannock, P. M., Ortmann, A. C., Moss, A. G., & Halanych, K. M. (2016). Metabarcoding reveals environmental factors influencing spatio-temporal variation in pelagic micro-eukaryotes. *Molecular Ecology*, *25*(15), 3593–3604.

Bray, J. R., & Curtis, J. T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs*, *27*(4), 325–349.

Breiman, L. (2001). Random forests. *Machine Learning*, *45*(1), 5–32.

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., Garçon, V., Gilbert, D., Gutiérrez, D., & Isensee, K. (2018). Declining oxygen in the global ocean and coastal waters. *Science*, *359*(6371).

Brennan, G. L., Colegrave, N., & Collins, S. (2017). Evolutionary consequences of multidriver environmental change in an aquatic primary producer. *Proceedings of the National Academy of Sciences*, *114*(37), 9930–9935.

Briceño, H. O., & Boyer, J. N. (2010). Climatic controls on phytoplankton biomass in a sub-tropical estuary, Florida Bay, USA. *Estuaries and Coasts*, *33*(2), 541–553.

Brierley, A. S., & Kingsford, M. J. (2009). Impacts of climate change on marine organisms and ecosystems. *Current Biology*, *19*(14), R602–R614.

Brito, A. C., Moita, T., Gameiro, C., Silva, T., Anselmo, T., & Brotas, V. (2015). Changes in the Phytoplankton Composition in a Temperate Estuarine System (1960 to 2010). *Estuaries and Coasts*, *38*(5), 1678–1691. https://doi.org/10.1007/s12237-014-9900-8

Brun, P., Kiørboe, T., & Payne, M. R. (2015a). Measuring evolutionary adaptation of phytoplankton with local field observations. *Proceedings of the National Academy of Sciences*, *112*(38), E5223–E5224. https://doi.org/10.1073/pnas.1513353112

Brun, P., Vogt, M., Payne, M. R., Gruber, N., O'brien, C. J., Buitenhuis, E. T., Le Quéré, C., Leblanc, K., & Luo, Y.-W. (2015b). Ecological niches of open ocean phytoplankton taxa. *Limnology and Oceanography*, *60*(3), 1020–1038.

Bunse, C., & Pinhassi, J. (2017). Marine Bacterioplankton Seasonal Succession Dynamics. *Trends in Microbiology*, *25*(6), 494–505. https://doi.org/10.1016/j.tim.2016.12.013

Burson, A., Stomp, M., Greenwell, E., Grosse, J., & Huisman, J. (2018). Competition for nutrients and light: Testing advances in resource competition with a natural phytoplankton community. *Ecology*, *99*(5), 1108–1118.

Burson, A., Stomp, M., Mekkes, L., & Huisman, J. (2019). Stable coexistence of equivalent nutrient competitors through niche differentiation in the light spectrum. *Ecology*, *100*(12), e02873.

Byun, D.-S., Wang, X. H., Hart, D. E., & Cho, Y.-K. (2005). Modeling the effect of freshwater inflows on the development of spring blooms in an estuarine embayment. *Estuarine, Coastal and Shelf Science*, *65*(1–2), 351–360.

Cabrini, M., Fornasaro, D., Cossarini, G., Lipizer, M., & Virgilio, D. (2012). Phytoplankton temporal changes in a coastal northern Adriatic site during the last 25 years. *Estuarine, Coastal and Shelf Science*, *115*, 113–124.

Cailliez, F. (1983). The analytical solution of the additive constant problem. *Psychometrika*, *48*(2), 305–308.

Caloiero, T., Coscarelli, R., Ferrari, E., & Mancini, M. (2011). Precipitation change in Southern Italy linked to global scale oscillation indexes. *Natural Hazards and Earth System Sciences*, *11*(6), 1683–1694.

Carey, C. C., Hanson, P. C., Lathrop, R. C., & St. Amand, A. L. (2016). Using wavelet analyses to examine variability in phytoplankton seasonal succession and annual periodicity. *Journal of Plankton Research*, *38*(1), 27–40.

Carrada, G. C., Fresi, E., Marino, D., Modigh, M., & D'Alcalà, M. R. (1981). Structural analysis of winter phytoplankton in the Gulf of Naples. *Journal of Plankton Research*, *3*(2), 291–314.

Carrada, G. C., Hopkins, T. S., Bonaduce, G., Ianora, A., Marino, D., Modigh, M., Ribera, D. M., & di Scotto, C. B. (1980). Variability in the hydrographic and biological features of the Gulf of Naples. *Marine Ecology*, *1*(2), 105–120.

Carstensen, J., Klais, R., & Cloern, J. E. (2015). Phytoplankton blooms in estuarine and coastal waters: Seasonal patterns and key species. *Estuarine, Coastal and Shelf Science*, *162*, 98–109. https://doi.org/10.1016/j.ecss.2015.05.005

Charalampous, E., Matthiessen, B., & Sommer, U. (2018). Light effects on phytoplankton morphometric traits influence nutrient utilization ability. *Journal of Plankton Research*, *40*(5), 568–579.

Chavez, F. P., Messié, M., & Pennington, J. T. (2011). Marine Primary Production in Relation to Climate Variability and Change. *Annu. Rev. Mar. Sci*, *3*, 227–260.

Chavez, F. P., Strutton, P. G., Friederich, G. E., Feely, R. A., Feldman, G. C., Foley, D. G., & McPhaden, M. J. (1999). Biological and chemical response of the equatorial Pacific Ocean to the 1997-98 El Niño. *Science*, *286*(5447), 2126–2131.

CHISHOLM, S. W. (1981). Temporal patterns of cell division of unicellualar algae. *Can. Bull. Fish. Aquat. Sci.*, *210*, 150–181.

Chivers, W. J., Edwards, M., & Hays, G. C. (2020). Phenological shuffling of major marine phytoplankton groups over the last six decades. *Diversity and Distributions*, *26*(5), 536–548.

Chivers, W. J., Walne, A. W., & Hays, G. C. (2017). Mismatch between marine plankton range movements and the velocity of climate change. *Nature Communications*, *8*, 14434. https://doi.org/10.1038/ncomms14434

Cianelli, D., D'Alelio, D., Uttieri, M., Sarno, D., Zingone, A., Zambianchi, E., & d'Alcalà, M. R. (2017). Disentangling physical and biological drivers of phytoplankton dynamics in a coastal system. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-15880-x

Cleveland, R. B., Cleveland, W. S., McRae, J. E., & Terpenning, I. (1990). STL: A seasonal-trend decomposition. *J. Off. Stat*, *6*(1), 3–73.

Cloern, J. (2001). Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series*, *210*, 223–253. https://doi.org/10.3354/meps210223

Cloern, J., & Dufford, R. (2005). Phytoplankton community ecology: Principles applied in San Francisco Bay. *Marine Ecology Progress Series*, *285*, 11–28. https://doi.org/10.3354/meps285011

Cloern, J. E. (1987). Turbidity as a control on phytoplankton biomass and productivity in estuaries. *Continental Shelf Research*, *7*(11–12), 1367–1381.

Cloern, J. E., Abreu, P. C., Carstensen, J., Chauvaud, L., Elmgren, R., Grall, J., Greening, H., Johansson, J. O. R., Kahru, M., & Sherwood, E. T. (2016). Human activities and climate variability drive fast-paced change across the world's estuarine–coastal ecosystems. *Global Change Biology*, *22*(2), 513–529.

Cloern, J. E., & Jassby, A. D. (2008). Complex seasonal patterns of primary producers at the land–sea interface. *Ecology Letters*, *11*(12), 1294–1303.

Cloern, J. E., & Jassby, A. D. (2010). Patterns and scales of phytoplankton variability in estuarine–coastal ecosystems. *Estuaries and Coasts*, *33*(2), 230–241.

Cloern, J. E., Jassby, A. D., Thompson, J. K., & Hieb, K. A. (2007). A cold phase of the East Pacific triggers new phytoplankton blooms in San Francisco Bay. *Proceedings of the National Academy of Sciences*, *104*(47), 18561–18565.

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Ben Rais Lasram, F., Aguzzi, J., Ballesteros, E., Bianchi, C. N., Corbera, J., Dailianis, T., Danovaro, R., Estrada, M., Froglia, C., Galil, B. S., Gasol, J. M., Gertwagen, R., Gil, J., Guilhaumon, F., Kesner-Reyes, K., … Voultsiadou, E. (2010). The Biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats. *PLoS ONE*, *5*(8), e11842. https://doi.org/10.1371/journal.pone.0011842

Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W. J., Johns, T., & Krinner, G. (2013). Long-term climate change: Projections, commitments and irreversibility. In *Climate Change 2013-The Physical Science Basis: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 1029–1136). Cambridge University Press.

Collins, S., Rost, B., & Rynearson, T. A. (2014). Evolutionary potential of marine phytoplankton under ocean acidification. *Evolutionary Applications*, *7*(1), 140–155.

Conte, M., Giuffrida, A., & Tedesco, S. (1989). *The Mediterranean oscillation: Impact on precipitation and hydrology in Italy*.

Conversi, A., Fonda Umani, S., Peluso, T., Molinero, J. C., Santojanni, A., & Edwards, M. (2010). The Mediterranean Sea regime shift at the end of the 1980s, and intriguing parallelisms with other European basins. *Plos One*, *5*(5), e10633.

Costa, O. G. (1836). *Fauna del Regno di Napoli, ossia enumerazione di tutti gli animali che abitano le diverse regioni di questo regno e le acque che le bagnano contenente la descrizione de'nuovi o poco esattamente conosciuti...: Crostacei ed aracneidi* (Vol. 6). dai torchi di Azzolino e compagno.

Countway, P. D., Vigil, P. D., Schnetzer, A., Moorthi, S. D., & Caron, D. A. (2010). Seasonal analysis of protistan community structure and diversity at the USC Microbial Observatory (San Pedro Channel, North Pacific Ocean). *Limnology and Oceanography*, *55*(6), 2381–2396.

Cram, J. A., Chow, C.-E. T., Sachdeva, R., Needham, D. M., Parada, A. E., Steele, J. A., & Fuhrman, J. A. (2015). Seasonal and interannual variability of the marine bacterioplankton community

throughout the water column over ten years. *The ISME Journal*, *9*(3), 563–580. https://doi.org/10.1038/ismej.2014.153

Cushing, D. H. (1959). The seasonal variation in oceanic production as a problem in population dynamics. *ICES Journal of Marine Science*, *24*(3), 455–464.

Dale, V. H., & Beyeler, S. C. (2001). Challenges in the development and use of ecological indicators. *Ecological Indicators*, *1*(1), 3–10.

D'Alelio, D., d'Alcala, M. R., Dubroca, L., Zingone, A., & Montresor, M. (2010). The time for sex: A biennial life cycle in a marine planktonic diatom. *Limnology and Oceanography*, *55*(1), 106–114.

D'Alelio, D., Libralato, S., Wyatt, T., & d'Alcalà, M. R. (2016). Ecological-network models link diversity, structure and function in the plankton food-web. *Scientific Reports*, *6*(1), 1–13.

D'Alelio, D., Mazzocchi, M. G., Montresor, M., Sarno, D., Zingone, A., Di Capua, I., Franzè, G., Margiotta, F., Saggiomo, V., & Ribera d'Alcalà, M. (2015). The green–blue swing: Plasticity of plankton food-webs in response to coastal oceanographic dynamics. *Marine Ecology*, *36*(4), 1155–1170.

D'Alelio, D., Rampone, S., Cusano, L. M., Morfino, V., Russo, L., Sanseverino, N., Cloern, J. E., & Lomas, M. W. (2020). Machine learning identifies a strong association between warming and reduced primary productivity in an oligotrophic ocean gyre. *Scientific Reports*, *10*(1), 1–12.

Darwin, C. (1859). *On the Origin of Species by Means of Natural Selection Or the Preservation of Favoured Races in the Struggle for Life.* International Book Company.

Daufresne, M., Lengfellner, K., & Sommer, U. (2009). Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences*, *106*(31), 12788–12793.

Davies, R. B. (1987). Hypothesis testing when a nuisance parameter is present only under the alternative. *Biometrika*, *74*(1), 33–43.

De Angelis, C. M. (1958). Seasonal variation of plankton collected in the Gulf of Naples during 1954–1955. *Rapp Comm Int Mer Médit*, *14*, 245–254.

De Clerck, O., Bogaert, K. A., & Leliaert, F. (2012). Diversity and evolution of algae: Primary endosymbiosis. *Advances in Botanical Research*, *64*, 55–86.

De Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., Lara, E., Berney, C., Le Bescot, N., & Probert, I. (2015). Eukaryotic plankton diversity in the sunlit ocean. *Science*, *348*(6237).

Degerlund, M., Huseby, S., Zingone, A., Sarno, D., & Landfald, B. (2012). Functional diversity in cryptic species of Chaetoceros socialis Lauder (Bacillariophyceae). *Journal of Plankton Research*, *34*(5), 416–431.

Depauw, F. A., Rogato, A., Ribera d'Alcalá, M., & Falciatore, A. (2012). Exploring the molecular basis of responses to light in marine diatoms. *Journal of Experimental Botany*, *63*(4), 1575–1591.

Deutsch, C., & Weber, T. (2012). Nutrient ratios as a tracer and driver of ocean biogeochemistry. *Annual Review of Marine Science*, *4*, 113–141.

Devlin, M., Best, M., Coates, D., Bresnan, E., O'Boyle, S., Park, R., Silke, J., Cusack, C., & Skeats, J. (2007). Establishing boundary classes for the classification of UK marine waters using phytoplankton communities. *Marine Pollution Bulletin*, *55*(1–6), 91–103.

Dickey, T. D. (2003). Emerging ocean observations for interdisciplinary data assimilation systems. *Journal of Marine Systems*, *40*, 5–48.

Dinsmore, R. P. (1996). Alpha, Bravo, Charlie. *Oceanus*, *39*(2), 9–11.

Directive, S. F. (2008). Directive 2008/56/EC of the European Parliament and of the Council. *Journal). Council Decision Of*.

Dolédec, S., Chessel, D., & Gimaret-Carpentier, C. (2000). Niche separation in community analysis: A new method. *Ecology*, *81*(10), 2914–2927.

Doney, S. C. (2006). Plankton in a warmer world. *Nature*, *444*(7120), 695–696.

Dray, S., Chessel, D., & Thioulouse, J. (2003). Co-inertia analysis and the linking of ecological data tables. *Ecology*, *84*(11), 3078–3089.

Duarte, C. M., Cebrián, J., & Marbá, N. (1992). Uncertainty of detecting sea change. *Nature*, *356*(6366), 190–190.

Dufrêne, M., & Legendre, P. (1997). SPECIES ASSEMBLAGES AND INDICATOR SPECIES:THE NEED FOR A FLEXIBLE ASYMMETRICAL APPROACH. *Ecological Monographs*, *67*(3), 345–366. https://doi.org/10.1890/0012-9615(1997)067[0345:SAAIST]2.0.CO;2

Edwards, K. F. (2016). Community trait structure in phytoplankton: Seasonal dynamics from a method for sparse trait data. *Ecology*, *97*(12), 3441–3451.

Edwards, K. F., Litchman, E., & Klausmeier, C. A. (2013). Functional traits explain phytoplankton community structure and seasonal dynamics in a marine ecosystem. *Ecology Letters*, *16*(1), 56–63. https://doi.org/10.1111/ele.12012

Edwards, M., & Richardson, A. J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature*, *430*(7002), 881–884.

Eilertsen, H. C., Sandberg, S., & Tøllefsen, H. (1995). Photoperiodic control of diatom spore growth: A theory to explain the onset of phytoplankton blooms. *Marine Ecology Progress Series. Oldendorf*, *116*(1), 303–307.

Eilertsen, H. C., & Wyatt, T. (2000). Phytoplankton models and life history strategies. *South African Journal of Marine Science*, *22*(1), 323–338.

Elton, C. (1927). *Animal ecology*. Sidgwick, & Jackson.

Enfield, D. B., Mestas-Nuñez, A. M., & Trimble, P. J. (2001). The Atlantic multidecadal oscillation and its relation to rainfall and river flows in the continental US. *Geophysical Research Letters*, *28*(10), 2077–2080.

Escoufier, Y. (1973). Le traitement des variables vectorielles. *Biometrics*, 751–760.

Falkowski, P. (2012). Ocean science: The power of plankton. *Nature*, *483*(7387), S17–S20.

Falkowski, P. G., Barber, R. T., & Smetacek, V. (1998). Biogeochemical controls and feedbacks on ocean primary production. *Science*, *281*(5374), 200–206.

Feuchtmayr, H., Thackeray, S. J., Jones, I. D., De Ville, M., Fletcher, J., James, B. E. N., & Kelly, J. (2012). Spring phytoplankton phenology–are patterns and drivers of change consistent among lakes in the same climatological region? *Freshwater Biology*, *57*(2), 331–344.

Field, C. B., Behrenfeld, M. J., Randerson, J. T., & Falkowski, P. (1998). Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science*, *281*(5374), 237–240.

Finkel, Z. V., Katz, M. E., Wright, J. D., Schofield, O. M., & Falkowski, P. G. (2005). Climatically driven macroevolutionary patterns in the size of marine diatoms over the Cenozoic. *Proceedings of the National Academy of Sciences*, *102*(25), 8927–8932.

Finkel, Z. V., Sebbo, J., Feist-Burkhardt, S., Irwin, A. J., Katz, M. E., Schofield, O. M. E., Young, J. R., & Falkowski, P. G. (2007). A universal driver of macroevolutionary change in the size of marine phytoplankton over the Cenozoic. *Proceedings of the National Academy of Sciences*, *104*(51), 20416–20420.

Finney, J. C., Pettay, D. T., Sampayo, E. M., Warner, M. E., Oxenford, H. A., & LaJeunesse, T. C. (2010). The relative significance of host–habitat, depth, and geography on the ecology, endemism, and speciation of coral endosymbionts in the genus Symbiodinium. *Microbial Ecology*, *60*(1), 250–263.

Flombaum, P., Gallegos, J. L., Gordillo, R. A., Rincón, J., Zabala, L. L., Jiao, N., Karl, D. M., Li, W. K., Lomas, M. W., & Veneziano, D. (2013). Present and future global distributions of the marine

Cyanobacteria Prochlorococcus and Synechococcus. *Proceedings of the National Academy of Sciences*, *110*(24), 9824–9829.

Forrest, J., & Miller-Rushing, A. J. (2010a). Introduction: Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions: Biological Sciences*, 3101–3112.

Forrest, J., & Miller-Rushing, A. J. (2010b). *Toward a synthetic understanding of the role of phenology in ecology and evolution*. The Royal Society.

Franke, H.-D., Buchholz, F., & Wiltshire, K. H. (2004). Ecological long-term research at Helgoland (German Bight, North Sea): Retrospect and prospect—An introduction. *Helgoland Marine Research*, *58*(4), 223–229.

Friedland, K. D., Mouw, C. B., Asch, R. G., Ferreira, A. S. A., Henson, S., Hyde, K. J., Morse, R. E., Thomas, A. C., & Brady, D. C. (2018). Phenology and time series trends of the dominant seasonal phytoplankton bloom across global scales. *Global Ecology and Biogeography*, *27*(5), 551–569.

Fu, W., Randerson, J. T., & Moore, J. K. (2016). Climate change impacts on net primary production (NPP) and export production (EP) regulated by increasing stratification and phytoplankton community structure in the CMIP5 models. *Biogeosciences*, *13*(18), 5151–5170.

Fuhrman, J. A., Cram, J. A., & Needham, D. M. (2015). Marine microbial community dynamics and their ecological interpretation. *Nature Reviews Microbiology*, *13*(3), 133–146.

Fuhrman, J. A., Hewson, I., Schwalbach, M. S., Steele, J. A., Brown, M. V., & Naeem, S. (2006). Annually reoccurring bacterial communities are predictable from ocean conditions. *Proceedings of the National Academy of Sciences*, *103*(35), 13104–13109. https://doi.org/10.1073/pnas.0602399103

Galand, P. E., Gutiérrez-Provecho, C., Massana, R., Gasol, J. M., & Casamayor, E. O. (2010). Inter-annual recurrence of archaeal assemblages in the coastal NW Mediterranean Sea (Blanes Bay Microbial Observatory). *Limnology and Oceanography*, *55*(5), 2117–2125.

Gallagher, J. C. (1982). Physiological variation and electrophoretic banding patterns of genetically different seasonal populations of Skeletonema costatum (bacillariophyceae) 1. *Journal of Phycology*, *18*(1), 148–162.

Gelaro, R., McCarty, W., Suárez, M. J., Todling, R., Molod, A., Takacs, L., Randles, C. A., Darmenov, A., Bosilovich, M. G., & Reichle, R. (2017). The modern-era retrospective analysis for research and applications, version 2 (MERRA-2). *Journal of Climate*, *30*(14), 5419–5454.

Gemmell, B. J., Oh, G., Buskey, E. J., & Villareal, T. A. (2016). Dynamic sinking behaviour in marine phytoplankton: Rapid changes in buoyancy may aid in nutrient uptake. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1840), 20161126.

Genitsaris, S., Monchy, S., Viscogliosi, E., Sime-Ngando, T., Ferreira, S., & Christaki, U. (2015). Seasonal variations of marine protist community structure based on taxon-specific traits using the eastern English Channel as a model coastal system. *FEMS Microbiology Ecology*, *91*(5). https://doi.org/10.1093/femsec/fiv034

Giesbrecht, W. (1892). *Systematik und Faunistik der pelagischen Copepoden des Golfes von Neapel und der angrenzenden Meeres-Abschnitte* (Vol. 1). Friedländer.

Gilbert, J. A., Steele, J. A., Caporaso, J. G., Steinbrück, L., Reeder, J., Temperton, B., Huse, S., McHardy, A. C., Knight, R., Joint, I., Somerfield, P., Fuhrman, J. A., & Field, D. (2012). Defining seasonal marine microbial community dynamics. *The ISME Journal*, *6*(2), 298–308. https://doi.org/10.1038/ismej.2011.107

Giner, C. R., Balagué, V., Krabberød, A. K., Ferrera, I., Reñé, A., Garcés, E., Gasol, J. M., Logares, R., & Massana, R. (2019). Quantifying long-term recurrence in planktonic microbial eukaryotes. *Molecular Ecology*. https://doi.org/10.1111/mec.14929

Giron-Nava, A., James, C., Johnson, A., Dannecker, D., Kolody, B., Lee, A., Nagarkar, M., Pao, G., Ye, H., Johns, D., & Sugihara, G. (2017). Quantitative argument for long-term ecological monitoring. *Marine Ecology Progress Series*, *572*, 269–274. https://doi.org/10.3354/meps12149

Glibert, P. M. (2016). Margalef revisited: A new phytoplankton mandala incorporating twelve dimensions, including nutritional physiology. *Harmful Algae*, *55*, 25–30. https://doi.org/10.1016/j.hal.2016.01.008

Goberville, E., Beaugrand, G., Sautour, B., Tréguer, P., & Team, S. (2010). Climate-driven changes in coastal marine systems of western Europe. *Marine Ecology Progress Series*, *408*, 129–148.

Gowen, R. J., McQuatters-Gollop, A., Tett, P., Best, M., Bresnan, E., Castellani, C., Cook, K., Forster, R., Scherer, C., & Mckinney, A. (2011). The development of UK pelagic (Plankton) indicators and targets for the MSFD. *Workshop Report, Belfast*.

Gower, J. C., & Legendre, P. (1986). Metric and Euclidean properties of dissimilarity coefficients. *Journal of Classification*, *3*(1), 5–48.

Gran, H. H. (1931). On the conditions for the production of plankton in the sea. *Conseil Perm. Internat. Pour l'Explor. de La Mer. Rapp. et Proces-Verb.*, *75*, 37–46.

Gran, H. H., & Braarud, T. (1935). A quantitative study of the phytoplankton in the Bay of Fundy and the Gulf of Maine (including observations on hydrography, chemistry and turbidity). *Journal of the Biological Board of Canada*, *1*(5), 279–467.

Grbec, B., MOROVIĆ, M., MATIĆ, F., NINČEVIĆ GLADAN, Ž., MARASOVIĆ, I., VIdJAK, O., BOJANIĆ, N., ČIKEŠ KEČ, V., KUŠPILIĆ, G., & MATIĆ-SKOKO, S. (2015). Climate regime shifts and multi-decadal

variability of the Adriatic Sea pelagic ecosystem. *Acta Adriatica: International Journal of Marine Sciences*, *56*(1), 47–66.

Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist*, *111*(982), 1169–1194.

Grime, J. P. (1979). Primary strategies in plants. *Transactions of the Botanical Society of Edinburgh*, *43*(2), 151–160.

Grinnell, J. (1917). The niche-relationships of the California Thrasher. *The Auk*, *34*(4), 427–433.

Groemping, U., & Matthias, L. (2018). Package 'relaimpo'. *Relative Importance of Regressors in Linear Models (R Package Version)*.

Grömping, U. (2006). Relative importance for linear regression in R: the package relaimpo. *Journal of Statistical Software*, *17*(1), 1–27.

Grüner, N., Gebühr, C., Boersma, M., Feudel, U., Wiltshire, K. H., & Freund, J. A. (2011). Reconstructing the realized niche of phytoplankton species from environmental data: Fitness versus abundance approach. *Limnology and Oceanography: Methods*, *9*(10), 432–442.

Guadayol, Ò., Peters, F., Marrasé, C., Gasol, J. M., Roldán, C., Berdalet, E., Massana, R., & Sabata, A. (2009). Episodic meteorological and nutrient-load events as drivers of coastal planktonic ecosystem dynamics: A time-series analysis. *Marine Ecology Progress Series*, *381*, 139–155.

Guinder, V. A., Popovich, C. A., Molinero, J. C., & Perillo, G. M. (2010). Long-term changes in phytoplankton phenology and community structure in the Bahía Blanca Estuary, Argentina. *Marine Biology*, *157*(12), 2703–2716.

Gutiérrez-Rodríguez, A., Latasa, M., Scharek, R., Massana, R., Vila, G., & Gasol, J. M. (2011). Growth and grazing rate dynamics of major phytoplankton groups in an oligotrophic coastal site. *Estuarine, Coastal and Shelf Science*, *95*(1), 77–87. https://doi.org/10.1016/j.ecss.2011.08.008

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., Bruno, J. F., Casey, K. S., Ebert, C., & Fox, H. E. (2008). A global map of human impact on marine ecosystems. *Science*, *319*(5865), 948–952.

Hansen, H. P., & Grasshoff, K. (1983). Automated chemical analysis. *Methods of Seawater Analysis*, 347–395.

Hardin, G. (1960). The competitive exclusion principle. *Science*, *131*(3409), 1292–1297.

Harding Jr, L. W., Adolf, J. E., Mallonee, M. E., Miller, W. D., Gallegos, C. L., Perry, E. S., Johnson, J. M., Sellner, K. G., & Paerl, H. W. (2015). Climate effects on phytoplankton floral composition in Chesapeake Bay. *Estuarine, Coastal and Shelf Science*, *162*, 53–68.

Harding, L. W., Gallegos, C. L., Perry, E. S., Miller, W. D., Adolf, J. E., Mallonee, M. E., & Paerl, H. W. (2016). Long-term trends of nutrients and phytoplankton in Chesapeake Bay. *Estuaries and Coasts*, *39*(3), 664–681.

Hardy, A. C. (1953). Some problems of pelagic life. *Essays in Marine Biology*, 101–121.

Harley, C. D., Randall Hughes, A., Hultgren, K. M., Miner, B. G., Sorte, C. J., Thornber, C. S., Rodriguez, L. F., Tomanek, L., & Williams, S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology Letters*, *9*(2), 228–241.

Harris, G. P. (1980). Temporal and Spatial Scales in Phytoplankton Ecology. Mechanisms, Methods, Models, and Management. *Canadian Journal of Fisheries and Aquatic Sciences*, *37*(5), 877–900. https://doi.org/10.1139/f80-117

Harris, V., Edwards, M., & Olhede, S. C. (2014). Multidecadal Atlantic climate variability and its impact on marine pelagic communities. *Journal of Marine Systems*, *133*, 55–69. https://doi.org/10.1016/j.jmarsys.2013.07.001

Harvey, P. H., & Pagel, M. D. (1991). *The comparative method in evolutionary biology* (Vol. 239). Oxford university press Oxford.

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., Dortch, Q., Gobler, C. J., Heil, C. A., & Humphries, E. (2008). Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae*, *8*(1), 3–13.

Henson, S. A., Beaulieu, C., & Lampitt, R. (2016). Observing climate change trends in ocean biogeochemistry: When and where. *Global Change Biology*, *22*(4), 1561–1571. https://doi.org/10.1111/gcb.13152

Hernández-Fariñas, T., Soudant, D., Barillé, L., Belin, C., Lefebvre, A., & Bacher, C. (2014). Temporal changes in the phytoplankton community along the French coast of the eastern English Channel and the southern Bight of the North Sea. *ICES Journal of Marine Science*, *71*(4), 821–833. https://doi.org/10.1093/icesjms/fst192

Hiltz, M., Bates, S. S., & Kaczmarska, I. (2000). Effect of light: Dark cycles and cell apical length on the sexual reproduction of the pennate diatom Pseudo-nitzschia multiseries (Bacillariophyceae) in culture. *Phycologia*, *39*(1), 59–66.

Hoagland, P., & Scatasta, S. (2006). The economic effects of harmful algal blooms. In *Ecology of harmful algae* (pp. 391–402). Springer.

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. *Science*, *328*(5985), 1523–1528.

Holt, R. D. (2009). Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proceedings of the National Academy of Sciences*, *106*(Supplement 2), 19659–19665.

Hughes, B. B., Beas-Luna, R., Barner, A. K., Brewitt, K., Brumbaugh, D. R., Cerny-Chipman, E. B., Close, S. L., Coblentz, K. E., de Nesnera, K. L., Drobnitch, S. T., Figurski, J. D., Focht, B., Friedman, M., Freiwald, J., Heady, K. K., Heady, W. N., Hettinger, A., Johnson, A., Karr, K. A., … Carr, M. H. (2017). Long-Term Studies Contribute Disproportionately to Ecology and Policy. *BioScience*, *67*(3), 271–281. https://doi.org/10.1093/biosci/biw185

Hunter-Cevera, K. R., Neubert, M. G., Olson, R. J., Solow, A. R., Shalapyonok, A., & Sosik, H. M. (2016). Physiological and ecological drivers of early spring blooms of a coastal phytoplankter. *Science*, *354*(6310), 326–329.

Hure, J., & di Carlo, B. S. (1974). New patterns of diurnal vertical migration of some deep-water copepods in the Tyrrhenian and Adriatis Seas. *Marine Biology*, *28*(3), 179–184.

Hutchinson, G. E. (1961). The paradox of the plankton. *The American Naturalist*, *95*(882), 137–145.

HUTCHINSON, G. E. (1957). The multivariate niche. *Cold Spring Harbor Symposia on Quantitative Biology*, *22*, 415–421.

Indelli, E. (1945). II microplancton di superficie del Golfo di Napoli. *Acta Pontif. Acaod. Sc*, *7*(11), 91–100.

Irwin, A. J., Finkel, Z. V., Müller-Karger, F. E., & Troccoli Ghinaglia, L. (2015a). Phytoplankton adapt to changing ocean environments. *Proceedings of the National Academy of Sciences*, *112*(18), 5762–5766. https://doi.org/10.1073/pnas.1414752112

Irwin, A. J., Finkel, Z. V., Müller-Karger, F., & Ghinaglia, L. T. (2015b). Reply to Brun et al.: Fingerprint of evolution revealed by shifts in realized phytoplankton niches in natural populations. *Proceedings of the National Academy of Sciences*, *112*(38), E5225–E5225.

Irwin, A. J., Nelles, A. M., & Finkel, Z. V. (2012). Phytoplankton niches estimated from field data. *Limnology and Oceanography*, *57*(3), 787–797.

Islam, M. S., & Tanaka, M. (2004). Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: A review and synthesis. *Marine Pollution Bulletin*, *48*(7–8), 624–649.

Jaanus, A., Andersson, A., Olenina, I., Toming, K., & Kaljurand, K. (2011). Changes in phytoplankton communities along a north–south gradient in the Baltic Sea between 1990 and 2008. *Boreal Environment Research*, *16*, 191–208.

Jackson, J. B., Kirby, M. X., Berger, W. H., Bjorndal, K. A., Botsford, L. W., Bourque, B. J., Bradbury, R. H., Cooke, R., Erlandson, J., & Estes, J. A. (2001). Historical overfishing and the recent collapse of coastal ecosystems. *Science*, *293*(5530), 629–637.

Jassby, A. D., & Cloern, J. E. (2015). wq: Exploring water quality monitoring data. *R Package Version 0.4*, *5*.

Jaubert, M., Bouly, J.-P., d'Alcalà, M. R., & Falciatore, A. (2017). Light sensing and responses in marine microalgae. *Current Opinion in Plant Biology*, *37*, 70–77.

Jefferson, R., & Hawkins, S. J. (2006). The evaluation of time series: Their scientific value and contribution to policy needs. *Occasional Publication. Marine Biological Association of the United Kingdom*, *22*, 94.

Ji, R., Edwards, M., Mackas, D. L., Runge, J. A., & Thomas, A. C. (2010). Marine plankton phenology and life history in a changing climate: Current research and future directions. *Journal of Plankton Research*, *32*(10), 1355–1368.

Jin, P., & Agustí, S. (2018). Fast adaptation of tropical diatoms to increased warming with trade-offs. *Scientific Reports*, *8*(1), 1–10.

Jin, X., Gruber, N., Dunne, J. P., Sarmiento, J. L., & Armstrong, R. A. (2006). Diagnosing the contribution of phytoplankton functional groups to the production and export of particulate organic carbon, CaCO3, and opal from global nutrient and alkalinity distributions. *Global Biogeochemical Cycles*, *20*(2).

Johnson, M. D. (2015). Inducible mixotrophy in the dinoflagellate Prorocentrum minimum. *Journal of Eukaryotic Microbiology*, *62*(4), 431–443.

Jones, K. R., Klein, C. J., Halpern, B. S., Venter, O., Grantham, H., Kuempel, C. D., Shumway, N., Friedlander, A. M., Possingham, H. P., & Watson, J. E. (2018). The location and protection status of Earth's diminishing marine wilderness. *Current Biology*, *28*(15), 2506-2512. e3.

Jones, P. D., Jónsson, T., & Wheeler, D. (1997). Extension to the North Atlantic Oscillation using early instrumental pressure observations from Gibraltar and south-west Iceland. *International Journal of Climatology: A Journal of the Royal Meteorological Society*, *17*(13), 1433–1450.

Kaczmarska, I., Mather, L., Luddington, I. A., Muise, F., & Ehrman, J. M. (2014). Cryptic diversity in a cosmopolitan diatom known as Asterionellopsis glacialis (Fragilariaceae): Implications for ecology, biogeography, and taxonomy. *American Journal of Botany*, *101*(2), 267–286.

Kahru, M., Leppanen, J.-M., & Rud, O. (1993). Cyanobacterial blooms cause heating of the sea surface. *Marine Ecology Progress Series*, 1–7.

Karasiewicz, S., Breton, E., Lefebvre, A., Hernández Fariñas, T., & Lefebvre, S. (2018). Realized niche analysis of phytoplankton communities involving HAB: Phaeocystis spp. as a case study. *Harmful Algae*, *72*, 1–13. https://doi.org/10.1016/j.hal.2017.12.005

Karentz, D., & Smayda, T. J. (1984). Temperature and seasonal occurrence patterns of 30 dominant phytoplankton species in Narragansett Bay over a 22-year period (1959–1980). *Mar. Ecol. Prog. Ser*, *18*, 277–293.

Karl, D. M. (2010). Oceanic ecosystem time-series programs: Ten lessons learned. *Oceanography*, *23*(3), 104–125.

Karl, D. M., Bidigare, R. R., & Letelier, R. M. (2001). Long-term changes in plankton community structure and productivity in the North Pacific Subtropical Gyre: The domain shift hypothesis. *Deep Sea Research Part II: Topical Studies in Oceanography*, *48*(8–9), 1449–1470.

Kemp, A. E. S., & Villareal, T. A. (2018). The case of the diatoms and the muddled mandalas: Time to recognize diatom adaptations to stratified waters. *Progress in Oceanography*, *167*, 138–149. https://doi.org/10.1016/j.pocean.2018.08.002

Kendall, M. G. (1975). Kendall rank correlation methods. *Griffin, London*.

Kim, D. Y., Countway, P. D., Jones, A. C., Schnetzer, A., Yamashita, W., Tung, C., & Caron, D. A. (2014). Monthly to interannual variability of microbial eukaryote assemblages at four depths in the eastern North Pacific. *The ISME Journal*, *8*(3), 515–530.

Kiørboe, T. (1993). Turbulence, phytoplankton cell size, and the structure of pelagic food webs. *Advances in Marine Biology*, *29*, 1–72.

Klais, R., Tamminen, T., Kremp, A., Spilling, K., & Olli, K. (2011). Decadal-scale changes of dinoflagellates and diatoms in the anomalous Baltic Sea spring bloom. *PloS One*, *6*(6), e21567.

Koslow, J. A., & Couture, J. (2013). Ocean sciences: Follow the fish. *Nature News*, *502*(7470), 163.

KRISHNA KUMARI, K., RAJESH, G., SUDHIR, A., & BINU, M. (1998). SEVERE FISH MORTALITY ASSOCIATED WITH'RED TIDE'OBSERVED IN THE SEA OFF COCHIN. *Current Science*, *75*(6), 543–544.

Kromkamp, J. C., & Van Engeland, T. (2010). Changes in phytoplankton biomass in the Western Scheldt Estuary during the period 1978–2006. *Estuaries and Coasts*, *33*(2), 270–285.

Kruk, C., Devercelli, M., Huszar, V. L. M., Hernández, E., Beamud, G., Diaz, M., Silva, L. H. S., & Segura, A. M. (2017). Classification of Reynolds phytoplankton functional groups using individual traits and machine learning techniques. *Freshwater Biology*, *62*(10), 1681–1692. https://doi.org/10.1111/fwb.12968

Kruk, C., Huszar, V. L. M., Peeters, E. T. H. M., Bonilla, S., Costa, L., LüRling, M., Reynolds, C. S., & Scheffer, M. (2010). A morphological classification capturing functional variation in phytoplankton. *Freshwater Biology*, *55*(3), 614–627. https://doi.org/10.1111/j.1365-2427.2009.02298.x

Kruk, C., Peeters, E. T. H. M., Van Nes, E. H., Huszar, V. L. M., Costa, L. S., & Scheffer, M. (2011). Phytoplankton community composition can be predicted best in terms of morphological groups. *Limnology and Oceanography*, *56*(1), 110–118. https://doi.org/10.4319/lo.2011.56.1.0110

Kruskal, W. (1987). Relative importance by averaging over orderings. *The American Statistician*, *41*(1), 6–10.

Lakeman, M. B., Von Dassow, P., & Cattolico, R. A. (2009). The strain concept in phytoplankton ecology. *Harmful Algae*, *8*(5), 746–758.

Lambert, S., Tragin, M., Lozano, J.-C., Ghiglione, J.-F., Vaulot, D., Bouget, F.-Y., & Galand, P. E. (2019). Rhythmicity of coastal marine picoeukaryotes, bacteria and archaea despite irregular environmental perturbations. *The ISME Journal*, *13*(2), 388–401.

Lauritano, C., Ferrante, M. I., & Rogato, A. (2019). Marine natural products from microalgae: An-omics overview. *Marine Drugs*, *17*(5), 269.

Laws, E. A. (2013). Evaluation of in situ phytoplankton growth rates: A synthesis of data from varied approaches. *Annual Review of Marine Science*, *5*, 247–268.

Legendre, L., Frechette, M., & Legendre, P. (1981). The contingency periodogram: A method of identifying rhythms in series of nonmetric ecological data. *The Journal of Ecology*, 965–979.

Legendre, P., & Legendre, L. (2012). *Numerical ecology*. Elsevier.

Lejeusne, C., Chevaldonné, P., Pergent-Martini, C., Boudouresque, C. F., & Pérez, T. (2010). Climate change effects on a miniature ocean: The highly diverse, highly impacted Mediterranean Sea. *Trends in Ecology & Evolution*, *25*(4), 250–260. https://doi.org/10.1016/j.tree.2009.10.009

Lelong, A., Hégaret, H., Soudant, P., & Bates, S. S. (2012). Pseudo-nitzschia (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: Revisiting previous paradigms. *Phycologia*, *51*(2), 168–216.

Li, W. K., & Glen Harrison, W. (2008). Propagation of an atmospheric climate signal to phytoplankton in a small marine basin. *Limnology and Oceanography*, *53*(5), 1734–1745.

Lindeman, R. H., Merenda, P. F., & Gold, R. Z. (1980). Introduction to bivariate and multivariate analysis, Glenview, IL. *Scott: Foresman and Company*, *119*.

Lingoes, J. C. (1971). Some boundary conditions for a monotone analysis of symmetric matrices. *Psychometrika*, *36*(2), 195–203.

Litchman, E., de Tezanos Pinto, P., Edwards, K. F., Klausmeier, C. A., Kremer, C. T., & Thomas, M. K. (2015). Global biogeochemical impacts of phytoplankton: A trait-based perspective. *Journal of Ecology*, *103*(6), 1384–1396.

Litchman, E., Edwards, K. F., Klausmeier, C. A., & Thomas, M. K. (2012). Phytoplankton niches, traits and eco-evolutionary responses to global environmental change. *Marine Ecology Progress Series*, *470*, 235–248.

Litchman, E., & Klausmeier, C. A. (2008). Trait-based community ecology of phytoplankton. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 615–639.

Litchman, E., Klausmeier, C. A., Miller, J. R., Schofield, O. M., & Falkowski, P. G. (2006). Multi-nutrient, multi-group model of present and future oceanic phytoplankton communities. *Biogeosciences*, *3*(4), 585–606.

Litchman, E., Klausmeier, C. A., Schofield, O. M., & Falkowski, P. G. (2007). The role of functional traits and trade-offs in structuring phytoplankton communities: Scaling from cellular to ecosystem level. *Ecology Letters*, *10*(12), 1170–1181.

Liu, C., Chan, Y., Alam Kazmi, S. H., & Fu, H. (2015). Financial fraud detection model: Based on random forest. *International Journal of Economics and Finance*, *7*(7).

Löder, M. G., Meunier, C., Wiltshire, K. H., Boersma, M., & Aberle, N. (2011). The role of ciliates, heterotrophic dinoflagellates and copepods in structuring spring plankton communities at Helgoland Roads, North Sea. *Marine Biology*, *158*(7), 1551–1580.

Lohbeck, K. T., Riebesell, U., & Reusch, T. B. (2012). Adaptive evolution of a key phytoplankton species to ocean acidification. *Nature Geoscience*, *5*(5), 346–351.

Lomb, N. R. (1976). Least-squares frequency analysis of unequally spaced data. *Astrophysics and Space Science*, *39*(2), 447–462.

Magnuson, J. J. (1990). Long-term ecological research and the invisible present. *BioScience*, *40*(7), 495–501.

Maguire Jr, B. (1973). Niche response structure and the analytical potentials of its relationship to the habitat. *The American Naturalist*, *107*(954), 213–246.

Mann, H. B. (1945). Nonparametric tests against trend. *Econometrica: Journal of the Econometric Society*, 245–259.

Margalef, R. (1978). Life-forms of phytoplankton as survival alternatives in an unstable environment. *Oceanologica Acta*, *1*(4), 493–509.

Margalef, R. (1979). Functional morphology of organisms involved in red tides, as adapted to decaying turbulence. *Toxic Dinoflagellate Blooms*, 89–94.

Margalef, R. (1997). Our BioSphere: Excellence in ecology. *Ecology Institute, Oldendorf-Luhe, Germany*.

Marino, D., Modigh, M., & Zingone, A. (1984). General features of phytoplankton communities and primary production in the Gulf of Naples and adjacent waters. In *Marine phytoplankton and productivity* (pp. 89–100). Springer.

Marquardt, M., Vader, A., Stübner, E. I., Reigstad, M., & Gabrielsen, T. M. (2016). Strong seasonality of marine microbial eukaryotes in a high-Arctic fjord (Isfjorden, in West Spitsbergen, Norway). *Applied and Environmental Microbiology*, *82*(6), 1868–1880.

Martin-Vide, J., & Lopez-Bustins, J.-A. (2006). The western Mediterranean oscillation and rainfall in the Iberian Peninsula. *International Journal of Climatology: A Journal of the Royal Meteorological Society*, *26*(11), 1455–1475.

Mat, A. M. (2019). Chronobiology and the design of marine biology experiments. *ICES Journal of Marine Science*, *76*(1), 60–65. https://doi.org/10.1093/icesjms/fsy131

Mazzocchi, M. G., Dubroca, L., García-Comas, C., Capua, I. D., & Ribera d'Alcalà, M. (2012). Stability and resilience in coastal copepod assemblages: The case of the Mediterranean long-term ecological research at Station MC (LTER-MC). *Progress in Oceanography*, *97–100*, 135–151. https://doi.org/10.1016/j.pocean.2011.11.003

McQuatters-Gollop, A., Johns, D. G., Bresnan, E., Skinner, J., Rombouts, I., Stern, R., Aubert, A., Johansen, M., Bedford, J., & Knights, A. (2017). From microscope to management: The critical value of plankton taxonomy to marine policy and biodiversity conservation. *Marine Policy*, *83*, 1–10. https://doi.org/10.1016/j.marpol.2017.05.022

McQuatters-Gollop, A., Reid, P. C., Edwards, M., Burkill, P. H., Castellani, C., Batten, S., Gieskes, W., Beare, D., Bidigare, R. R., & Head, E. (2011). Is there a decline in marine phytoplankton? *Nature*, *472*(7342), E6–E7.

Mee, L. D. (1992). The Black Sea in crisis: A need for concerted international action. *Ambio (Sweden)*.

Meunier, C. L., Boersma, M., Wiltshire, K. H., & Malzahn, A. M. (2016). Zooplankton eat what they need: Copepod selective feeding and potential consequences for marine systems. *Oikos*, *125*(1), 50–58.

Meunier, C. L., Schulz, K., Boersma, M., & Malzahn, A. M. (2013). Impact of swimming behaviour and nutrient limitation on predator–prey interactions in pelagic microbial food webs. *Journal of Experimental Marine Biology and Ecology*, *446*, 29–35.

Meyer, J., & Riebesell, U. (2015). Reviews and Syntheses: Responses of coccolithophores to ocean acidification: A meta-analysis. *Biogeosciences*, *12*(6), 1671–1682.

Mieruch, S., Freund, J. A., Feudel, U., Boersma, M., Janisch, S., & Wiltshire, K. H. (2010). A new method of describing phytoplankton blooms: Examples from Helgoland Roads. *Journal of Marine Systems*, *79*(1–2), 36–43.

Mills, E. L. (2012). *Biological oceanography: An early history, 1870-1960*. University of Toronto Press.

Montresor, M., Vitale, L., D'Alelio, D., & Ferrante, M. I. (2016). Sex in marine planktonic diatoms: Insights and challenges. *Perspect. Phycol*, *3*(2), 61–75.

Mori, T., & Johnson, C. H. (2000). Circadian control of cell division in unicellular organisms. *Progress in Cell Cycle Research*, 185–192.

Morin, P. J. (2009). *Community ecology*. John Wiley & Sons.

Mouget, J.-L., Gastineau, R., Davidovich, O., Gaudin, P., & Davidovich, N. A. (2009). Light is a key factor in triggering sexual reproduction in the pennate diatom Haslea ostrearia. *FEMS Microbiology Ecology*, *69*(2), 194–201.

Mozetič, P., Francé, J., Kogovšek, T., Talaber, I., & Malej, A. (2012). Plankton trends and community changes in a coastal sea (northern Adriatic): Bottom-up vs. Top-down control in relation to environmental drivers. *Estuarine, Coastal and Shelf Science*, *115*, 138–148.

Nagarkar, M., Countway, P. D., Du Yoo, Y., Daniels, E., Poulton, N. J., & Palenik, B. (2018). Temporal dynamics of eukaryotic microbial diversity at a coastal Pacific site. *The ISME Journal*, *12*(9), 2278–2291.

Nanjappa, D., Kooistra, W. H., & Zingone, A. (2013). A reappraisal of the genus L eptocylindrus (B acillariophyta), with the addition of three species and the erection of T enuicylindrus gen. Nov. *Journal of Phycology*, *49*(5), 917–936.

Naselli-Flores, L., Padisák, J., & Albay, M. (2007). Shape and size in phytoplankton ecology: Do they matter? *Hydrobiologia*, *578*(1), 157–161.

Needham, D. M., Fichot, E. B., Wang, E., Berdjeb, L., Cram, J. A., Fichot, C. G., & Fuhrman, J. A. (2017). Dynamics of finely resolved, abundant symbiotic marine plankton and other interacting microbes via automated high-frequency sampling. *BioRxiv*, 216978.

Needham, D. M., & Fuhrman, J. A. (2016). Pronounced daily succession of phytoplankton, archaea and bacteria following a spring bloom. *Nature Microbiology*, *1*(4), 16005. https://doi.org/10.1038/nmicrobiol.2016.5

Nelson, R. J., Denlinger, D. L., & Somers, D. E. (2010). *Photoperiodism: The biological calendar*. Oxford University Press.

Nixon, S. W. (1995). Coastal marine eutrophication: A definition, social causes, and future concerns. *Ophelia*, *41*(1), 199–219.

Not, F., Siano, R., Kooistra, W. H., Simon, N., Vaulot, D., & Probert, I. (2012). Diversity and ecology of eukaryotic marine phytoplankton. *Advances in Botanical Research*, *64*, 1–53.

Nunes, S., Latasa, M., Gasol, J. M., & Estrada, M. (2018). Seasonal and interannual variability of phytoplankton community structure in a Mediterranean coastal site. *Marine Ecology Progress Series*, *592*, 57–75.

O'brien, R. M. (2007). A caution regarding rules of thumb for variance inflation factors. *Quality & Quantity*, *41*(5), 673–690.

Odebrecht, C., Abreu, P. C., & Carstensen, J. (2015). Retention time generates short-term phytoplankton blooms in a shallow microtidal subtropical estuary. *Estuarine, Coastal and Shelf Science*, *162*, 35–44.

Odebrecht, C., Bergesch, M., Rörig, L. R., & Abreu, P. C. (2010). Phytoplankton interannual variability at Cassino Beach, Southern Brazil (1992–2007), with emphasis on the surf zone diatom Asterionellopsis glacialis. *Estuaries and Coasts*, *33*(2), 570–583.

Ohman, M. D., & Venrick, E. L. (2003). CalCOFI in a changing ocean. *Oceanography*, *16*(SPL. ISS. 3), 76–85.

Okamoto, O. K., & Hastings, J. W. (2003). NOVEL DINOFLAGELLATE CLOCK-RELATED GENES IDENTIFIED THROUGH MICROARRAY ANALYSIS 1. *Journal of Phycology*, *39*(3), 519–526.

Olli, K., Paerl, H. W., & Klais, R. (2015). Diversity of coastal phytoplankton assemblages–cross ecosystem comparison. *Estuarine, Coastal and Shelf Science*, *162*, 110–118.

Paerl, H. W., & Huisman, J. (2008). Blooms like it hot. *Science*, *320*(5872), 57–58.

Paerl, H. W., & Otten, T. G. (2013). Harmful cyanobacterial blooms: Causes, consequences, and controls. *Microbial Ecology*, *65*(4), 995–1010.

Pančić, M., & Kiørboe, T. (2018). Phytoplankton defence mechanisms: Traits and trade-offs. *Biological Reviews*, *93*(2), 1269–1303.

Pearman, P. B., Guisan, A., Broennimann, O., & Randin, C. F. (2008). Niche dynamics in space and time. *Trends in Ecology & Evolution*, *23*(3), 149–158.

Petersen, J. K., Hansen, J. W., Laursen, M. B., Clausen, P., Carstensen, J., & Conley, D. J. (2008). Regime shift in a coastal marine ecosystem. *Ecological Applications*, *18*(2), 497–510.

Piredda, R., Tomasino, M. P., D'Erchia, A. M., Manzari, C., Pesole, G., Montresor, M., Kooistra, W. H. C. F., Sarno, D., & Zingone, A. (2017). Diversity and temporal patterns of planktonic protist assemblages at a Mediterranean Long Term Ecological Research site. *FEMS Microbiology Ecology*, *93*(1), fiw200. https://doi.org/10.1093/femsec/fiw200

Platt, T., Fuentes-Yaco, C., & Frank, K. T. (2003). Spring algal bloom and larval fish survival. *Nature*, *423*(6938), 398–399.

Potapov, A. M., Brose, U., Scheu, S., & Tiunov, A. V. (2019). Trophic position of consumers and size structure of food webs across aquatic and terrestrial ecosystems. *The American Naturalist*, *194*(6), 823–839.

Putterill, J., Laurie, R., & Macknight, R. (2004). It's time to flower: The genetic control of flowering time. *Bioessays*, *26*(4), 363–373.

Rabalais, N. N., Turner, R. E., Díaz, R. J., & Justić, D. (2009). Global change and eutrophication of coastal waters. *ICES Journal of Marine Science*, *66*(7), 1528–1537.

Rahmstorf, S., Perrette, M., & Vermeer, M. (2012). Testing the robustness of semi-empirical sea level projections. *Climate Dynamics*, *39*(3–4), 861–875.

Raven, J. A., & Geider, R. J. (1988). Temperature and algal growth. *New Phytologist*, *110*(4), 441–461.

Regier, P., Briceño, H., & Boyer, J. N. (2019). Analyzing and comparing complex environmental time series using a cumulative sums approach. *MethodsX*, *6*, 779–787.

Reid, P. C., Colebrook, J. M., Matthews, J. B. L., & Aiken, J. (2003). The Continuous Plankton Recorder: Concepts and history, from Plankton Indicator to undulating recorders. *Progress in Oceanography*, *58*(2–4), 117–173. https://doi.org/10.1016/j.pocean.2003.08.002

Reusch, T. B., & Boyd, P. W. (2013). Experimental evolution meets marine phytoplankton. *Evolution*, *67*(7), 1849–1859.

Reynolds, C. S. (1980). Phytoplankton assemblages and their periodicity in stratifying lake systems. *Ecography*, *3*(3), 141–159.

Reynolds, C. S. (1984). Phytoplankton periodicity: The interactions of form, function and environmental variability. *Freshwater Biology*, *14*(2), 111–142.

Reynolds, C. S. (1988). The concept of ecological succession applied to seasonal periodicity of freshwater phytoplankton: With 4 figures in the text. *Internationale Vereinigung Für Theoretische Und Angewandte Limnologie: Verhandlungen*, *23*(2), 683–691.

Reynolds, C. S. (1998). What factors influence the species composition of phytoplankton in lakes of different trophic status? *Hydrobiologia*, *369*, 11–26.

Reynolds, C. S. (2006). *The ecology of phytoplankton*. Cambridge University Press.

Ribera d'Alcalà, M., Conversano, F., Corato, F., Licandro, P., Mangoni, O., Marino, D., Mazzocchi, M. G., Modigh, M., Montresor, M., & Nardella, M. (2004). Seasonal patterns in plankton communities in a pluriannual time series at a coastal Mediterranean site (Gulf of Naples): An attempt to discern recurrences and trends. *Scientia Marina*, *68*(S1), 65–83.

Richerson, P., Armstrong, R., & Goldman, C. R. (1970). Contemporaneous disequilibrium, a new hypothesis to explain the „Paradox of the Plankton". *Proceedings of the National Academy of Sciences*, *67*(4), 1710–1714.

Riegman, R., Kuipers, B. R., Noordeloos, A. A., & Witte, H. J. (1993). Size-differential control of phytoplankton and the structure of plankton communities. *Netherlands Journal of Sea Research*, *31*(3), 255–265.

Rienecker, M. M., Suarez, M. J., Gelaro, R., Todling, R., Bacmeister, J., Liu, E., Bosilovich, M. G., Schubert, S. D., Takacs, L., & Kim, G.-K. (2011). MERRA: NASA's modern-era retrospective analysis for research and applications. *Journal of Climate*, *24*(14), 3624–3648.

Rivkin, R. B. (1989). Influence of irradiance and spectral quality on the carbon metabolism of phytoplankton. I. Photosynthesis, chemical composition and growth. *Marine Ecology Progress Series. Oldendorf*, *55*(2), 291–304.

Rolinski, S., Horn, H., Petzoldt, T., & Paul, L. (2007). Identifying cardinal dates in phytoplankton time series to enable the analysis of long-term trends. *Oecologia*, *153*(4), 997–1008.

Romagnan, J.-B., Legendre, L., Guidi, L., Jamet, J.-L., Jamet, D., Mousseau, L., Pedrotti, M.-L., Picheral, M., Gorsky, G., Sardet, C., & Stemmann, L. (2015). Comprehensive Model of Annual Plankton Succession Based on the Whole-Plankton Time Series Approach. *PLOS ONE*, *10*(3), e0119219. https://doi.org/10.1371/journal.pone.0119219

Rönnberg, C., & Bonsdorff, E. (2004). Baltic Sea eutrophication: Area-specific ecological consequences. *Hydrobiologia*, *514*(1), 227–241.

Rosbash, M. (2009). The implications of multiple circadian clock origins. *PLoS Biol*, *7*(3), e1000062.

Rost, B., & Riebesell, U. (2004). Coccolithophores and the biological pump: Responses to environmental changes. In *Coccolithophores* (pp. 99–125). Springer.

Rost, B., Riebesell, U., Burkhardt, S., & Sültemeyer, D. (2003). Carbon acquisition of bloom-forming marine phytoplankton. *Limnology and Oceanography*, *48*(1), 55–67.

Rost, B., Riebesell, U., & Sültemeyer, D. (2006). Carbon acquisition of marine phytoplankton: Effect of photoperiod length. *Limnology and Oceanography*, *51*(1), 12–20. https://doi.org/10.4319/lo.2006.51.1.0012

Roxy, M. K., Modi, A., Murtugudde, R., Valsala, V., Panickal, S., Prasanna Kumar, S., Ravichandran, M., Vichi, M., & Lévy, M. (2016). A reduction in marine primary productivity driven by rapid warming over the tropical Indian Ocean. *Geophysical Research Letters*, *43*(2), 826–833.

Roy, S., & Chattopadhyay, J. (2007). Towards a resolution of 'the paradox of the plankton': A brief overview of the proposed mechanisms. *Ecological Complexity*, *4*(1–2), 26–33.

Ruf, T. (1999). The Lomb-Scargle periodogram in biological rhythm research: Analysis of incomplete and unequally spaced time-series. *Biological Rhythm Research*, *30*(2), 178–201.

Ruf, T., & Ruf, M. T. (2013). *Package 'lomb'*.

Russo, G. F. (1995). Ambienti marini del Golfo di Napoli e dell'isola d'Ischia: Particolarità e paradossi. *Scheria*, *4*(10), 58–72.

Rynearson, T. A., Flickinger, S. A., & Fontaine, D. N. (2020). Metabarcoding reveals temporal patterns of community composition and realized thermal niches of Thalassiosira spp.(bacillariophyceae) from the Narragansett bay long-term plankton time series. *Biology*, *9*(1), 19.

Sabia, L., Costanzo, A., D'ALCALÀ, M. R., Saggiomo, V., Zingone, A., & Margiotta, F. (2019). Assessing the quality of biogeochemical coastal data: A step-wise procedure. *Mediterranean Marine Science*, *20*(1), 56–73.

Sarno, D., Kooistra, W. H., Balzano, S., Hargraves, P. E., & Zingone, A. (2007). Diversity in the genus Skeletonema (BACILLARIOPHYCEAE): III. Phylogenetic position and morphological variability of Skeletonema costatum and Skeletonema grevillei, with the description of Skeletonema ardens sp. NOV. 1. *Journal of Phycology*, *43*(1), 156–170.

Sarno, D., Kooistra, W. H., Medlin, L. K., Percopo, I., & Zingone, A. (2005). Diversity in the genus Skeletonema (Bacillariophyceae). II. An assessment of the taxonomy of S. costatum-like species with the description of four new species 1. *Journal of Phycology*, *41*(1), 151–176.

Savoca, M. S., & Nevitt, G. A. (2014). Evidence that dimethyl sulfide facilitates a tritrophic mutualism between marine primary producers and top predators. *Proceedings of the National Academy of Sciences*, *111*(11), 4157–4161.

Scargle, J. D. (1982). Studies in astronomical time series analysis. II-Statistical aspects of spectral analysis of unevenly spaced data. *The Astrophysical Journal*, *263*, 835–853.

Scharfe, M., & Wiltshire, K. H. (2019). Modeling of intra-annual abundance distributions: Constancy and variation in the phenology of marine phytoplankton species over five decades at Helgoland Roads (North Sea). *Ecological Modelling*, *404*, 46–60.

Schaum, C.-E., Barton, S., Bestion, E., Buckling, A., Garcia-Carreras, B., Lopez, P., Lowe, C., Pawar, S., Smirnoff, N., & Trimmer, M. (2017). Adaptation of phytoplankton to a decade of experimental warming linked to increased photosynthesis. *Nature Ecology & Evolution*, *1*(4), 1–7.

Schaum, C.-E., Buckling, A., Smirnoff, N., Studholme, D. J., & Yvon-Durocher, G. (2018). Environmental fluctuations accelerate molecular evolution of thermal tolerance in a marine diatom. *Nature Communications*, *9*(1), 1–14.

Schaum, C.-E., Rost, B., & Collins, S. (2016). Environmental stability affects phenotypic evolution in a globally distributed marine picoplankton. *The ISME Journal*, *10*(1), 75–84.

Scheffers, B. R., De Meester, L., Bridge, T. C., Hoffmann, A. A., Pandolfi, J. M., Corlett, R. T., Butchart, S. H., Pearce-Kelly, P., Kovacs, K. M., & Dudgeon, D. (2016). The broad footprint of climate change from genes to biomes to people. *Science*, *354*(6313).

Schlüter, M. H., Kraberg, A., & Wiltshire, K. H. (2012). Long-term changes in the seasonality of selected diatoms related to grazers and environmental conditions. *Journal of Sea Research*, *67*(1), 91–97. https://doi.org/10.1016/j.seares.2011.11.001

Scotto di Carlo, B., Tomas, C. R., Ianora, A., Marino, D., Mazzocchi, M. G., Modigh, M., Montresor, M., Petrillo, L., Ribera d'Alcalà, M., & Saggiomo, V. (1985). Uno studio integrato dell'ecosistema pelagico costiero del Golfo di Napoli. *Nova Thalassia*, *7*(suppl 3), 99–128.

Sen, P. K. (1968). Estimates of the regression coefficient based on Kendall's tau. *Journal of the American Statistical Association*, *63*(324), 1379–1389.

Simon, M., López-García, P., Deschamps, P., Moreira, D., Restoux, G., Bertolino, P., & Jardillier, L. (2015). Marked seasonality and high spatial variability of protist communities in shallow freshwater systems. *The ISME Journal*, *9*(9), 1941–1953.

Siokou-Frangou, I., Christaki, U., Mazzocchi, M. G., Montresor, M., Ribera d'Alcalá, M., Vaqué, D., & Zingone, A. (2010). Plankton in the open Mediterranean Sea: A review. *Biogeosciences*, *7*(5), 1543–1586. https://doi.org/10.5194/bg-7-1543-2010

Smalley, G. W., Coats, D. W., & Stoecker, D. K. (2003). Feeding in the mixotrophic dinoflagellate Ceratium furca is influenced by intracellular nutrient concentrations. *Marine Ecology Progress Series*, *262*, 137–151.

Smayda, T. J. (1980). Phytoplankton species succession. *The Physiological Ecology of Phytoplakton.-*, 493–570.

Smayda, T. J. (2001). Community Assembly in Marine Phytoplankton: Application of Recent Models to Harmful Dinoflagellate Blooms. *Journal of Plankton Research*, *23*(5), 447–461. https://doi.org/10.1093/plankt/23.5.447

Smayda, T. J. (2004). Ecological effects of climate variation in the North Atlantic: Phytoplankton. *Marine Ecosystems and Climate Variation-the North Atlantic*, 49–58.

Smayda, T. J. (2011). Cryptic planktonic diatom challenges phytoplankton ecologists. *Proceedings of the National Academy of Sciences*, *108*(11), 4269–4270.

Smetacek, V. (2001). A watery arms race. *Nature*, *411*(6839), 745–745. https://doi.org/10.1038/35081210

Smetacek, V., Montresor, M., & Verity, P. (2002). *Marine Productivity: Footprints of the past and steps into the future*. Blackwell Science, Ltd., Oxford.

Smith, S. J., Edmonds, J., Hartin, C. A., Mundra, A., & Calvin, K. (2015). Near-term acceleration in the rate of temperature change. *Nature Climate Change*, *5*(4), 333–336.

Soberón, J. (2007). Grinnellian and Eltonian niches and geographic distributions of species. *Ecology Letters*, *10*(12), 1115–1123.

Sommer, U., Adrian, R., De Senerpont Domis, L., Elser, J. J., Gaedke, U., Ibelings, B., Jeppesen, E., Lürling, M., Molinero, J. C., Mooij, W. M., van Donk, E., & Winder, M. (2012). Beyond the Plankton Ecology Group (PEG) Model: Mechanisms Driving Plankton Succession. *Annual Review of Ecology, Evolution, and Systematics*, *43*(1), 429–448. https://doi.org/10.1146/annurev-ecolsys-110411-160251

Sommer, U., Charalampous, E., Genitsaris, S., & Moustaka-Gouni, M. (2017). Benefits, costs and taxonomic distribution of marine phytoplankton body size. *Journal of Plankton Research*, *39*(3), 494–508.

Sommer, U., Gliwicz, Z. M., Lampert, W., & Duncan, A. (1986). The PEG-model of seasonal succession of planktonic events in fresh waters. *Arch. Hydrobiol*, *106*(4), 433–471.

Sommer, U., & Lengfellner, K. (2008). Climate change and the timing, magnitude, and composition of the phytoplankton spring bloom. *Global Change Biology*, *14*(6), 1199–1208.

Sommer, U., Padisák, J., Reynolds, C. S., & Juhász-Nagy, P. (1993). Hutchinson's heritage: The diversity-disturbance relationship in phytoplankton. *Hydrobiologia*, *249*(1), 1–7.

Sonntag, S., & Hense, I. (2011). Phytoplankton behavior affects ocean mixed layer dynamics through biological-physical feedback mechanisms. *Geophysical Research Letters*, *38*(15).

Southwood, T. R. (1977). Habitat, the templet for ecological strategies? *Journal of Animal Ecology*, *46*(2), 337–365.

Spatharis, S., Lamprinou, V., Meziti, A., Kormas, K. A., Danielidis, D. D., Smeti, E., Roelke, D. L., Mancy, R., & Tsirtsis, G. (2019). Everything is not everywhere: Can marine compartments shape phytoplankton assemblages? *Proceedings of the Royal Society B*, *286*(1914), 20191890.

Spilling, K., Olli, K., Lehtoranta, J., Kremp, A., Tedesco, L., Tamelander, T., Klais, R., Peltonen, H., & Tamminen, T. (2018). Shifting diatom—Dinoflagellate dominance during spring bloom in the Baltic Sea and its potential effects on biogeochemical cycling. *Frontiers in Marine Science*, *5*, 327.

Stackhouse, P. W., Zhang, T., Westberg, D., Barnett, A. J., Bristow, T., Macpherson, B., & Hoell, J. M. (2018). *POWER Release 8 (with GIS Applications) Methodology (Data Parameters, Sources, & Validation) Documentation Date May 1, 2018*.

Steinke, M., Malin, G., & Liss, P. S. (2002). Trophic interactions in the sea: An ecological role for climate relevant volatiles? 1. *Journal of Phycology*, *38*(4), 630–638.

Stenseth, N. C., Ottersen, G., Hurrell, J. W., Mysterud, A., Lima, M., Chan, K.-S., Yoccoz, N. G., & Ådlandsvik, B. (2003). Studying climate effects on ecology through the use of climate indices: The North Atlantic Oscillation, El Nino Southern Oscillation and beyond. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1529), 2087–2096.

Stocker, T. F., Qin, D., Plattner, G.-K., Alexander, L. V., Allen, S. K., Bindoff, N. L., Bréon, F.-M., Church, J. A., Cubasch, U., & Emori, S. (2013). Technical summary. In *Climate change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 33–115). Cambridge University Press.

Suikkanen, S., Laamanen, M., & Huttunen, M. (2007). Long-term changes in summer phytoplankton communities of the open northern Baltic Sea. *Estuarine, Coastal and Shelf Science*, *71*(3–4), 580–592. https://doi.org/10.1016/j.ecss.2006.09.004

Suzuki, L., & Johnson, C. H. (2001). Algae know the time of day: Circadian and photoperiodic programs. *Journal of Phycology*, *37*(6), 933–942.

Sverdrup, H. U. (1953). On conditions for the vernal blooming of phytoplankton. *J. Cons. Int. Explor. Mer*, *18*(3), 287–295.

Sweeney, B. M. (1984). Circadian rhythmicity in dinoflagellates. In *Dinoflagellates* (pp. 343–364). Academic Press New York.

Tanhua, T., Orr, J. C., Lorenzoni, L., & Hansson, L. (2015). Monitoring ocean carbon and ocean acidification. *WMO Bulletin*, *64*(1).

Team, R. C. (2013). *R: A language and environment for statistical computing*.

Teeling, H., Fuchs, B. M., Bennke, C. M., Krüger, K., Chafee, M., Kappelmann, L., Reintjes, G., Waldmann, J., Quast, C., Glöckner, F. O., Lucas, J., Wichels, A., Gerdts, G., Wiltshire, K. H., & Amann, R. I. (2016). Recurring patterns in bacterioplankton dynamics during coastal spring algae blooms. *ELife*, *5*, e11888. https://doi.org/10.7554/eLife.11888

Tett, P., Gowen, R., Mills, D., Fernandes, T., Gilpin, L., Huxham, M., Kennington, K., Read, P., Service, M., Wilkinson, M., & Malcolm, S. (2007). Defining and detecting undesirable disturbance in the context of marine eutrophication. *Marine Pollution Bulletin*, *55*(1–6), 282–297. https://doi.org/10.1016/j.marpolbul.2006.08.028

Thackeray, S. J. (2012). Mismatch revisited: What is trophic mismatching from the perspective of the plankton? *Journal of Plankton Research*, *34*(12), 1001–1010.

Thackeray, S. J., Henrys, P. A., Hemming, D., Bell, J. R., Botham, M. S., Burthe, S., Helaouet, P., Johns, D. G., Jones, I. D., & Leech, D. I. (2016). Phenological sensitivity to climate across taxa and trophic levels. *Nature*, *535*(7611), 241–245.

Thackeray, S. J., Jones, I. D., & Maberly, S. C. (2008). Long-term change in the phenology of spring phytoplankton: Species-specific responses to nutrient enrichment and climatic change. *Journal of Ecology*, *96*(3), 523–535.

Thingstad, T. F. (2000). Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnology and Oceanography*, *45*(6), 1320–1328.

Thingstad, T. F., & Lignell, R. (1997). Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. *Aquatic Microbial Ecology*, *13*(1), 19–27.

Thioulouse, J., & Chessel, D. (1987). Les analyses multitableaux en écologie factorielle. I. De la typologie d'état à la typologie de fonctionnement par l'analyse triadique. *Acta Oecologica. Série Oecologia Generalis*, *8*(4), 463–480.

Thioulouse, J., Simier, M., & Chessel, D. (2004). SIMULTANEOUS ANALYSIS OF A SEQUENCE OF PAIRED ECOLOGICAL TABLES. *Ecology*, *85*(1), 272–283. https://doi.org/10.1890/02-0605

Thomas, M. K., Kremer, C. T., Klausmeier, C. A., & Litchman, E. (2012). A global pattern of thermal adaptation in marine phytoplankton. *Science*, *338*(6110), 1085–1088.

Thompson, P. A., O'Brien, T. D., Paerl, H. W., Peierls, B. L., Harrison, P. J., & Robb, M. (2015). Precipitation as a driver of phytoplankton ecology in coastal waters: A climatic perspective. *Estuarine, Coastal and Shelf Science*, *162*, 119–129.

Tian, W. (2013). A review of sensitivity analysis methods in building energy analysis. *Renewable and Sustainable Energy Reviews*, *20*, 411–419.

Tibshirani, R., Walther, G., & Hastie, T. (2001). Estimating the number of clusters in a data set via the gap statistic. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *63*(2), 411–423.

Tilman, D. (1987). Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. *Ecological Monographs*, *57*(3), 189–214.

Tonkin, J. D., Bogan, M. T., Bonada, N., Rios-Touma, B., & Lytle, D. A. (2017). Seasonality and predictability shape temporal species diversity. *Ecology*, *98*(5), 1201–1216. https://doi.org/10.1002/ecy.1761

Tornero, V., & d'Alcalà, M. R. (2014). Contamination by hazardous substances in the Gulf of Naples and nearby coastal areas: A review of sources, environmental levels and potential impacts in the MSFD perspective. *Science of the Total Environment*, *466*, 820–840.

Toseland, A., Daines, S. J., Clark, J. R., Kirkham, A., Strauss, J., Uhlig, C., Lenton, T. M., Valentin, K., Pearson, G. A., & Moulton, V. (2013). The impact of temperature on marine phytoplankton resource allocation and metabolism. *Nature Climate Change*, *3*(11), 979–984.

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

Tréguer, P. J., & De La Rocha, C. L. (2013). The world ocean silica cycle. *Annual Review of Marine Science*, *5*, 477–501.

Trenberth, K. E. (2011). Changes in precipitation with climate change. *Climate Research*, *47*(1–2), 123–138.

Trombetta, T., Vidussi, F., Mas, S., Parin, D., Simier, M., & Mostajir, B. (2019). Water temperature drives phytoplankton blooms in coastal waters. *PLoS One*, *14*(4), e0214933.

Vantrepotte, V., & Melin, F. (2009). Temporal variability of 10-year global SeaWiFS time-series of phytoplankton chlorophyll a concentration. *ICES Journal of Marine Science*, *66*(7), 1547–1556. https://doi.org/10.1093/icesjms/fsp107

Vasseur, D. A., Gaedke, U., & McCann, K. S. (2005). A seasonal alternation of coherent and compensatory dynamics occurs in phytoplankton. *Oikos*, *110*(3), 507–514. https://doi.org/10.1111/j.0030-1299.2005.14006.x

Vita, P. D., Allocca, V., Manna, F., & Fabbrocino, S. (2012). Coupled decadal variability of the North Atlantic Oscillation, regional rainfall and karst spring discharges in the Campania region (southern Italy). *Hydrology and Earth System Sciences*, *16*(5), 1389–1399.

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science*, *277*(5325), 494–499.

Ward, C. S., Yung, C.-M., Davis, K. M., Blinebry, S. K., Williams, T. C., Johnson, Z. I., & Hunt, D. E. (2017). Annual community patterns are driven by seasonal switching between closely related marine bacteria. *The ISME Journal*, *11*(6), 1412–1422. https://doi.org/10.1038/ismej.2017.4

Warner, A. J., & Hays, G. C. (1994). Sampling by the continuous plankton recorder survey. *Progress in Oceanography*, *34*(2–3), 237–256.

Wasmund, N., Tuimala, J., Suikkanen, S., Vandepitte, L., & Kraberg, A. (2011). Long-term trends in phytoplankton composition in the western and central Baltic Sea. *Journal of Marine Systems*, *87*(2), 145–159. https://doi.org/10.1016/j.jmarsys.2011.03.010

Webster, I. T., & Hutchinson, P. A. (1994). Effect of wind on the distribution of phytoplankton cells in lakes revisited. *Limnology and Oceanography*, *39*(2), 365–373.

Wentzky, V. C., Tittel, J., Jäger, C. G., Bruggeman, J., & Rinke, K. (2020). Seasonal succession of functional traits in phytoplankton communities and their interaction with trophic state. *Journal of Ecology*, *108*(4), 1649–1663.

White, E. R. (2019). Minimum time required to detect population trends: The need for long-term monitoring programs. *BioScience*, *69*(1), 40–46.

Whyte, C., Davidson, K., Gilpin, L., Mitchell, E., Moschonas, G., McNeill, S., & Tett, P. (2017). Tracking changes to a microplankton community in a North Atlantic sea loch using the microplankton index PI (mp). *ICES Journal of Marine Science*, *74*(1), 311–325.

Widdicombe, C. E., Eloire, D., Harbour, D., Harris, R. P., & Somerfield, P. J. (2010). Long-term phytoplankton community dynamics in the Western English Channel. *Journal of Plankton Research*, *32*(5), 643–655.

Wilkinson, G. M., Walter, J., Fleck, R., & Pace, M. L. (2020). Beyond the trends: The need to understand multiannual dynamics in aquatic ecosystems. *Limnology and Oceanography Letters*, *5*(4), 281–286.

Wiltshire, K. H., Boersma, M., Carstens, K., Kraberg, A. C., Peters, S., & Scharfe, M. (2015). Control of phytoplankton in a shelf sea: Determination of the main drivers based on the Helgoland Roads Time Series. *Journal of Sea Research*, *105*, 42–52. https://doi.org/10.1016/j.seares.2015.06.022

Wiltshire, K. H., Malzahn, A. M., Wirtz, K., Greve, W., Janisch, S., Mangelsdorf, P., Manly, B. F., & Boersma, M. (2008). Resilience of North Sea phytoplankton spring bloom dynamics: An analysis of long-term data at Helgoland Roads. *Limnology and Oceanography*, *53*(4), 1294–1302.

Winder, M., Berger, S. A., Lewandowska, A., Aberle, N., Lengfellner, K., Sommer, U., & Diehl, S. (2012). Spring phenological responses of marine and freshwater plankton to changing temperature and light conditions. *Marine Biology*, *159*(11), 2491–2501.

Winder, M., & Cloern, J. E. (2010). The annual cycles of phytoplankton biomass. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1555), 3215–3226.

Winder, M., & Sommer, U. (2012). Phytoplankton response to a changing climate. *Hydrobiologia*, *698*(1), 5–16.

Wood, A. M., & Leatham, T. (1992). THE SPECIES CONCEPT IN PHYTOPLANKTON ECOLOGY 1. *Journal of Phycology*, *28*(6), 723–729.

Woods, S., & Villareal, T. A. (2008). Intracellular ion concentrations and cell sap density in positively buoyant oceanic phytoplankton. *Nova Hedwigia*, 131–145.

Wyatt, T. (2014). Margalef's mandala and phytoplankton bloom strategies. *Deep Sea Research Part II: Topical Studies in Oceanography*, *101*, 32–49. https://doi.org/10.1016/j.dsr2.2012.12.006

Zhou, M., Shen, Z., & Yu, R. (2008). Responses of a coastal phytoplankton community to increased nutrient input from the Changjiang (Yangtze) River. *Continental Shelf Research*, *28*(12), 1483–1489. https://doi.org/10.1016/j.csr.2007.02.009

Zingone, A., Casotti, R., d'Alcala, M. R., Scardi, M., & Marino, D. (1995). 'St Martin's Summer': The case of an autumn phytoplankton bloom in the Gulf of Naples (Mediterranean Sea). *Journal of Plankton Research*, *17*(3), 575–593.

Zingone, A., D'Alelio, D., Mazzocchi, M. G., Montresor, M., Sarno, D., & team, L.-M. (2019). Time series and beyond: Multifaceted plankton research at a marine Mediterranean LTER site. *Nature Conservation*, *34*, 273–310. https://doi.org/10.3897/natureconservation.34.30789

Zingone, A., Dubroca, L., Iudicone, D., Margiotta, F., Corato, F., d'Alcalà, M. R., Saggiomo, V., & Sarno, D. (2010a). Coastal phytoplankton do not rest in winter. *Estuaries and Coasts*, *33*(2), 342–361.

Zingone, A., & Enevoldsen, H. O. (2000). The diversity of harmful algal blooms: A challenge for science and management. *Ocean & Coastal Management*, *43*(8–9), 725–748.

Zingone, A., Harrison, P. J., Kraberg, A., Lehtinen, S., McQuatters-Gollop, A., O'Brien, T., Sun, J., & Jakobsen, H. H. (2015). Increasing the quality, comparability and accessibility of phytoplankton species composition time-series data. *Estuarine, Coastal and Shelf Science*, *162*, 151–160.

Zingone, A., Licandro, P., & Sarno, D. (2003). Revising paradigms and myths of phytoplankton ecology using biological time series. *Mediterranean Biological Time Series. CIESM Workshop Monographs*, *22*, 109–114.

Zingone, A., Montresor, M., & Marino, D. (1990). Summer Phytoplankton Physiognomy in Coastal Waters of the Gulf of Naples. *Marine Ecology*, *11*(2), 157–172. https://doi.org/10.1111/j.1439-0485.1990.tb00236.x

Zingone, A., Phlips, E. J., & Harrison, P. J. (2010b). Multiscale Variability of Twenty-Two Coastal Phytoplankton Time Series: A Global Scale Comparison. *Estuaries and Coasts*, *33*(2), 224.

Zingone, A., & Sarno, D. (2001). Recurrent patterns in coastal phytoplankton from the Gulf of Naples. *Archivio Di Oceanografia e Limnologia*, *22*, 113–118.

Zingone, A., & Wyatt, T. (2005). Harmful algal blooms: Keys to the understanding of phytoplankton ecology. *The Sea. Harvard University Press, Harvard*, 867–926.