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Species concepts and functional aspects of cold-water diatoms (Bacillariophyceae)

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PREFACE

This thesis is a tribute to the slow, but truly beautiful, science of phytoplankton taxonomy. In the 1970s, Wilson (1971) drew attention to the ’plight of taxonomy‘, and the challenges sketched by him are still highly relevant. Focus had shifted towards more ecological research, to the disadvantage of not only taxonomic research and teaching, but also of the maintenance of systematic collections. And yet, taxonomic diversity forms a foundation for all other fields of biology, and is essential to the understanding of the structure and function of natural species communities. Physiology is performed by species, not by grams of carbon! Moreover, the introduction of advanced microscopic and molecular techniques into taxonomic research has dramatically changed the conceptions of species delimitation and species diversity, a process which will also bear consequences for ecological research. Likewise, functional traits of species can contribute to delineating taxonomic diversity, and the technological advancements within the fields of e.g. metabolomics or transcriptomics are immense. Integrative approaches within ecological and taxonomic research should therefore be favourable to future development of both research fields.

In recent years, some progress has been made. Public awareness of taxonomic issues has momentarily increased through the anniversaries of Carl von Linné and Charles Darwin, the father figures of systematic and evolutionary research. Also, the heavy focus on climate change in ecological research has drawn renewed attention to the risks of change or loss of species diversity. Sweden and Norway have invested resources in taxonomic initiatives (e.g. Artsdatabanken run by the Norwegian Biodiversity Information Centre, Trondheim) and launched a comprehensive programme for a proper species inventory of natural habitats, the results of which are also made available to the general public (e.g. Encyclopedia of the Swedish Flora and Fauna). Per today, these inventories do not include microscopic (single-celled) organisms. Efforts for the successful and continuous recruitment of qualified taxonomists have also been made, e.g. in terms of research schools such as the Swedish-Norwegian Research School on Biosystematics (ForBio), or through specific taxonomic training opportunities such as the Advanced Phytoplankton Course (Stazione Zoologica Anton Dohrn, Naples) and the courses arranged for the identification of Harmful Algal Bloom species (IOC, Copenhagen).

These initiatives are absolutely necessary for a future maintenance and development of taxonomic knowledge at universities as well as at other institutions in society. New focus on taxonomic expertise has emerged with the advance of various bioprospecting projects, in particular in marine environments. Needless to say, accurate and updated taxonomic knowledge in this field is crucial. The need for integrating taxonomic knowledge with functional aspects of a species is slowly being realized and a call for functional or integrative taxonomic concepts has been made by several. This thesis contributes to this process, through integrating traditional biogeographic investigations with experimental studies on phytoplankton species by combining descriptive and analytic tools.

TABLE OF CONTENTS

ORIGINAL PAPERS

ABSTRACT

The choice of species concept when delineating phytoplankton species is decisive for our knowledge of species diversity and distribution, but will also affect our understanding of the functioning of marine ecosystems. This is particularly true for so-called cryptic species, which may show high degree of genetic and physiological heterogeneity although being morphologically similar. A precise species delimitation procedure appreciating the value of genotypic as well as phenotypic traits is therefore important.

In this thesis, species concepts and functional aspects in abundant cold-water diatoms were investigated using biogeographic (species presence and abundance), taxonomic (morphological and molecular data), and functional (experimental physiological and metabolomics data) tools. A compilation of multiannual phytoplankton species abundance data gathered during the spring bloom period from north-east Atlantic and Arctic waters (68-80°N) revealed high similarities in associations of the most abundant species. Spring associations were dominated by the diatom *Chaetoceros socialis* and the prymnesiophyte *Phaeocystis pouchetii*. A corresponding investigation of the winter period in this area could not identify associations of actively growing, autotrophic phytoplankton species. Change in species composition and abundance in time could not be assessed due to lack of consistency and regularity in sampling. A high-resolution time series of phytoplankton distribution is therefore needed in this area.

A case study of the so-called cosmopolitan diatom *C. socialis* was performed using strains from north-east Atlantic/Arctic and from Mediterranean waters. By applying a phylo-phenetic species concept the two geographical populations were found to be pseudo-cryptic. They were genetically distinct, but only slight morphological differences were observed, in this case in resting spore morphology. Furthermore, when cultivated at 2.5, 8 and 13°C, the two groups of strains were functionally different in terms of growth rates (doublings day^{-1}), photosynthetic efficiency (maximum quantum yield), and metabolic profiles. Our results do not support the assumed cosmopolitan distribution of *C. socialis* and therefore the present taxonomy of this species will need revision. A taxonomic update of the pseudo-cryptic diatom formerly known as *Skeletonema costatum* indicated that the species present in northern Norwegian and Barents Sea waters is identical to *S. marinoi*. Due to reports of high genetic, metabolic, and physiological diversity within this species, a functional approach would be valuable for understanding the ecology of *S. marinoi* in this area.

Integrated approaches combining taxonomic and functional variables are recommended for future taxonomic work on phytoplankton species. Similarly, phytoplankton ecologists are urged to acknowledge the high degree of hidden taxonomic and functional diversity present in several phytoplankton species. Functional diversity studies are suggested to be a useful spot for integration and eventual modelling phenotypic, genotypic and ecological species data. Ultimately, such an approach should be beneficial also at the ecosystem level.

LIST OF PAPERS

This thesis is based on the following original papers, which are referred to by their Roman numerals I-IV.

Paper I

Degerlund M, Eilertsen HC (2010) Main species characteristics of phytoplankton spring blooms in NE Atlantic and Arctic waters (68-80^{o}N). Estuaries and Coasts 33: 242-269.

Paper II

Eilertsen HC, Degerlund M (2010) Phytoplankton and light during the northern high-latitude winter. Journal of Plankton Research 32: 899-912.

Paper III

Degerlund M, Huseby S, Zingone A, Sarno D, Landfald B (submitted to J Plank Res) Functional diversity in cryptic species of *Chaetoceros socialis* Lauder (Bacillariophyceae)

Paper IV

Huseby S, Degerlund M, Zingone A, Hansen, E (manuscript) Metabolite fingerprinting and physiology of the cryptic diatom *Chaetoceros socialis* Lauder

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INTRODUCTION

Species diversity in marine diatoms

In ecology, species diversity is defined as a function of the number of species present (species richness) and the evenness with which the individuals are distributed among these species (species evenness) (Margalef, 1958). From a systematic or taxonomic diversity perspective, the focus is often on the first part of the aforementioned definition, i.e., on the number (and the identity) of species. The methods and conceptions applied in the identification process are decisive in estimating the taxonomic diversity of an ecosystem. This is particularly true for marine microorganisms including marine diatoms, the nominal diversity of which has risen immensely over the past century (Pedrós-Alió, 2006; Armbrust, 2009). The diatoms comprise a highly diverse group with ca. 10 000 known species (Guillard and Kilham, 1977), but estimates of total diversity (including unknown species) are in the range 100 000–200 000 species (Mann and Droop, 1996; Armbrust, 2009). The proportion of marine pelagic diatoms is estimated to ca. 5000 (Katz et al., 2004) or even 10 000 species (Sournia, 1995), which is several orders of magnitude higher than the diversity reported for other marine planktonic algae (Kooistra et al., 2007). The evolutionary success of the diatoms as a group has been related to the chimeric nature of the diatom genome (Armbrust et al., 2004; Bowler et al., 2008) and the subsequent ability to diversify in a plethora of different environments.

The nearly exponential increase in microbial diversity and the high variability in reported estimates of diatom diversity are largely dependent on the type and the width of the species concepts applied (Box 1). Moreover, the taxonomic methodology has greatly improved, leading to a higher resolution in the delimitation process. As a result, considerable hidden or cryptic diversity (Box 2) has been disclosed in recent studies (Sarno et al., 2005; 2007; Lundholm et al., 2006; Kooistra et al., 2010). In fact, in state-of-the-art taxonomy, knowledge of several other fields is necessary in order to properly understand the nature and the phylogeny of the taxonomic units under examination. In conjunction with the traditional morphological approach leaning on advanced microscope analyses, the development of molecular techniques has introduced a phylogenetic species concept which often resolves phylogenetic relationships at a finer resolution than the morphological concept does (Alverson, 2008). Chemotaxonomic approaches are still not in regular use in diatom taxonomy (e.g., Chauton and Størseth, 2008), but have been useful for the identification of bacterial diversity not discernible by molecular techniques (Rosselló-Mora et al., 2008). In addition to advanced taxonomical skills, both metabolomics and molecular data demand extensive use of bioinformatics, which will also be important for the future advancement of these methods (Macel et al., 2010). Knowledge of algal physiology and life cycles is essential for taxonomists employing the biological species concept (e.g. Amato et al., 2007), and is obviously useful also when applying the morphological species concept so as to avoid incorrect descriptions of "new", morphologically delineated species that are merely separate life cycle stages of species already known to science (e.g. Edlund and Stoermer, 1997). Functional or ecological species information is also needed in taxonomy when confronted with e.g. morphologically different sympatric populations (Cerino et al., 2005) or allochronic, but morphologically similar populations (Proschkina-Lavrenko, 1953; Paper III). In conclusion, a functional or more holistic species concept would be useful and could result in a better understanding of not only the species richness or taxonomic diversity of a community, but also of the functioning of the taxonomic units described as ecologically important actors in marine ecosystems.

Box 1. *What is a species?*

Morphological species concept; a species differing from all other species according to a set of morphological traits (the number of which is not defined).
Phylogenetic species concept; a species which is monophyletic (belonging to one clade) and forms a genetically coherent cluster of individual organisms or in the case of algae: separate strains.
Phylo-phenetic species concept; a species which is monophyletic, but is diagnosable also by a discriminative phenotypic property.
Biological species concept; a species which is not able to interbreed with other populations, nor produce fertile descendants as the result of interbreeding.
Ecological species concept; a species separated from others on the basis of its ecological niche, i.e., occupying different niches.
Functional species concept; a species which is phylo-phenetic, but diagnosable also in terms of its functional properties, e.g. by physiological (Paper III) or metabolic traits (Paper IV).
Lineage-based species concept; a species constituting a single evolutionary line from direct ancestor to descendant, or a single branch on a phylogenetic tree.
Operational taxonomic units (OTU); a species or taxon defined by statistical treatment of many characters, often also applied to taxa defined in a pragmatic way, separated by e.g. number of DNA bands in a denaturing gradient gel electrophoresis.

For references, see e.g. Pedrós-Alió (2006), de Quieroz (2007), Alverson (2008).

The cosmopolitan species concept

A fundamental topic in biological research is the knowledge of species distribution in space and time. This biogeographic approach also aims at understanding *why* certain species occur in a habitat, at a specific abundance and sometimes with distinct seasonalities, but this aspect of biogeography is often overlooked (Martiny et al., 2006). The much debated hypothesis on ubiquitous dispersal or cosmopolitan distribution of microorganisms (Finlay, 2002; Fenchel, 2005) focuses on species abundance and size-related dispersal mechanisms, but restricts the explanatory dimension of species distribution in defining a cosmopolitan species as “one that thrives wherever its required habitat is realised” (Finlay et al., 2004). However, colonization and diversification is dependant also on past, present and future characteristics of the environment as well as on functional characteristics of the organisms involved (Martiny et al., 2006; Faurby and Funch, 2011). In other words, distribution is not only related to the dispersal abilities of the organisms and planktonic species are not necessarily able to colonize every pleasant spot. Traditional biogeography studies (Smayda, 1958; Braarud, 1962; Hasle, 1976) are also largely descriptive, sometimes relating the reported species distributions to contemporary environmental characteristics such as temperature or salinity, but rarely to physiological characteristics of the species in question. Exceptions are found e.g. within research trying to connect species distribution to phenological or life history traits (Eilertsen et al., 1995; Edwards and Richardson, 2004; D'Alelio et al., 2010; Sarno et al., 2010). Attempts have also been made to replace the species unit with functional plankton types (Thingstad et al. 2010; Allen and Polimene, 2011) or so-called ecotypes (Fraser et al., 2009) in ecosystem descriptions. In modelling studies (e.g. Wassmann et al., 2006), the aim is often to generalize in order to see large-scale structures, but there are also endeavours claiming that “traits, not taxon names, are the fundamental units of biodiversity and biogeography” (Weiher and Keddy, 1995).

When it comes to so-called cosmopolitan species, a considerable range of phenotypic plasticity is required by the species showing such wide distribution patterns. Though somewhat controversial, such plasticity is plausible, but the high degree of intra-specific variation and the seemingly ubiquitous dispersal of many microorganisms may often also be explained by a correspondingly high degree of intra-specific genetic diversity in allopatric or sympatric populations (e.g. Kooistra et al., 2008). Again, the species concept applied will be decisive for the outcome of a biogeographic study, and it is clear that the dictum of microbial biogeography “everything is everywhere – the environment selects” is founded on a morphological species concept. In other words, a large proportion of the species diversity is left unconsidered, or simply not discovered due to inefficient sampling or study techniques (e.g. Pedrós-Alió, 2006). This methodological problem actually works both in favour of the ubiquitous dispersal hypothesis and the contrary. Would it hence be easier to change the unit, and to see biogeography as a function of some other operational unit such as the above-mentioned functional plankton types or trait-based approach? As soon as we broaden our field of view, however, we lose resolution. An immediate challenge here would be to efficiently narrow down the range of traits chosen, so as not to overlook the high degree of functional diversity known to exist within so-called functional groups (Zak et al., 1994; Green et al., 2008), also on the species level (e.g. Thornton, 2002; Rynearson and Armbrust, 2004; Koester et al., 2010; von Dassow and Montresor, 2010). It is thus possible to continue advocating the need for autecological studies and the development of a functional species approach in future biogeographic work. This is a laborious approach indeed, probably requiring extensive experimental studies to complement biogeographic data from natural populations, but it seems necessary in order to depict the processes determining the distribution of microbial species.

Box 2. *Protist diversity is different?*

Hidden diversity; unexpected diversity revealed by molecular analyses.
Cryptic diversity; genetic diversity within taxa not separated by morphological traits.
Pseudo-cryptic diversity; genetic diversity in taxa showing only subtle morphological differentiation. The pseudo-cryptic diversity is often accompanied by ecological or functional diversity. Also referred to as "semi-cryptic", or "previously undetected" diversity.
Functional diversity; diversity in taxa separated by various functional or ecological traits, e.g., often also adaptive strategies.
Allopatric speciation; speciation occurring in geographically (and reproductively) isolated populations.
Sympatric speciation; speciation occurring in populations not geographically, but reproductively, isolated. This can be brought about e.g. by temporal separation of populations occurring over multiple seasons in the same habitat.

For references, see e.g. Amato et al., 2007; McDonald et al., 2007; Alverson, 2008.

Functional aspects of cold-water diatoms

In contrast to the potentially broad biogeographic ranges discussed above, many species seem to have more specific and narrow environmental requirements. Some microorganisms are even suggested to be endemic, though this is a topic equally controversial as the ubiquitous dispersal hypothesis (Sharma and Rai, 2011). From a taxonomic as well as an ecological perspective, diatoms often dominate the microalgal communities of polar waters during peak periods of production, in particular the communities within or in close connection to sea ice (Lizotte, 2003). Compared to diatoms which generally are easily sampled, cultured or preserved, other algal groups have been thoroughly investigated in terms of diversity only more recently (e.g. Lovejoy et al., 2006; Lovejoy and Potvin, 2011), and the contribution of small-celled phytoplankton to primary production is particularly significant prior to and after peak bloom periods (Wassmann et al., 2006; Hodal and Kristiansen, 2008). Many diatom species are if not endemic, at least particularly abundant in cold waters (Priddle and Fryxell, 1985; Medlin and Priddle, 1990). This has been explained by the presence of higher silicate concentrations in such waters (Southern Ocean: Zentara and Kamykowski, 1981), and a successful adaptation to pronounced vertical mixing, irradiance seasonality, low temperatures, and extreme salinities (Cota, 1985; Boyd, 2002; Ryan et al., 2004; Krell et al., 2007). Polar species may show a high tolerance for strong light and long days in combination with low temperature, i.e., conditions prevailing during the midnight sun period (Sakshaug and Slagstad, 1991). Molecular adaptations indicate the presence of specific enzymes and hence the potential for physiological adaptations which enable growth under extreme conditions (Mock and Thomas, 2004). More specifically, up-regulation of stress-response genes and genes for protein turnover has been observed during high light conditions in the polar diatom *Fragilariopsis cylindrus* (Grunow) Krieger (Mock and Valentin, 2004). Furthermore, specificity factors of RUBISCO (Ribulose-1.5-bisphosphate carboxylase oxygenase), the enzyme involved in carbon fixation during photosynthesis, can be higher in the cold-water diatoms *Chaetoceros socialis* Lauder, *Thalassiosira hyalina* (Grunow) Gran, and *Thalassiosira antarctica* Comber (Haslam et al., 2005), or at least, function differently than in species from lower latitudes (Li et al., 1984; Smith and Platt, 1985).

Descriptions of characteristic cold-water diatom species date back to the late nineteenth century (Cleve, 1873; 1896; Gran, 1902; 1904) and biogeographic literature has categorized these species either by their association to specific water masses, water depths, geographic regions or life histories of the species (Box 3). Numerous reports exist on spatial and temporal distribution of cold-water phytoplankton (Gaarder, 1938; Eilertsen et al., 1981; Syvertsen, 1991; Wassmann et al., 1999; Hegseth, 1992; 1997; von Quillfeldt 2000; Rat'kova and Wassmann, 2002), but summative analyses linking species distribution with environmental variables (e.g. Evensen, 1994) are very scarce. The harsh conditions prevailing during winter may perhaps explain the low number of studies dealing with cold-water phytoplankton during this period (Khromov and Salakhutdinov, 1985; Seuthe et al., 2010). Modelling studies (Wassmann et al., 2006) tend to be large-scale and hence only include higher taxonomic levels or functional groups and the functional species perspective becomes missing. Hidden diversity is also to be expected in cold-waters, and as a matter of fact, considerable phenotypic plasticity has been observed in diatoms from north-east Atlantic and Arctic waters (von Quillfeldt, 1996; Donnelly, 2006). There is hence a need for detailed taxonomic investigations employing a functional species approach. Knowledge of diversity on the autecological (species) level should be of great value when extrapolated to a larger (species community or ecosystem) scale.

Box 3. *Floristic and biogeographical categories of cold-water phytoplankton*

Life-history or water depth-related categories suggested by Haeckel (1890) and redefined by Gran (1902):
Neritic; a species confined to coastal or inshore waters, possessing a sedentary (resting) stage
Oceanic; a species occupying open ocean/deep-sea waters in a continuously pelagic state
Holoplanktonic; a species possessing a purely pelagic life cycle
Meroplanktonic; a species possessing benthic as well as pelagic life cycle stages
N.B. A neritic species need not be meroplanktonic, and vice versa! This applies also to the terms oceanic/ holoplanktonic (Smayda, 1958).

Plankton types or species assemblages named after key species (Cleve, 1896):
A. Oceanic
1. Triposplankton (high abundance of cilioflagellates, i.e. dinoflagellates such as *Ceratium tripos*, few diatoms, dominating in the summer)
2. Styliplankton (abundance of diatoms, e.g. *Rhizosolenia styliformis, R. alata, R. gracillima*)
3. Chaetoplankton (*Chaetoceros* spp., *C. decipiens*, *C. borealis*, and *C. constrictus* in particular)
4. Desmoplankton (*Trichodesmium* spp.,)
5. Trichoplankton (*Rhizosolenia semispina*, *Thalassiothrix longissima*)
6. Siraplankton (*Thalassiosira nordenskioeldii* key species)
B. Neritic
1. Didymusplankton (e.g. *Chaetoceros didymus*, *C. curvisetus*, *C. danicus*, *Ditylum brightwellii*, *Eucampia zodiacus*)
2. Northern neritic plankton (*Leptocylindrus danicus*, *Skeletonema costatum*, *Lauderia annulata*)
3. Arctic neritic plankton (many species in common with the oceanic Siraplankton)
4. Concinnusplankton (*Coscinodiscus* spp. *C. concinnus*)
5. Halospaeraplankton (*Halosphaera viridis*)

Plankton elements or assemblages named after biogeographic region (Gran, 1902):
A. Neritic
1. Arctic-neritic plankton element (*Chaetoceros furcellatum*)
2. Boreal-neritic plankton element (*Thalassiosira nordenskiöldii, Chaetoceros debile*)
3. Temperate-Atlantic-neritic plankton element (*Chaetoceros didymum, Evadne Nordmanni*)
A. Oceanic
1. Arctic-oceanic plankton element (*Ceratium arcticum*)
2. Boreal-oceanic plankton element (*Ceratium longipes*)
3. Temperate-Atlantic, oceanic plankton element (*Ceratium macroceros, C. tripos, C. fusus*)

Categories related to environmental tolerance (Smayda, 1958; Hasle, 1973):
Stenothermal; a species possessing a narrow temperature tolerance, common in species adapted to cold-waters (e.g. Atlantic, Arctic, Polar, Bipolar species)
Eurythermal; a species possessing a wide temperature tolerance, common in warm-water (e.g. tropical) species
Stenohaline; a species possessing a narrow salinity tolerance, common in cold-water and warm-water species
Euryhaline; a species possessing a wide salinity tolerance
Cosmopolitan; a widely distributed, or circumglobal species, being at least eurythermal in its environmental tolerance, but preferably also euryhaline. So-called bipolar species are assumed to be cosmopolitan.

OBJECTIVES

The overarching goal of this thesis is to evaluate the species concepts and functional aspects of common cold-water diatoms. This was achieved by examining the variability of biogeographic phytoplankton species data and environmental variables over several years in northern waters, and by closer examination of taxonomic and functional (physiological and biochemical) diversity in selected species. The approach chosen is integrative in that it combines several different tools to examine taxonomy (morphology, molecular data) and physiology (growth rate, photosynthetic performance and biochemistry). It is hypothesized that the traditional species concept defined by morphological species characteristics may not be applied to all mitotic dividing organisms in a dynamic environment or in environmentally far apart areas. It is therefore anticipated that phylogenetic and functional analyses will revise the taxonomy of several phytoplankton species.

To address this objective, the following research questions were posed:

1) What are the main phytoplankton species associations in northern waters and how do they relate to environmental variables?
2) What is the validity of the species concepts in ecologically plastic or so-called cosmopolitan diatoms, dominant in large geographical areas and in areas far apart?
3) Can functional diversity studies elucidate the taxonomic and ecological identity of cryptic, i.e., morphologically similar, but phylogenetically distinct, phytoplankton species?
4) What are the consequences of cryptic or hidden diversity for predicting physiology and productivity of phytoplankton stocks?

STUDY AREA

The present study was performed on compiled data from publications, monographs and reports, and on own samples from north-east Atlantic and Arctic waters (Paper I-IV). For comparison, an experimental study was also performed on corresponding diatom species from the Mediterranean Sea (Gulf of Naples, Tyrrhenian Sea, Paper III-IV).

High-latitude areas are characterized by pronounced seasonal variations in the geophysical variables light, temperature and salinity (affecting vertical stability). The seasonal variations in light (irradiance and photoperiod) are particularly distinct north of the Arctic Circle (66^{o} 33' 44" N), where winter darkness and midnight sun prevail during large parts of the year. This seasonality is naturally reflected in the annual pattern of primary production and species succession. In open north-east Atlantic (Barents Sea) and Arctic waters, seasonal changes in water temperature and salinity are closely linked to the spatial and temporal extent of the annual ice-cover, which also governs under-water light conditions as well as the stability of the water column. Hence, the spring bloom has often been observed to follow the annual retreat of the ice in this region (e.g. Rey and Loeng, 1985; Strass and Nöthig, 1996; Engelsen, 2002). Due to the inflow of Atlantic water in to the Barents Sea, the hydrophysical and biological features of this region are also governed by large-scale variability in atmospheric processes over the Atlantic Ocean (Ottersen and Stenseth, 2001). This inflow is highly variable and is governed by the so-called North Atlantic Oscillation (NAO), which is an atmospheric change in the pressure distribution affecting the whole ocean basin (Hurrell, 1996). This variability has profound effects on dynamics in the northern marine ecosystems (Dickson, 1999).

Closer to the coast in the north Norwegian fjords, local climatic processes increase in importance, e.g. for total heat exchange (Eilertsen and Skarðhamar, 2006), which is generally positive (heating of surface waters) from May to August. During the spring bloom period in April-May, sea surface temperature anomalies in these fjords may be as large as ± 2^{o}C (ibid.). Sea ice is present only in the innermost parts of more isolated fjords, and freshwater runoff affecting water column stability normally sets in after the spring bloom peak in April. In the pre-bloom period, the radiation dose increases, and the magnitude of this increase is larger the further north one gets (Fig. 1). Since waters are clear (low k, i.e., extinction coefficient for solar irradiance), the critical depths deepen fast and may get excessively deep (Platt et al., 1991; Eilertsen and Franzen, 2007). Consequently, the onset of spring blooms often take place in non-stratified (and non-heated) surface waters (Eilertsen and Taasen, 1984; Eilertsen, 1993;

Wassmann et al., 1996). Stratification and regeneration of nutrients grow more important in regulating primary production during summer, both along the northern Norwegian coast and in the Barents Sea (Eilertsen and Taasen, 1984; Eilertsen et al., 1989a; Sakshaug and Skjoldal, 1989; Kristiansen et al., 1994), whereas the decrease in incoming light limits growth towards the end of the productive season (Hegseth, 1992). Grazing does not normally influence the spring bloom onset in northern areas where the main herbivore grazers (i.e., mesozooplankton) only have one generation, appearing late in the spring (Eilertsen et al., 1989b; Båmstedt et al., 1992). Microzooplankton grazers are present already at the onset of the spring bloom and may hence efficiently mediate the energy flux to higher trophic levels during this period (Seuthe et al., 2010).

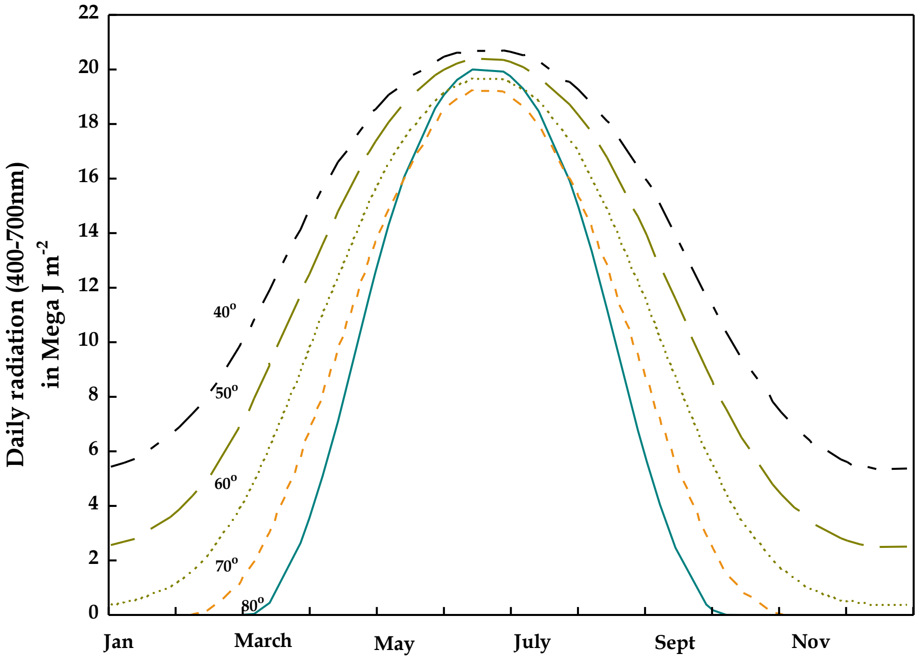

Figure 1. Seasonal variation in diurnal radiation PAR dose (400-700 nm) for latitudes 80, 70, 60, 50 and 40^{o}N. Values are theoretical and modelled according to the procedure described in Eilertsen and Holm-Hansen (2000) using a cloud cover of 1.0 (scale 0-9 where 9= full cloud/fog cover).

From a primary production perspective, irradiance and photoperiod therefore appear to be the most important environmental variables. Two to four months of winter darkness with close to zero levels of irradiance and photoperiod (Paper II) are followed by an intense increase in integrated light (photoperiod and irradiance) after the vernal equinox (Fig. 1, 2), which culminates in two to four months of midnight sun with continuous light. During this period, diurnal variations in irradiance are mainly governed by the elevation of the sun

(Jerlov, 1976). In fact, due to the compensating effect of the midnight sun period, integrated annual irradiance in Tromsø amounts to 85–90% of that in e.g. Oslo (60°N, Fig. 1). Primary production closely follows this seasonality in northern waters, and phytoplankton life history strategies are tightly linked to the seasonal changes in irradiance and photoperiod (Eilertsen and Wyatt, 2000). Initial stocks for the spring bloom are largely provided by the photoregulated germination of re-suspended resting stages and spores (Eilertsen et al., 1995; von Quillfeldt, 1996) produced towards the end of the previous productive season. This is the apparent overwintering strategy for many of the neritic diatom species dominating during the spring bloom in the north-east Atlantic (Hegseth et al., 1995; Paper II). In ice-covered waters, algae connected to the sea ice can also provide inoculum for the spring bloom (Syvertsen, 1991; Rat'kova and Wassmann, 2002). Heterotrophic or mixotrophic life history strategies allow other groups of the plankton to sustain their growth also outside the relatively short productive season. Mixotrophic dinoflagellates and ciliates are primarily active as autotrophs during the spring and summer, while they may act as heterotrophs during the rest of the year (Seuthe et al., 2010). Likewise, heterotrophic bacteria together with heterotrophic and autotrophic picoplankton and nanoflagellates are abundant during spring and summer, and are present during winter though with reduced activity levels (Iversen and Seuthe, 2010). The life history of one of the most important phytoplankton species in northern waters, the prymnesiophyte *Phaeocystis pouchetii* (Hariot) Lagerheim, is to a considerable degree as yet not completely resolved (e.g. Rousseau et al., 1994; Peperzak et al., 2000; Gæbler-Schwarz et al., 2010).

Table 1. Annual range (min-max) of geophysical variables in surface waters of the north-east (NE) Atlantic coastal fjords (Eilertsen & Skarðhamar 2006, Paper I), the Barents Sea (BS) (Sakshaug & Slagstad 1991, Paper I), and the Mediterranean (Garrett et al., 1993; Zingone et al. 2010).

	NE Atlantic / Barents Sea	**Mediterranean**
Temperature, ^{o}C	-1.8 – +9.4	12.4 – 28.5
Salinity	ca. 22 – 38	36.5 – 38.3
Heat flux, W m^{-2}	-181 – +155[1]	-100[2] – +ca.120[3]
Photoperiod, h	0 – 24	ca. 9 – 15

[1] Heat flux values only for NE Atlantic waters. Annual values for the Barents Sea are not available.

[2] Mean minimum heat flux for the period 1985/1986–1998/1999. After 2000, the mean minimum heat flux has changed to -140 W m^{-2} (Zingone et al., 2010).

[3] Monthly means for May-July, when total heat flux is maximal during the summer period (Garrett et al., 1993).

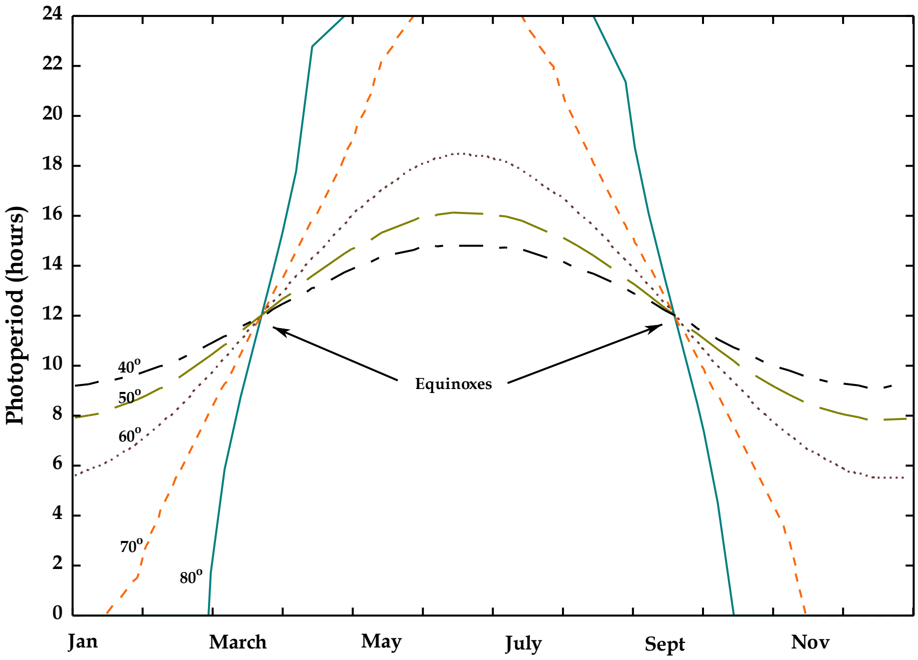

Figure 2. Seasonal variation in photoperiod for latitudes 80, 70, 60, 50 and 40°N. Values are theoretical and modelled according to the procedure described in Eilertsen and Holm-Hansen (2000).

In contrast to the northern waters, seasonality in Mediterranean waters is much less pronounced. Temperature variability is greater, and even though the total heat exchange range is smaller compared to that of northern waters (Table 1), the length of e.g. the period with positive heat flux is considerably longer in the Mediterranean (lasting from late March to November, Zingone et al., 2010). Consequently, variations in irradiance and photoperiod are by far not as extreme as in northern waters (Table 1, Fig. 1, 2). Differences in the production patterns are therefore also less pronounced, as conditions favourable to photosynthetic growth are present during larger parts of the year (Zingone et al., 1995; Modigh, 2001; Ribera d'Alcalá et al., 2004; Zingone et al., 2010). Calculations on energy flux and critical depths over the whole water column have indicated light conditions sufficient for net growth during most of the winter period (Zingone et al., 2010).

The Gulf of Naples is a coastal embayment on the SW Italian peninsula with an average depth of 170 m. The area is heavily influenced by runoff from land but is also affected by advection from more open Tyrrhenian Sea waters (Zingone et al., 1995). Autotrophic biomass usually shows an increase in winter, followed by a peak in late spring-summer, and a new increase during the autumn (Ribera d'Alcalá et al., 2004). Diatoms generally dominate during the growth peaks, whereas flagellated species (prymnesiophytes and other nanoflagellates) appear more regularly, often in deeper water layers (Zingone et al., 1990). Dinoflagellates peak during summer and produce cysts from late spring to early autumn (Montresor et al., 1998). Production of resting stages is in other words not restricted to high-latitude areas, and the percentage of cyst-forming dinoflagellates does not necessarily differ significantly from that observed in northern waters (Dale, 1976). Winter season in the Gulf of Naples is mainly characterized by an association of flagellates and coccolithophores, alternating with occasional peaks of small-celled diatoms. Autotrophic biomass is generally smaller during winter and is often more dispersed in the water column, but depth-integrated biomass levels during the occasional peaks can be in the range of spring or summer values (Zingone et al., 2010). Grazing in these areas will have a more profound impact on spring blooms and other peaks of autotrophic biomass due to the presence of larger zooplankton biomasses during winter and early spring (Mazzocchi et al., 2011).

The two geographical regions are in other words very different in terms of the seasonal variation of geophysical variables and primary production. And yet, there are many phytoplankton species occurring in both regions (WeMP - Website on Mediterranean Phytoplankton; Paper I; Throndsen and Kristiansen, 1991; Rat'kova and Wassmann, 2002; Iversen and Seuthe, 2010; Seuthe et al., 2010). Most species groups are represented, i.e., diatoms (e.g. *Chaetoceros affinis*, *C. brevis*, *C. decipiens*, *C. diadema*, *C. curvisetus*, *C. socialis*, *C. tenuissimus*, *Cylindrotheca closertium*, *Dactyliosolen fragilissimus*, *Leptocylindrus danicus*, *L. minimus*, *Proboscia alata*, *Pseudo-nitzschia delicatissima*, *Skeletonema marinoi*, *Thalassionema nitzschioides*, *Thalassiosira rotula*/*gravida*), dinoflagellates (e.g. *Alexandrium minutum*, *A. tamarense*, *Ceratium furca*, *C. fusus*, *C. tripos*, *Gonyaulax spinifera*, *G. verior*, *Gymnodinium pulchellum*, *Gyrodinium spirale*, *Protoperidinium divergens*, *P. steinii*, *Scrippsiella trochoidea*), dictyochophyseans (*Dictyocha fibula*, *D. speculum*), coccolithophores (*Emiliania huxleyi*), prasinophyceans (*Halosphaera viridis*, *Micromonas pusilla*), cryptophyceans (*Teleaulax acuta*), chrysophyceans (*Dinobryon faculiferum*), and raphidophyceans (*Fibrocapsa japonica*). Not all of the above-mentioned species are ecologically equally important in both regions, but nevertheless, they apparently manage to cope with such different growth conditions as depicted above. This conclusion assumes that these species are true functional species, i.e., phylo-phenetic species diagnosable also in terms of their functional traits, and in that case, showing broad ecological tolerance.

METHODS

Field data

Field data on phytoplankton species abundance and environmental variables were gathered in May 2006, 2007 (Paper I), in February 2001, 2005, and in December 2008 (Paper II). A database on species abundance from published and unpublished sources was compiled, to form the basis of an extensive review on species presence, abundance and succession in north-east Atlantic and Arctic waters during periods of high (Paper I) and low (Paper II) phytoplankton abundances. These data sets laid the foundation for subsequent experimental physiological and taxonomic studies (Paper III, IV) performed in the laboratory.

Monocultures

The establishment of algal material in culture is necessary for detailed taxonomic and molecular analyses, even though the techniques for screening natural populations in terms of their genetic composition are becoming more and more advanced (e.g. Hamsher et al. 2011). Monocultures of selected cold-water diatoms were isolated from north-east Atlantic and Arctic waters (Table 1 in Paper III, with the addition of e.g. *Skeletonema costatum sensu lato*). Several isolates were established of each species in order to include a larger proportion of the genetic variability observed within natural populations of phytoplankton (e.g. Brand, 1981; Thornton, 2002). Isolations were performed manually, transferring single cells or colonies from diluted net samples or germinated spore samples using micropipettes in inverted microscopes. Cultures were then maintained in f/10 medium (Guillard and Ryther, 1962) with additional silicate (12.3 µmol $Si(OH)_4$ l^{-1}) in irradiance and temperature controlled rooms at ambient light and temperature conditions until further analysis was undertaken. Additional cultures of corresponding species were isolated from Mediterranean waters (Gulf of Naples, Tyrrhenian Sea) for intra-specific comparisons. When possible, morphological examinations of natural populations in field samples were conducted (e.g. *Chaetoceros socialis*, *Skeletonema* sp., *Thalassiosira* spp.). Strains were considered to be unique when isolated from different geographical locations or at different times of the year (see methods Paper III).

To minimize selection within cultivated strains (e.g. Wood and Leatham, 1992; Lakeman et al., 2009), strains from culture collections were not used and the majority of the strains used for experimental purpose were recent isolations. Strains from different locations were maintained under similar conditions, with the exception of the Mediterranean strains which were initially cultivated at ambient conditions of their original location and then gradually acclimatized to cold-water conditions prior to the experiments. The potential for in-culture evolution could in other words not be excluded. Detection of such evolutionary distances between strains would probably require the analysis of another gene region, e.g. a protein-encoding gene region, since the ribosomal RNA region (partially sequenced for Paper III) is considered a so-called conditionally neutral phylogenetic marker (Petrov and Aleshin, 2002), which largely diverges as a result of passive genetic drift rather than adaptation to environmental forcing exerted by e.g. continuous cultivation.

Morphological studies and taxonomic nomenclature

Traditional morphological examinations still form a regular part of state-of the-art taxonomic phytoplankton research, and the methodology in use remains largely unchanged even though microscope technology has revolutionarily improved in course of the 20th century. In this study, general morphological characters and morphometric features of the cells were examined in light microscopes (LM) whereas ultrastructural details of the diatom frustules were further studied in scanning (SEM) and/or transmission (TEM) electron microscopes. Diagnostic features were documented with microscope cameras. Prior to microscopy, cleaning of diatom cells was performed in order to remove organic material. This was done either by the use of hydrogen peroxide (H_2O_2) and heating, without exposure to UV radiation, or using acids (HNO_3, H_2SO_4), after which permanent slides for LM or samples for EM could be prepared. The systematic nomenclature generally followed Hasle and Syvertsen (1997) or recommendations given by the taxonomical database ERMS 2.0 of MarBEF, the EU Network of Excellence for Marine Biodiversity and Ecosystem Functioning (http://www.marbef.org/data/erms.php), comparable to the World Register of Marine Species, WoRMS (http://www.marinespecies.org). In the case of cryptic or pseudo-cryptic species such as *Skeletonema* spp., biogeographical species data (Paper I, II) was gathered under the name *Skeletonema costatum sensu lato*, indicating the species which was formerly described as *S. costatum* (Zingone et al., 2005).

Molecular analysis

For phylogenetic purpose, partial sequences of 28S or the large subunit (LSU) of the ribosomal RNA gene region were retrieved from the centric diatoms *Chaetoceros socialis* (Paper III) and *S. costatum sensu lato*. The resolution of this gene region is different from that of e.g. the small subunit (18S or SSU rRNA), i.e., the gene region for which the largest number of sequences are available in public databases. Similar in resolution to SSU, the COI-5P gene region has been suggested for standard use in barcoding of diatoms (Moniz and Kaczmarska, 2009). SSU and COI-5P are mainly able to detect inter-specific variability (Evans et al., 2007), but COI-5P is less universal due to its restricted ability to amplify in certain species (Hamsher et al., 2011). The ribosomal LSU gene region is often able to resolve phylogenetic relationships on the intra-specific level in diatoms and has been demonstrated to detect cryptic diversity within morphologically delineated species (Sarno et al., 2005; 2007; Amato et al., 2007; Kooistra et al., 2010).

Universal primers for the LSU rRNA gene region were used following Leaners et al. (1989) and Scholin et al. (1994). Sequence products were obtained using the BigDye® v3.1 Sequencing Protocol (PE Biosystems, Foster City, USA). Sequence alignments were carried out by the Clustal W algorithm (Higgins et al., 1994), and consecutive phylogenetic inferences were performed by the MEGA 5.0 (Tamura et al., 2011) implementation of maximum likelihood (ML) analysis, and by employing the Bayesian analysis program MrBayes 3.1 (Ronquist & Huelsenbeck 2003).

Physiological studies

In order to investigate the functional diversity in strains of *C. socialis*, a series of experiments was run, where physiological characteristics in terms of growth rate and photosynthetic efficiency were estimated as a function of temperature (Paper III, IV). The estimation of algal growth rates (e.g. Eppley, 1972) is usually reported either as (maximum) specific growth rates (day^{-1}), or as division rates (doublings day^{-1}), usually referred to as μ or μ_{max}, but the application of these two different estimates in the literature is sometimes confusing, inconsistent, or not satisfactorily reported (e.g. Eppley, 1972; Gilstad and Sakshaug, 1990; Leu et al., 2007). Growth rates in Paper III and IV were determined as doublings day^{-1} using the formula $\mu = \log_2 (N_2 / N_1) / (t_2 - t_1)$, where N_1 *and* N_2 designate cell abundance (cells l^{-1}) or chlorophyll *a* concentrations (µg l^{-1}) measured at the start (t_1) and end (t_2) of each experimental run, and *t* is time in days.

Photosynthetic activity in microalgae can be estimated through e.g. oxygen evolution, Photo System (PSII) fluorescence kinetics, or ^{14}C assimilation, all of which are employed to assess primary production in aquatic environments (e.g. Hancke et al., 2008). PSII fluorescence measured by PAM fluorometry gives an approximation of the operational or maximum quantum yield in PSII (Φ_{PSII}). As such, the quantum yield describes the fraction of open and closed reaction centres in PSII (Schreiber et al. 1986), and may hence be applied as an estimate of the photosynthetic efficiency of the algal cells. In this study, PAM fluorometry was used as a means of monitoring the physiological status of the strains in culture (Paper III, IV). Φ_{PSII} was measured using a Water-PAM (Pulse Amplitude Modulated) fluorometer (Water-ED/B, Heinz Waltz GmbH). Φ_{PSII} was calculated as $\Phi_{\mathrm{PSII}} = (F_m - F_0) / F_m$, where F_m is the maximum fluorescence from the saturating light pulse (660 nm, modulated at a frequency of 20 kHz) in dark acclimated cells, and F_0 is the initial fluorescence (from the measuring light) in dark acclimated cells.

Biochemical analysis

As an additional functional phenotypic character, metabolite profiles were analysed in material collected during the experiments performed with strains of *C. socialis*. A wide array of metabolites can be analysed with different types of nuclear magnetic resonance (NMR) and mass spectrometry (MS) methods (e.g. Chauton and Størseth, 2008; Beckmann et al., 2008), even though no single technique can cover all metabolites in an organism (Macel et al., 2010). In the present study (Paper IV), ultra-performance liquid chromatography (UPLC, Waters) and high resolution mass spectrometry (HR-MS) was used in combination with specific data analytical tools (details given in Paper IV). This method has proven useful for the classification of filamentous fungi and yeast (Frisvad et al., 2008; Smedsgaard and Nielsen, 2005). The application of metabolomics fingerprinting in ecological algal research is limited to a few studies (Bölling and Fiehn, 2005; Chauton and Størseth, 2008; Barofsky et al., 2009; 2010), and the chemotaxonomic dimension in phytoplankton is as yet not fully explored (Chauton et al., 2003). Paper IV is an attempt to elucidate the chemotaxonomic application of metabolite fingerprinting to cryptic diatom species.

RESULTS AND DISCUSSION

Phytoplankton species associations in northern waters

The pioneers of phytoplankton biogeography in the nineteenth century did not refrain from defining the less studied waters as “unknown” in terms of species composition or so-called plankton types (e.g. Cleve, 1897). On the other hand, they also readily assigned particular assemblages of phytoplankton species to specific “biogeographic” categories, which resulted in a high degree of inconsistency and overlapping of categories when more data consecutively was added (Box 3). In contrast, contemporary studies often tend to overlook the problem of missing data when extrapolating sporadic data sets to large-scale ecosystem descriptions or models of future system change. The compilation of phytoplankton species abundance data from periods of high (Paper I) and low (Paper II) biomass presented in this thesis attempted to synthesize existing data and connect the species data to environmental variables. Due to an abundance of data rather than consistency in the sampling methods, we concluded with certain trends in species distribution in time and space. Therefore, one of the main conclusions of our study was that neither the phytoplankton species data per se nor the methods used to collect the data, was satisfactory in terms of consistency, spatial or temporal coverage so as to be able to conclude anything on potential long-term changes in species composition or environmental variables of the area studied (Paper I). However, we were able to say something on the methods used to define phytoplankton species associations and the ecological relevance of such approaches.

Earlier characterizations of phytoplankton species associations were mainly based on direct registrations of species found together at the time of sampling in a particular location (Box 3, Table 2 in Paper I). If repeated over several years and growth seasons, the observations get more representative, but very few studies are performed on such a temporal scale. This direct observational approach (Table 3) renders a “general multiannual association” of spring (March-May) species for north-east Atlantic and Arctic waters, consisting mainly of the prymnesiophyte *Phaeocystis pouchetii* and the diatoms *Fragilariopsis oceanica*, *Chaetoceros socialis*, *C. furcellatus*, *C. compressus*, *C. debilis, Skeletonema costatum sensu lato*, *Thalassiosira* spp., and *Bacterosira bathyomphala* (Paper I). This association is largely coherent with earlier observations from these waters (Heimdal, 1974; Evensen, 1994; von Quillfeldt, 2000), even with the so-called *Sira* plankton described by Cleve (1899) at the turn of the nineteenth century (See Box 3). The question is how representative such a characterization is, given the dynamic nature of phytoplankton community succession (Smayda, 1980) and of the water masses hosting the plankton. When based on statistical correlations between species assumed to grow together instead of direct registration of the most abundant species, the corresponding multiannual spring association of best correlated species for the whole region is highly similar (Table 3 in Paper I), but some of the most important spring species (in terms of abundance and frequency in reported observations) are left out. *Phaeocystis pouchetii* appears highly dynamic, being able to appear early as well as late in the succession of spring species, and is therefore not well correlated to other species. *Skeletonema costatum sensu lato* is in our compilation restricted to Atlantic waters (Paper I), which may explain the weak correlation to other abundant species in the whole data set. A statistical approach applying multivariate PCA ordination analysis seems more able to catch the stochastic nature of *P. pouchetii* and gives a better overview of how the most abundant species occur together in relation to different environmental variables (Fig. 5, 6 in Paper 1). Also, the splitting of the PCA analysis into two separate geographic regions affected the ordination of *S. costatum sensu lato*, in particular its distribution in relation to environmental variables in the Barents Sea. When run on data for the whole region, such information in the PCA plot was lost (Table 3).

Table 3. Phytoplankton species associations suggested for north-east Atlantic and Arctic waters applying different methodological approaches. Species abundance data from all locations is compiled in one geographical region.

Method	**Association**	**Predominant species**
Direct registration	Spring species (March-May)	*P. pouchetii, F. oceanica, C. socialis, C. furcellatus, C. compressus, C. debilis, S. costatum sensu lato, Thalassiosira* spp., *B. bathyomphala*
Spearman rank correlation	Spring species (March-May)	*C. socialis, C. furcellatus, T. nordenskioeldii, F. oceanica, C. compressus, C. debilis,*
PCA ordination	Early spring species	*P. pouchetii*[1] , *S. costatum sensu lato*[1], *F. oceanica, C. socialis, T. nordenskioeldii, C. compressus, C. debilis*
	Late spring species	*C. furcellatus, C. diadema, T. gravida*/*T. antarctica* var. *borealis, B. bathyomphala*[1]

[1] The ordinations of *P. pouchetii, S. costatum sensu lato* and *B. bathyomphala* change when ordinated separately for the coastal region and the Barents Sea. *P. pouchetii* was a late spring species, separated from the diatoms, in the analysis on coastal data, whereas *S. costatum sensu lato* appeared as a late, and *B. bathyomphala* as an early spring species in the Barents Sea data.

What is then the ecological relevance of depicting a phytoplankton species association? And how do we deal with the information or diversity which is lost when concentrating on the most abundant species? When based on solid time series data related to a sufficient number of environmental variables and combined with physiological information of the species constituting the association, knowledge of the composition of a species association may render information on the ecological state of the system in question. Such information may be bloom phase, productivity and carbon flux potential, nutritional quality, toxicity risk, etc. If this was to be achieved, real functional groups could be described. The compilation presented here (Paper I) does not meet these demands e.g. in that it lacks resolution in terms of temporal and spatial coverage of phytoplankton species abundance data. In addition, the statistical analysis was run on the most abundant species only, which may have contributed to the high similarity observed between species associations in the whole region. Nevertheless, the information available on species composition allows identifying successional groups during the spring in relation to predictable environmental factors such as water column stability, irradiance (PAR), and photoperiod.

Concerning the winter period (December-February) along the north Norwegian coast, no species associations were discernible due to the low numbers of vegetative cells detected in the water column (Table V in Paper II). *Skeletonema costatum sensu lato* was the only species observed repeatedly both during the winter months and in the spring. Also in this study, a higher taxonomic resolution could have rendered more refined results in terms of species important e.g. for the microbial loop, which is active throughout the period of winter darkness in northern waters (Iversen and Seuthe, 2010; Seuthe et al., 2010). Primary production measurements could be interesting to perform in relation to the low, but measurable irradiance registered by us during the dark winter period (Paper II). Net photosynthesis has been observed well below the 1 % compensation depth generally defining the lower limit of euphotic layers (e.g. Erga, 1989). During parts of the winter in northern waters, irradiance backscattered from clouds could possibly be enough to sustain status quo photosynthesis in cells close to the sea surface (Paper II).

In conclusion, knowledge on the generation, organisation, and distribution of taxonomic diversity such as phytoplankton species associations is useful when trying to understand the structure and function of an ecosystem. Species associations in north-east Atlantic and Arctic waters are highly similar during the spring and are dominated by the diatom Chaetoceros socialis *and the prymnesiophyte* Phaeocystis pouchetii. *Winter populations in the northern Norwegian fjords are absent in terms of actively growing, autotrophic microalgal cells. A high-resolution time series of phytoplankton abundance in this region would be very useful* *for an improved understanding of the community structure and for the eventual detection of future change.*

The cosmopolitan species concept revisited

The disclosure of cryptic or pseudo-cryptic diversity in microalgae has cast doubts on the presumed cosmopolitan distribution of many species. A scholarly example is provided by the centric diatom *Skeletonema costatum* (Greville) Cleve, a particularly abundant and fast-growing species, considered to be cosmopolitan, and commonly used in a number of community and autecological studies (e.g. Pratt, 1966; Serra et al., 1978; Sakshaug et al., 1989; Thornton and Thake, 1998; Ianora et al., 2004). Observations of a high degree of morphological variability within *S. costatum* and related species (Hasle, 1973), and recent morphological examinations combined with molecular analyses (Medlin et al., 1991; Sarno et al., 2005) discovered considerable genetic variation within the genus *Skeletonema*. This ultimately resulted in an emended description of the type material or the “real” *S. costatum sensu stricto,* i.e., *S. costatum* (Greville) Cleve emend. Zingone et Sarno (Zingone et al., 2005), and the description of several new species (ibid.; Medlin et al., 1991; Sarno et al., 2005; 2007) within the morphospecies previously referred to as *S. costatum*. The biogeography of the *Skeletonema* species was concomitantly revised (Kooistra et al., 2008), and taxonomic updating was carried out in geographical areas not covered by the revision (e.g. Naik et al., 2010). Based on morphological examinations of natural and cultivated material in EM, the *Skeletonema* species in north-east Atlantic waters referred to as *S. costatum sensu lato* (Paper I, II) is identical to *S. marinoi* Sarno et Zingone (Fig. 3; Paper I). A proper phylogenetic analysis has not been performed due to limited availability of high-quality sequences of *Skeletonema* isolates from northern Norwegian or Barents Sea waters, but a preliminary BLAST (Zhang et al., 2000) alignment of the partial sequences from the LSU rRNA gene region rendered the highest similarity (96 %) with the LSU sequences of *S. marinoi* available in the public nucleotide database GenBank.

However, considerable genetic heterogeneity is reported for *S. marinoi* (Ellegaard et al., 2008; Godhe and Härnström, 2010; Saravanan and Godhe, 2010). While phylogenetically distinct from its sibling species *S. dohrnii* Sarno et Kooistra (Sarno et al., 2005; Ellegaard et al., 2008), overlapping of the ultrastructural girdle band characters separating the two species on a morphological basis has been observed (Kooistra et al., 2008), even in *S. marinoi* strains isolated from the same net sample (Ellegaard et al., 2008). In this case, the morphological species concept on its own is not enough to properly delineate the diatom species. Rather, an integrated approach combining several delimitation parameters (a phylo-phenetic species concept) can prove useful. In particular in the case of ecologically plastic diatoms, such an approach can decrease the risk of overlooking the functional diversity obviously present within the taxa. In the case of *S. costatum sensu lato*, the change from a morphological species concept to a phylo-phenetic species concept combining genetic and morphological characters also bore consequences for the biogeographic status of the species.

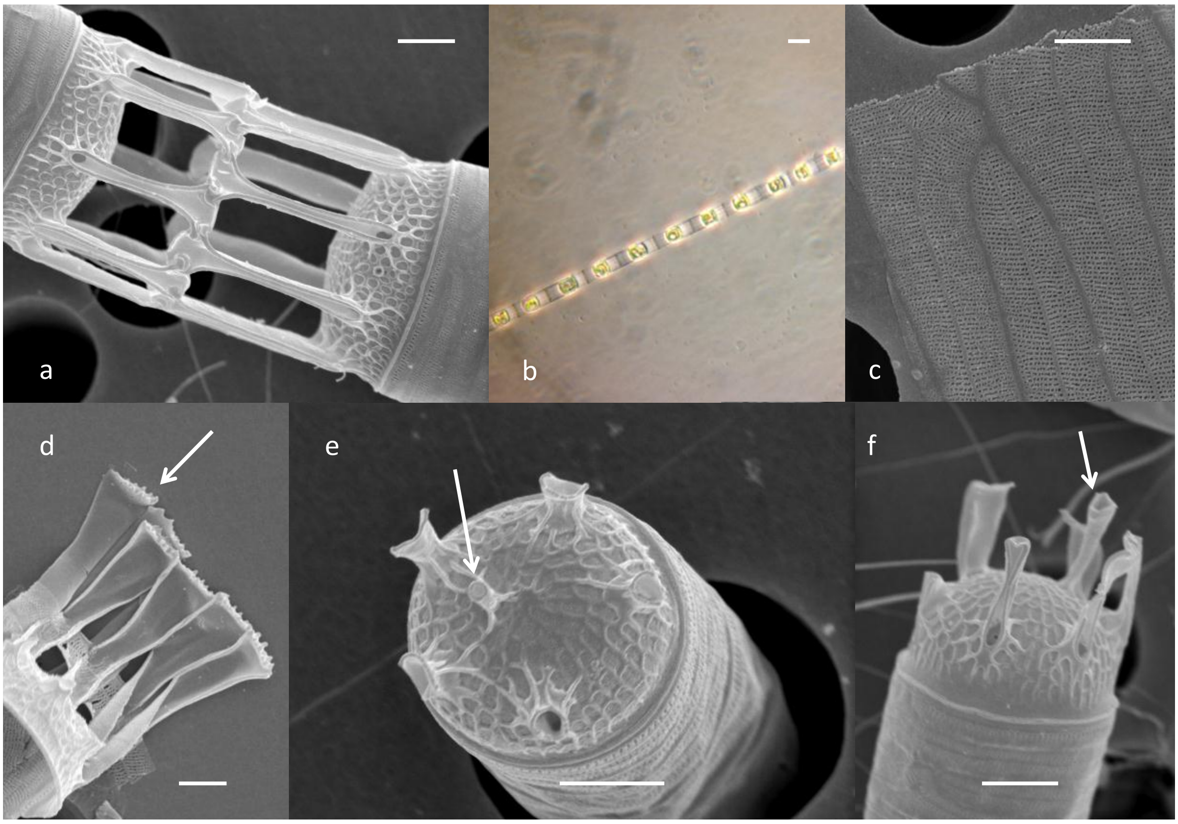

Figure 3. *Skeletonema marinoi* from north-east Atlantic waters, LM: b and SEM: a, c-f. (**a**) Strain AMB-39, showing intercalary valves with 1:2 intercalary fultoportula process junctions, IFPP, scale bar 1 µm. (**b**) Net sample from Vestfjord, northern Norway, showing colony of cells in girdle view, with one large chloroplast per cell, scale bar 5 µm. (**c**) Net sample from Ullsfjord, northern Norway, showing cingular band with uniseriate rows of pores, scale bar 1 µm. (**d**) Net sample from Ullsfjord, northern Norway, showing terminal valve with flare ends of terminal fultoportula processes, TFPPS (arrow), scale bar 1 µm. (**e, f**) Strain AMB-39, showing terminal valves with subcentral terminal rimoportula process, TRPP (arrows), scale bars 1 µm. Terminology according to Sarno et al., 2005.

A similar fate is predicted for another so-called cosmopolitan diatom, *Chaetoceros socialis* Lauder, the ecological plasticity (or functional diversity) of which was tested on strains isolated from north-east Atlantic/Arctic and Mediterranean (Gulf of Naples, Tyrrhenian Sea) waters (Paper III, IV). We found the morphological species concept to be valid, but only in non-vegetative resting stages or spores, the morphology of which was clearly different in the two allopatric populations (Fig.1 in Paper III). Spore morphology appears to be a character differentiating more easily than the characters of the vegetative cell when it comes to *C. socialis*. As many as four different spore types were observed by Hargraves (1979) in this species, but so far, delimitation into separate species on the grounds of spore morphology in *C. socialis* has not been proposed. Rather the opposite, the taxonomic status of *C. socialis* and the closely related *C. radians* Schütt was revised with reference to spore morphology (Proschkina-Lavrenko, 1953), the only morphological character separating the two species. Proschkina-Lavrenko (1953; 1963) observed identical spores in two seasonal populations from the Black Sea (Proschkina-Lavrenko, 1953) and the Sea of Azov (Proschkina-Lavrenko, 1963), and for some reason these populations were considered taxonomically different. Consequently, the taxonomic rank of *C. radians* was reduced to a form of *C. socialis* (*C. socialis* f. *radians* Proschkina Lavrenko 1963), whereas the "original" *C. socialis* Lauder gained status as *C. socialis* f. *socialis* (Proschkina-Lavrenko, 1963). In our study, the northern (north-east Atlantic/Arctic) strains were morphologically similar to *C. socialis* f. *socialis*, whereas the southern (Tyrrhenian Sea) strains matched the description of *C. socialis* f. *radians* (Paper III). However, our phylogenetic analysis clearly separated the two geographic groups of strains into distinct clades (Fig. 2 in Paper III). Also, a thorough examination (i.e., a translation) of the literature indicated that what Proschkina-Lavrenko (1953; 1963) probably observed was two seasonal populations of one and the same species, namely *C. radians*, even though the author assigned them to separate taxonomic categories due to their distinct seasonality, and to forms of *C. socialis* due to taxonomic nomenclature rules (Proschkina-Lavrenko, 1963).

In other words, also the use of a strict morphological species concept may sometimes be misleading and result in taxonomic changes not representative for the species in question. We believe that *C. radians* should be reinstated at the species level, but refrain from doing so in lack of more data on the morphospecies *C. socialis* from several geographic regions (Paper III). Nevertheless, it is to be expected that both the taxonomy and the biogeography of *C. socialis* will be updated in future research. The cosmopolitan status of *C. socialis* is not supported by our results (Paper III, IV), but more data is needed in order to gain knowledge on the number and distribution of cryptic, or pseudo-cryptic, species hiding within this morphospecies. It is important to note, however, that not all cosmopolitan diatoms are obligate generators of cryptic diversity, implying that they would ultimately lose their cosmopolitan biogeographic status. A cosmopolitan distribution pattern has been observed also in pseudo-cryptic species within the diatom genera *Pseudo-nitzschia* (Hasle, 2002) and

Navicula (Poulíčková et al., 2010). Also, the similarity in main phytoplankton species composition within the associations reported here from an extensive geographical area (Paper I) points in the direction of considerable ecological plasticity in many so-called cold-water species. From this perspective, the use of biogeographical categories such as Atlantic or Arctic does not make much sense, at least not when applied to spring bloom species in this area (Paper I).

In order to evaluate the species concepts applied for ecologically plastic or cosmopolitan species, an integrated approach is needed where several characters are used in the delimitation procedure. A phylo-phenetic species concept combining genetic and morphological or other phenotypic traits seems useful for resolving phylogenies within cryptic diatom taxa. The ultimate challenge of bringing taxonomic diversity closer to the field of phytoplankton ecology could be mediated by a functional approach, i.e., a delimitation procedure including also functional variables, in taxonomic and biogeographic studies.

Functional studies may decipher the hidden diversity of cryptic species

Traditional species descriptions tend to have a fair amount of ecological information attached, such as notes on life history strategy, seasonality or geophysical characteristics of the waters in which the species were found (e.g. Lauder, 1864; Cleve, 1873; Gran, 1897; Hasle et al., 1996). This perspective seems to have faded, perhaps as a result of the development of more refined study techniques rendering finer morphological resolution, but correspondingly requiring a higher degree of taxonomic expertise. The fairly recent development of molecular techniques has also largely focussed on discovering the “true dimension” of taxonomic diversity, i.e., species richness, whereas the functional roles of these taxonomic entities have largely been overlooked. To a very great extent, taxonomic research efforts have concentrated on developing an appropriate species concept capable of correctly describing the diversity and biogeography of microbial species, as new molecular tools have proven the traditional morphological concept insufficient for this purpose. Though useful for phylogenetic species delimitation, the genetic data as such does not reflect the phenotypic properties of the species. This will probably change when expression profiling techniques are developed and automatized to such a degree that they become standard tools in taxonomic and/or ecological research.

The understanding of ecosystem processes is also affected by the ground-breaking increase in taxonomic diversity brought about by the detection of cryptic species or rare taxa among marine microorganisms. Phytoplankton ecologists are not simply faced with new names for the taxonomic units generating or mediating the energy flow within the systems of interest, but also by a considerable degree of phenotypic variability accompanying these taxa (Cerino et al., 2005; Balzano et al., 2011; Barofsky et al. 2010). This brings further complexity into existing ecosystem models and the challenge lies in bridging the gap between revised taxonomic information and existing knowledge on species distribution and physiology, and the consequences for community structure and function. This work is proceeding more slowly. A change of perspective is needed, not only within the science of phytoplankton taxonomy, but also in ecological research. There is a call for an integrative approach to species delimitation (Dayrat, 2005; Valdecasas et al., 2008; Schlick-Steiner et al., 2010) or a functional species concept (Weithoff, 2003; Thingstad et al., 2010; Paper III, IV), and the need to examine species as parts of their habitats is growing more evident (Rodriguez et al., 2005; DeLong, 2009; Lennon and Jones, 2011). From the ecological point of view, micro-evolutionary processes acting upon the interplay between environmental and genetic heterogeneity expressed in phytoplankton populations (Gallagher, 1980; Rynearson et al., 2006; Saravanan and Godhe, 2010; Härnström et al., 2011) should be considered to a far greater extent (Smayda, 2011).

Until now, few investigations have been performed on functional diversity (e.g. Gallagher, 1982; Zak et al. 1994; Weithoff, 2003; Koester et al., 2010), and the theoretical foundations for this concept are poorly understood. Functional diversity results as a consequence of genetic variability within a taxon, environmental effects on gene expression, and ecological interactions among taxa (Zak et al., 1994). However, the degree to which genetic diversity is translated into taxonomic diversity is not fully understood, and even less is known about the manner in which genetic and taxonomic diversity affects functional diversity or ecosystem properties. In this thesis, an integrated approach was chosen in order to explore the extent of phenotypic variability and allopatric speciation expressed by cryptic diatom species (Paper III, IV). The taxonomic (morphological and molecular) analyses revealed clear differentiation in spore morphology between phylogenetically distinct populations of *C. socialis* (Fig. 1, 2 in Paper III). Furthermore, when cultivated at 2.5, 8 and 13^{o}C, the distinct clades corresponding to northern (north-east Atlantic/Arctic) and southern (Gulf of Naples, Tyrrhenian Sea) populations were functionally different in terms of growth rates (doublings day^{-1}), maximum quantum yields (Fig. 3, 4 in Paper III; Table III in Paper III and IV), and metabolite fingerprints (Fig. 1, 2, 3 in Paper IV). These results reflected adaptive strategies of both geographic groups, but also demonstrated a high degree of phenotypic plasticity, in particular within the southern group of strains, i.e., the form corresponding to *C. socialis* f. *radians* (Paper III). Metabolite fingerprints differed quantitatively as well as qualitatively between the two geographic groups of strains (Paper IV), but no detailed information on the exact character of these differences was obtained by the metabolomics method applied in our study. Nevertheless, the observed taxonomic and functional diversity in *C. socialis* (Paper III, IV) strongly indicated that the two geographic populations are distinct allopatric taxa. Corresponding taxonomic investigations on strains of *C. socialis* from a larger geographic area largely support our results and will probably lead to a revision of the morphospecies (personal communication, N. Lundholm, University of Copenhagen, Denmark).

Also in *S. marinoi,* remarkable physiological heterogeneity (growth rate) is reported together with genetic differentiation for seasonally separated populations (Saravanan and Godhe, 2010). Physiological plasticity (growth rate) in relation to salinity tolerance is also reported for the species (Balzano et al., 2011). Moreover, considerable variability in metabolic profiles has been observed during different growth phases in *S. marinoi* (Barofsky et al., 2009; Videoudez and Pohnert, 2011). A phylogenetically delineated species can in other words still be ecologically and genetically highly variable. Concerning *S.* cf. *marinoi* in north-east Atlantic waters, a thorough investigation of populations isolated during different times of the year (spring, summer/autumn, and if possible, winter) would be interesting to elucidate the genetic, morphological, and physiological differentiation within the species. In particular the cells present during the winter period (Paper II) are expected to be physiologically different from those growing in other seasons.

To understand the factors and processes acting upon functional diversity, other kinds of studies are needed. More specific metabolomics analysis such as gas chromatography (GC-MS) could contribute to a more detailed knowledge on e.g. the qualitative differences between the two allopatric *C. socialis* taxa (Paper IV). Different genomics-enabled approaches have been applied in exploring evolutionary ecophysiology in algae, to shed light on e.g. photosynthetic properties (reviewed by Finazzi et al., 2010), light responses (Bailleul et al., 2010), nitrogen metabolism (Allen et al., 2006; 2011; Bender et al., 2011), and stress surveillance systems important for cell signalling and fate during the succession of blooms (Vardi et al., 2006; 2008). Transcriptomic or expression-profiling genetic studies can give information on how the environment affects phytoplankton genomes, for selected species in autecological studies (Mock and Valentin, 2004; Maumus et al., 2009), or at a larger scale, where metatranscriptome sequencing can give information on gene expression in algal communities from larger geographical areas (e.g. Mitra et al., 2011). Such an approach can also be useful for clarifying evolutionary relationships between different algal groups (Tirichine and Bowler, 2011; Moustafa et al., 2009). Functional diversity lies at the heart of taxonomic, genetic and ecological research and should give rise to multi-disciplinary work on all sides (Fig. 4). This could mediate the incorporation of functional approaches into taxonomic work as well as the integration of hidden or unexpected microbial diversity into ecosystem studies.

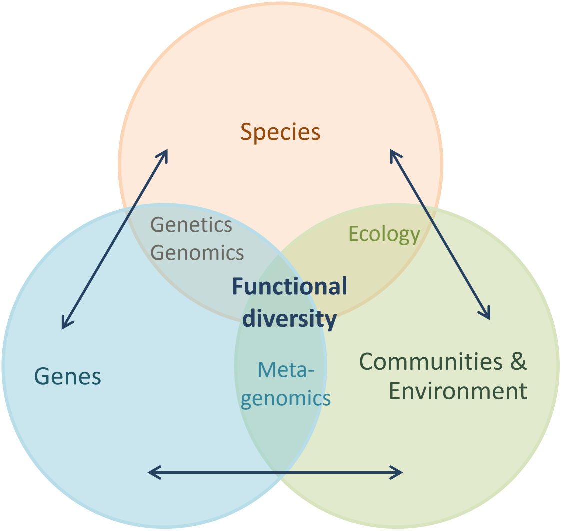

Figure 4. Schematic model for the interplay between genotype, phenotype, and the biotic and abiotic environment. Functional diversity lies at the centre of these three fields of research, and should be a valuable approach in genetic, taxonomic, as well as ecological work. Here, integration and modelling of information from culture-based studies, environmental surveys and metagenomics and/or metatranscriptomics projects can be performed. Modified from DeLong (2009).

To some extent, the emergence of functionally diverse associations of cryptic species is comparable to the introduction of new species to a community e.g. as the result of environmental change. This is not necessarily accompanied by changes to the quantity or quality of energy flow within the ecosystem (Caron and Countway, 2009), but it may bear consequences for our knowledge on trophic interactions or pathways of energy flow (Tamelander and Heiskanen, 2004; Tamelander et al., 2008; Iversen, 2010), and ultimately the function of the system. From this perspective, estimates of productivity could be affected and the functional diversity of cryptic species should also be recognized and included in physiological and ecosystem models.

Conclusively, functional approaches may contribute to clarifying the species concept for cryptic phytoplankton taxa, and will shed light on the ecological role of cryptic species in their respective habitats. In combination with taxonomic tools, functional studies can be useful for species delimitation. Integrated approaches are therefore recommended for future taxonomic work and phytoplankton ecologists are urged to acknowledge the dynamic nature of their species of interest in its interaction with the environment and other taxa.

CONCLUSION

This thesis has evaluated the species concepts and functional aspects of abundant cold-water diatoms. It was hypothesized that the traditional species concept defined by morphological species characteristics cannot necessarily be applied to all mitotic dividing organisms in a dynamic environment or in environmentally far apart areas. It was also anticipated that phylogenetic and functional analyses would revise the taxonomy of several species. These aniticipations were partially fulfilled, and the following conclusions could be drawn:

1) Phytoplankton species associations in north-east Atlantic and Arctic waters are highly dynamic, and the composition of the associations depicted will vary not only on a spatial and temporal scale, but also with the method chosen for describing the association. Taxonomic knowledge or resolution will also be decisive for the outcome of such work. Therefore, a high-resolution time series of phytoplankton abundance in this area is needed for an improved understanding of community structure and for detection of eventual future change to the ecosystem.
2) A high degree of similarity and ecological plasticity was observed in associations of the most abundant phytoplankton species during the spring in this area. This rendered biogeographical categories such as Atlantic or Arctic less useful for describing the environmental preference of the species.
3) A morphological species concept alone cannot properly identify phytoplankton species showing cryptic diversity. Using morphological and molecular tools, the pseudo-cryptic diatom *Skeletonema costatum sensu lato* in north-east Atlantic waters was identified as *S. marinoi* Sarno et Zingone, but the phylogeny needs to be properly inferred in future work.
4) Applying a phylo-phenetic species concept, pseudo-cryptic diversity was shown in strains of *Chaetoceros socialis* Lauder from north-east Atlantic/Arctic and Mediterranean waters. The cosmopolitan distribution reported for *C. socialis* is not supported by the results of this thesis. The taxonomic and biogeographic status of *C. socialis* (with the forms *C. socialis* f. *socialis* and *C. socialis* f. *radians*) will hence need revision.
5) Differences were also observed in several phenotypic or functional variables in the two allopatric populations of *C. socialis,* strengthening the conclusions from the

taxonomic investigation. Functional studies may in other words help decipher the hidden diversity of cryptic phytoplankton species.

6) Functional diversity is suggested as a melting pot approach, by which taxonomic, genetic and ecological species information could be integrated and eventually modeled. This multi-disciplinary approach would enhance the development of all fields and contribute to a better understanding of the dynamic nature of phytoplankton species and the interplay between these species and the environment.

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PAPER I

Degerlund M, Eilertsen HC (2010)
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PAPER II

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PAPER III

Degerlund M, Huseby S, Zingone A, Sarno D, Landfald B (in revision)
Functional diversity in cryptic species of *Chaetoceros socialis* Lauder (Bacillariophyceae)
Journal of Plankton Research

PAPER IV

Huseby S, Degerlund M, Zingone A, Hansen, E (manuscript)
Metabolite fingerprinting and physiology of the cryptic diatom *Chaetoceros socialis* Lauder

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