SPATIO TEMPORAL TURNOVER OF A PHYTO PLANKTON META COMMUNITY IN A NATURAL COASTAL SYSTEM

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INTRODUCTION

1. *GENERAL INTRODUCTION*

Coastal areas are highly populated. More than half of the world´s human population resides in this region, and it is expected that this proportion will increase to 75% by the year 2025 (Vitousek et al. 1997; Scavia et al. 2002; Paerl et al. 2006; Rullkötter 2009). The agricultural and urban development of coastal regions is accompanied by a multitude of anthropogenic stressors which directly and indirectly lead to severe changes in the coastal water quality and living resources (Cloern 2001; Paerl et al. 2006). The biodiversity in coastal ecosystems is directly threatened by overfishing (decreases in predator populations (Paerl et al. 2006)), pollutant discharges (Cloern 2001; Worm et al. 2006; Boyce et al. 2010), manipulation of freshwater flows, aquacultures, translocation of species (Cloern 2001), tourism, the oil industry as well as by wind and wave power installations (Rullkötter 2009). Beyond that, global warming affects coastal regions by a number of processes. The rising water level and the accelerated frequency and magnitude of extreme events (disturbances), such as extreme temperatures, precipitations and storms affect erosion, sediment dynamics, the current regime, the vertical mixing and thus the turbidity of the water column (Scavia et al. 2002; Zhang et al. 2004; Diffenbaugh et al. 2005). These changes are even reinforced by dyke buildings and shore line stabilizations on the one hand and by the destruction of natural breakwaters such as mangrove forests and coral reefs on the other hand. Moreover, the accumulation of vast amounts of plastic debris in the marine realm may lead to biodiversity loss, because many animals decease trapped in plastic litter or by accidental ingestion (Laist 1987; Derraik 2002). Some plastic substances (e.g. bisphenol-A) are also known as endocrine disrupting chemicals (Kwak et al. 2001).

A species group which currently is subject to severe changes in the coastal waters is the phytoplankton community. Phytoplankton species are characterized by small cell sizes, large population numbers and short generation times, and thus respond rapidly to human and natural perturbations (Paerl et al. 2006; Litchman and Klausmeier 2008). Therefore, the evaluation of the phytoplankton community structure represents a sensitive tool to detect environmental changes. On the global scale, a general decline in phytoplankton biomass was observed during the past century with an estimated global rate of ~1% of the global median per year due to climatic and oceanographic variability (Boyce et al. 2010). Such changes in the phytoplankton community may strongly affect ecosystem functioning (Chapin III et al. 2000; Hooper et al. 2012; Lürling and De Senerpont Domis 2013): phytoplankton species generate around 50% of the global primary production and mediate energy and nutrient fluxes, affecting all trophic levels (Cloern 2001; Boyce et al. 2010). These ecosystem functions drive ecosystem services (ecosystem processes that support human activity and sustain human life) such as fishery, water and air quality (Chapin III et al. 2000). Global long-term trends in the spatiotemporal structure of the phytoplankton community can be attributed to increasing sea surface temperatures (Boyce et al. 2010). As a consequence, warm-water species generally expand their spatial ranges towards higher latitudes at the expense of native species (Nehring 1998a; Stachowicz et al. 2002). The invasion probability is further strengthened by the world-wide shipping traffic and ballast water exchange (Carlton 1996; Nehring 1998b). Temporal shifts in species ranges, such as seasonal changes in life cycle events (e.g. earlier timing of phytoplankton blooms) may lead to a decoupling of predator-prey dynamics (trophic mismatch) (Chapin III et al. 2000; Daufresne et al. 2009; Hallegraeff 2010). Since higher trophic levels strongly depend on the synchronization with the pulsed planktonic primary production, temporal changes in the food web structure induce ecosystem-wide changes (Edwards and Richardson 2004).

Global warming may also contribute to an increased number of harmful algal blooms (HAB) formed by toxin-producing, warm-water dinoflagellates and cyanobacteria (Hallegraeff 2010; Lürling and De Senerpont Domis 2013). The incidence and intensity of (harmful) algal blooms is further enhanced by eutrophication of coastal areas (Lürling and De Senerpont Domis 2013) due to the riverine input of agricultural fertilizers (Vitousek et al. 1997; McQuatters-Gollop et al. 2007). These nutrient loads lead to increased phytoplankton productivity, affecting nutrient (C, N, P and Si) cycling and the water quality (Vitousek et al. 1997; Dippner 1998; Paerl et al. 2006). The microbial degradation of accumulated phytoplankton biomass is oxygen-consuming and might lead to hypoxia or anoxia, causing fish mortality (Vitousek et al. 1997; Paerl et al. 2006). Eutrophication is also associated with significant losses of biodiversity and marked changes in the community composition. Flagellates, for instance, are able to outcompete diatom species which do not profit from enhanced N and P values, if they suffer Si limitation, resulting in altered food quality for higher trophic levels (Reid et al. 1990; Vitousek et al. 1997; Cloern 2001; Tilman et al. 2001).

Besides global warming and the eutrophication of coastal areas, ocean acidification is a major threat for marine biodiversity. Approximately 25% of the anthropogenic emitted CO_2 enters the ocean mainly via the solubility pump, where carbonic acid is formed (Hoegh-Guldberg et al. 2007). This does not only affect coral reefs due to the reduced calcification rate of reef-building calcified organisms (Anthony et al. 2008), but also calcified phytoplankton species such as coccolithophores (Beaufort et al. 2011). However, the effect of increased carbon dioxide in the atmosphere for phytoplankton has to be differentiated between regions. Recent biogeochemical models for the North Sea indicate that only the open North Sea will draw down more carbon dioxide from the atmosphere and export carbon to deep waters and into the North Atlantic. In shallow coastal areas of the Southern North Sea, water tends to be (super-)saturated in CO_2 and response to additional atmospheric CO_2 will probably be negligible (Thomas et al. 2004).

Along with the global warming trend, average water temperatures in the North Sea have risen by 1.13 °C over the past 40 years since 1962 accompanied by a salinity rise of about 1.0 PSU (Wiltshire and Manly 2004b). While global long-term declining trends of phytoplankton biomass have been related to increasing sea surface temperatures (Boyce et al. 2010), this does not hold true for the North Sea, as this effect is probably superimposed by the effect of eutrophication on phytoplankton biomass. McQuatters-Gollop et al. (2007) report a double-digit increase in the phytoplankton biomass after a regime shift in the 1980s. After eutrophication had been perceived as international challenge (Nixon and Kristensen 2003; Grunwald et al. 2010), several programmes for the reduction of the nutrient input have been established. Since then N and P loads from industry and urban waste water were reduced at high (> 50%) percentages (Nixon and Kristensen 2003), resulting in a further regime shift in the phytoplankton community (Grunwald et al. 2010).

All above mentioned studies regarding the effects of eutrophication and climate change on the phytoplankton community are based on phenology studies, i.e. the analysis of annually recurring life cycle events (Edwards and Richardson 2004). Such phenology studies provide the empirical foundation for the assessment of the *status quo* and the prediction of the phytoplankton dynamics under future environmental conditions. Thereby, complex short- and long-term trends in phytoplankton communities can be identified and the underlying mechanisms driving community changes can be addressed to either stochastic variability or anthropogenic change, which is essential for the management and conservation of the marine realm (McQuatters-Gollop et al. 2007; Bresnan et al. 2009; Rullkötter 2009; Zingone et al. 2010; Edwards et al. 2013; Lürling and De Senerpont Domis 2013). To date, many time-series programmes are based on the analysis of satellite images (Reid et al. 1990) or pigment analyses (chlorophyll a, b, carotenoids, phycoerythrin) (Letelier et al. 1993; Bidigare and Ondrusek 1996; Lionard et al. 2008; Harris 2010). Yet, pigment analyses have a limited scope of application, as they only allow a differentiation between major phytoplankton groups. Furthermore, the cellular chlorophyll content not only varies among species but also among individuals (Harris 1980). Consequently, pigment analyses are not sensitive to changes in abundances of distinct phytoplankton species or groups, and thus cannot be utilized for the identification of harmful algae blooms and invasion events, which may be critical for the assessment of trophodynamics, emphasizing the importance of monitoring on species-level (Zingone et al. 2010).

Only few long-term monitoring data sets, providing species-level resolution, are available for the North Sea. Among others, observational studies are conducted at Helgoland Roads since 1962 (Wiltshire and Dürselen 2004b; Wiltshire et al. 2010), in the Marsdiep (Dutch coastal waters) (Cadée and Hegeman 2002), in Scottish coastal waters (Bresnan et al. 2009), while the longest time series is available for the central North Sea (Continuous Plankton Recorder since 1958) (Warner and Hays 1994). However, phytoplankton long-term monitoring has not been established in the East Frisian Wadden Sea so far. Therefore, in the scope of this thesis, I started a long-term monitoring of the phytoplankton community in the backbarrier tidal flats of Spiekeroog island, Wadden Sea, next to a permanently installed time-series station (TSS) (Grunwald et al. 2007a; Reuter et al. 2009), from which I obtained continuously recorded environmental variables (first Chapter). Hence, I was able to relate phytoplankton species composition to a comprehensive set of environmental data recorded by the TSS. This is an outstanding feature, as in most phytoplankton monitoring environmental variables are obtained only during sampling, so that (extreme) events causing change in the community composition might be missed. I followed the turnover of the phytoplankton community composition during 3.5 consecutive years from 2009 to 2012 at various scales in space (horizontal and vertical) and time (tidal, seasonal, inter-annual). The turnover in the phytoplankton community composition may be driven by various mechanisms such as the tidally induced horizontal exchange of water masses, benthopelagic coupling, zooplankton grazing, and, in particular, by seasonal changes in abiotic variables (Cloern et al. 1985). I further analyzed at which spatiotemporal resolution long-term monitoring should be conducted to gain sufficient insight into phytoplankton dynamics based on the evaluation of different biodiversity measures.

At the beginning of the observational study, in spring 2009, the invasive species *Mediopyxis helysia* was first observed in this region. This large centric diatom has bloomed every spring since then and was found in substantial abundance throughout three consecutive years (second Chapter). As this species has only recently been described (Kuehn et al. 2006), information about its autecological characteristics is scarce. Hence, complementing the observational study, I conducted a laboratory experiment (second Chapter), addressing the question, which environmental conditions facilitate *M. helysia´s* expansion. More precisely, I tested under which nutrient conditions *M. helysia* performs best and whether it also reaches dominance in an artificial community. Therefore, I cultured 11 species in a 2 x 3 orthogonal design (2 Si- x 3 N-concentrations in the presence as well as absence of *M. helysia*). Important findings were that I) relative abundance of *M. helysia* remained high throughout all treatments and was neither influenced by Si nor N availability and II*) M. helysia* was able to alter the community composition. Thus, cautious extrapolation of the results indicates that a successful establishment of *M. helysia* may have far-reaching consequences not only for the phytoplankton community of the Wadden Sea, but also for higher trophic levels, as it may only be partially ingested by copepods due to its large size.

In the third Chapter, I addressed the question whether the metacommunity concept (Leibold et al. 2004) is applicable to this open, highly dynamic coastal system, since it so far has mostly been applied to systems with patches confined by distinct boundaries (Kneitel and Miller 2003; Cadotte 2006; Vanschoenwinkel et al. 2007; Logue et al. 2011). In such enclosed systems, metacommunity studies have mainly assessed the regional influence on local patterns and processes. However, in open environments such as the marine realm, this research question has to be reversed, as there the role of local dynamics in ephemeral patch formations within the apparent homogeneous pelagic zone needs to be investigated. Significant temporal patchiness (tidal, seasonal, inter-annual) and an evident spatial separation in species number were observed, while the dominance structure of phytoplankton showed a non-significant spatial imprint during the most of the year. Further, the compositional turnover was significantly associated with seasonal changes in environmental conditions, reflecting especially trends in P, N, photosynthetically active radiation and temperature. Combined, the results suggest that ephemeral patch formation and species sorting mechanisms play an important role for community assembly in this highly dynamic system.

During the observational study, strong benthopelagic coupling in the backbarrier tidal flat has became apparent. High current velocities during half tide promote the resuspension of high abundances of living benthic microalgae into the water column. To quantify the degree of the benthopelagic coupling, the number of microphytobenthic species on the sediment surface has to be evaluated. Therefore, in the fourth Chapter, I developed a method for quantitative removal of microphytobenthic species from sediment particles via the application of an ultrasonic homogenizer and addition of a surface-active detergent for subsequent microscopical analysis.

1.1. BOX 1

BIODIVERSITY itself has a diversity of meanings, ranging from the genetic diversity within populations and the diversity between species to the diversity within ecosystems and landscapes, which all contribute to global biodiversity (Chapin III et al. 2000; Begon et al. 2009). Usually, most attention is paid to species richness.

ALPHA DIVERSITY: diversity within a community

BETA DIVERSITY: diversity between communities

GAMMA DIVERSITY: diversity between regions

A METACOMMUNITY is defined as a set of local communities that are linked by dispersal (Wilson 1992). The prevalence of the different metacommunity types is related to dispersal rates (Holyoak 2005). High dispersal rates and subsequent colonization may lead to MASS EFFECTS (including source-sink dynamics or rescue-effects) resulting in a homogenized metacommunity and relatively low beta diversity (Martiny et al. 2006). At intermediate dispersal, species can disperse to their most suitable patch, resulting in a conformance between environmental gradients and community composition (i.e. SPECIES SORTING) (Van der Gucht et al. 2007). However, if habitat patches are isolated and dispersal is limited, a colonization-competition trade-off can be observed, indicating PATCH DYNAMICS. In contrast, the NEUTRAL THEORY (Hubbell 2001) presents a null-model, assuming that all species have equal fitness, movement and competitive abilities, leading to random community assembly generated by species losses and gains (Leibold et al. 2004). Each of these four conceptual paradigms of the metacommunity framework emphasizes different community processes (Leibold et al. 2004; Logue et al. 2011).

CHAPTER 1

2. *TEMPORAL VARIABILITY OF THE PHYTOPLANKTON COMMUNITY IN THE BACKBARRIER TIDAL FLATS OF SPIEKEROOG*

2.1. ABSTRACT

A phytoplankton long-term monitoring in the backbarrier tidal flats of Spiekeroog island, Wadden Sea, has been established next to a permanently installed time-series station, from which continuously recorded environmental variables were obtained. Here, I report the development of the phytoplankton community from 2009 to 2012 at a range of spatial (horizontal and vertical) and temporal (tidal, seasonal, inter-annual) scales. Phytoplankton turnover was mainly driven by seasonal changes in abiotic variables, but was also influenced by the tidally induced horizontal exchange of water masses and benthopelagic coupling. Moreover, I evaluated different biodiversity measures to assess, at which resolution future monitoring should be conducted to capture most of the spatiotemporal variation of the phytoplankton community at lowest cost. Recommendations for future monitoring were given.

2.2. INTRODUCTION

2.2.1. THE NORTH SEA

The North Sea is a shallow (mean depth 74 m (Otto et al. 1990) semi-enclosed shelf sea, which covers an area of about 750000 km^2. It is connected to the Baltic Sea via the Skagerrak and Kattegat and to the Atlantic Ocean via the English Channel. The Atlantic Ocean and the Gulf Stream widely influence the physical conditions in the North Sea. The mean water temperature is 10 °C (Wiltshire and Manly 2004b). Yet, as a consequence of the climate change average water temperatures increased (Franke et al. 2004): at Helgoland, average water temperature is 1.13 °C higher than

1962 accompanied by a salinity rise of about 1.0 PSU (Wiltshire and Manly 2004b). Terrestrial and anthropogenic influences are high due to the densely populated coastal areas surrounding the North Sea (Halpern et al. 2008). River run-off mainly originates from the rivers Elbe, Rhine and Weser. Additionally, numerous smaller tidal streams and other run-offs significantly affect salinity and nutrient concentrations (Dippner 1998).

2.2.2. THE WADDEN SEA, THE BARRIER ISLANDS AND THE BACKBARRIER TIDAL FLATS

The Wadden Sea located in the southern North Sea is one of the biggest intertidal flats globally as it covers an area of 9300 km², reaching from Den Helder (Netherlands) to Esbjerg (Denmark) (Hild et al. 1999). There are different Wadden Sea National Parks and in 2009 parts of the Dutch and German Wadden Sea have been declared a UNESCO World Heritage.

18000 years BP, during the Weichselian cold stage, the sea level was approximately 110-130 m lower than today (Hild et al. 1999). The melting of glacial ice masses at the beginning of the Holocene caused a rapid sea level rise. During the North Sea transgression (5500 years BC), a chain of seven sandy barrier islands between the estuaries of the rivers Ems and Jade was formed. The present shape of the barrier islands and the coast is the combined result of continuous geomorphological processes during the current interglacial phase, tidally-driven sediment transport as well as measures for coastal protection and land reclamation such as dike constructions (Eitner 1996; Wolff and Flemming 2003). Between the chain of barrier islands and the coast lie the backbarrier tidal flats which form a transition zone (Dittmann 1999; Grunwald et al. 2007a): tidal basins are connected to the North Sea by tidal inlets, allowing an extensive bi-directional exchange of water masses and thereby the exchange of dissolved and particulate matter as well as transportation of plankton biomass (Lunau et al. 2006; Grunwald et al. 2007a; Reuter et al. 2009).

2.2.3. TIDAL WAVES IN THE NORTH SEA AND THE WADDEN SEA

Tidal currents move water masses horizontally and vertically. Tidal waves generated in the North Atlantic Ocean enter and exit the North Sea via the North Atlantic boundary and via the Dover Straight. The tidal signal, which propagates into the North Sea, is a co-oscillating response to tides generated in the North Atlantic (Wolff and Flemming 2003; Velema 2010) The most influential tidal constituent in the North Sea is the M_2-tidal wave (period 12.25 h), which flows counter-clockwise through the main basin. Spatially, the M_2-tide is characterized by three amphidromic points, one located in the Southern Bight and two in the main basin (Defant 1923; Velema 2010). The M_2-tide mainly controls the currents in the backbarrier tidal flats of the East Frisian Wadden Sea (Wolff and Flemming 2003; Velema 2010). Here, the morphology of the area increasingly influences the amplitude and direction of the tide because shear stresses are enhanced due to the shallow depth (Niesel and Günther 1999). Thereby, a tidal asymmetry can be observed in the inlets of the East Frisian Wadden Sea, i.e., the outgoing tide has a shorter duration and a slightly higher velocity than the incoming tide (ebb-dominance) (Fitzgerald and Penland 1987; Wolff and Flemming 2003).

2.2.4. THE BACKBARRIER TIDAL FLATS OF SPIEKEROOG ISLAND

The back barrier islands protect the inner Wadden Sea from the North Sea. Spiekeroog is located in the eastern part of the East Frisian barrier islands. The backbarrier tidal flats of Spiekeroog island are a well studied system (Dittmann 1999; Lunau et al. 2006; Grunwald et al. 2007a; Reuter et al. 2009), which encompasses an area of approximately 73.5 km^2 (Walther 1972). The morphology of the backbarrier tidal flats is mainly shaped by the influence of semidiurnal tidal currents within the mesotidal range (2.2 – 2.8 m). The tidal flats are connected to the North Sea via the tidal inlet “Otzumer Balje”, which separates the islands Spiekeroog and Langeoog. During a single tidal cycle (12.42 h), huge amounts of water masses (40 to $170*10^6$ m^3 (Stanev et al. 2003)) enter the backbarrier tidal flats in the south-east direction with current velocities of up to 1.5 $m * s^{-1}$ in the narrow inlets and up to 0.3 $m * s^{-1}$ on the tidal flats (Stanev et al. 2003). Current velocities are not only influenced by the morphology of the area but also by spring and neap tides, meteorological forces (wind intensity and direction), and freshwater inflow (Niesel and Günther 1999; Wolff and Flemming 2003). With the enormous exchange of water masses through the Otzumer Balje, huge amounts of plankton biomass, nutrients and sediments are transported: up to 7200 tons of mud and 4300 tons of sand are moved during one tidal cycle (Stanev et al. 2007).

Sediment particles are distributed along an energy gradient from the island to the mainland (Hild et al. 1999). According to a model developed by Wolff and Flemming (2003), the residence time of particles transported into the backbarrier tidal flat should lie in the order of 2 to 18 tidal cycles. The intertidal section, which is flooded regularly, covers 80% of the area. There is no river discharge and only two small tidal creeks lead into this area of the Wadden Sea. Thus, the terrestrial influence is relatively low (Niesel and Günther 1999).

2.2.5. THE TIME-SERIES STATION

A time-series station was installed within the research programme "BioGeoChemistry of Tidal Flats" of the University of Oldenburg in 2002. It is located at the tidal inlet "Otzumer Balje" between the islands of Langeoog and Spiekeroog at position 53°45´01.0´´ N, 007°40´16.3´´ E, to obtain a better understanding of the dynamical processes in the backbarrier tidal flats. The time-series station is equipped with a variety of sensors to continuously provide a wide range of data on physical, biological and chemical as well as meteorological and hydrographical variables (Reuter et al. 2009). The primary objective was to investigate the budget of matter fluxes between tidal flats and open coastal waters as well as to assess the biogeochemical material transformation during tidal cycles (Reuter et al. 2009). The advantage of a permanently installed station is that it can operate under extreme weather conditions providing data of the impact of storms, ice-winters, spring-tides and other external forces on these matter fluxes. This is very important, as such extreme events generally severely influence the distribution of particles and biogeochemical processes in the Wadden Sea (Wolff and Flemming 2003). One of my sampling stations was located at the time-series station. I obtained various environmental variables from the time-series station to explain phytoplankton community variation.

2.2.6. CYCLING OF NUTRIENTS BETWEEN THE NORTH SEA AND THE WADDEN SEA

In general, nutrient concentrations in the Wadden Sea are higher than in the adjacent North Sea (Postma 1981; Brockmann et al. 1990). Sources of nutrients in the backbarrier tidal flats are the incoming water from the North Sea, diffusion of pore water and the input of agricultural fertilizers from the mainland (Gätje et al. 1998; Liebezeit 2003). In the backbarrier tidal flats of Spiekeroog, nutrients (N, P, Si) are distributed very heterogeneously in time and space following biological production and decomposition. Seasonal variations are more pronounced than variations between different years. Nutrient loads depend also strongly on the tidal phase: during high tide nutrient concentrations almost reflect the conditions of the North Sea, whereas they increase significantly during low tide due to an enhanced benthic influence on the shallow water body (Grunwald et al. 2007a). The relative importance of processes such as resuspension of benthic particles, diffusion of pore water and microbenthic degradation is higher during low tide due to the increased sediment-water-interface in relation to the water column (Asmus and Asmus 1998; Asmus et al. 1998). Consequently, vertical differences in nutrient concentrations can also be observed in the water column: nutrient concentrations are higher close to the sea floor.

2.2.7. PHYTOPLANKTON DYNAMICS

The phytoplankton community in the backbarrier tidal flat of Spiekeroog is mainly composed of dinoflagellates and diatoms (Kohlmeier 2004). Governed by seasonal variability in abiotic factors (e.g. light, temperature, and nutrients) and biotic correlations (zooplankton grazing pressure), the phytoplankton community composition shows an annually recurring succession (Reid et al. 1990; Sommer

2005): dinoflagellates usually prevail during the summer months, whereas diatoms form extensive spring blooms and less pronounced autumn blooms (Reid et al. 1990). A strong benthopelagic coupling can be observed. Vertical resuspension of microphytobenthic species into the water column increases with increasing current velocities during half tide. Horizontally, the tidal inlets can be seen as boundary mixing zones for phytoplankton species from the from the North Sea and the Wadden Sea (Niesel 1997). Thereby holoplanktic species are imported into the backbarrier tidal flats, whereas meroplanktic species are exported into the North Sea (Gätje et al. 1998). The Wadden Sea can be described as a net-heterotrophic system and a sink for organic particulate material imported from the North Sea. Heterotrophic degradation mostly outnumbers primary production (Postma 1981).

2.2.8. SAMPLING STRATEGY

Phytoplankton communities track changes in abiotic conditions, thereby individual cells respond with a certain, species-specific time lag to environmental fluctuations. Yet, on which temporal scales do such fluctuations occur? Ecological processes shaping the spatiotemporal distribution of phytoplankton biomass are hierarchical (Harris 1980) (Fig.1).

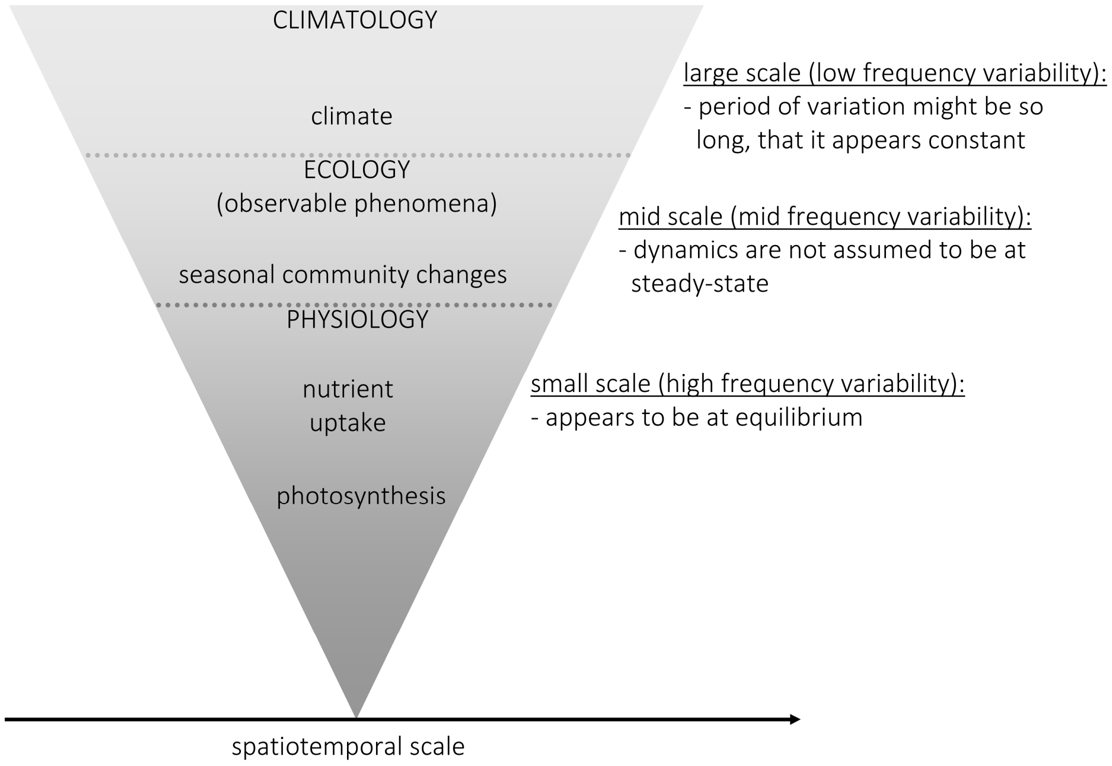


Fig. 1: Hierarchy of phytoplankton responses to fluctuations in environmental conditions (diagram modified after Harris (1980)).

Thus, temporal turnover of phytoplankton species occurs on a variety of scales from tidal cycles to inter-annual and decadal differences in the community composition. Highest variation in phytoplankton biomass and community composition is usually measured at time scales from weeks to months and over spatial scales from meters to kilometers (Lucas et al. 2001). However, short-term (i.e. hourly) and small-scale (centimeter to meter) variability may also be important particularly in coastal ecosystems (Harris 1980; Lucas et al. 2001). Exchange rates of benthic microalgae between the sediment and water column vary with tidal variations in current velocities (Lucas et al. 2001). Higher cell numbers of benthic and benthopelagic species are therefore expected in the bottom sample, especially during slack water, when the water column is assumed to be less mixed. At seasonal scales, re-suspension rates are likely to be enhanced in autumn and winter due to more frequent storm events during these seasons. At inter-annual scales, differences in the phytoplankton community composition are expected to be small as differences are superimposed by the seasonal phytoplankton succession (Korhonen et al. 2010).

Hence, sampling procedures have to be adjusted to gather underlying mechanisms shaping community dynamics at different spatiotemporal scales. However, sampling strategies are always a trade-off between the scientific ideal and the logistic reality, making accurate quantification of all aspects of the spatiotemporal variability difficult (Zingone et al. 2010). Therefore, I applied three different sampling strategies (Fig. 2) to test, how much effort is needed to gain a comprehensive understanding of the phytoplankton dynamics.

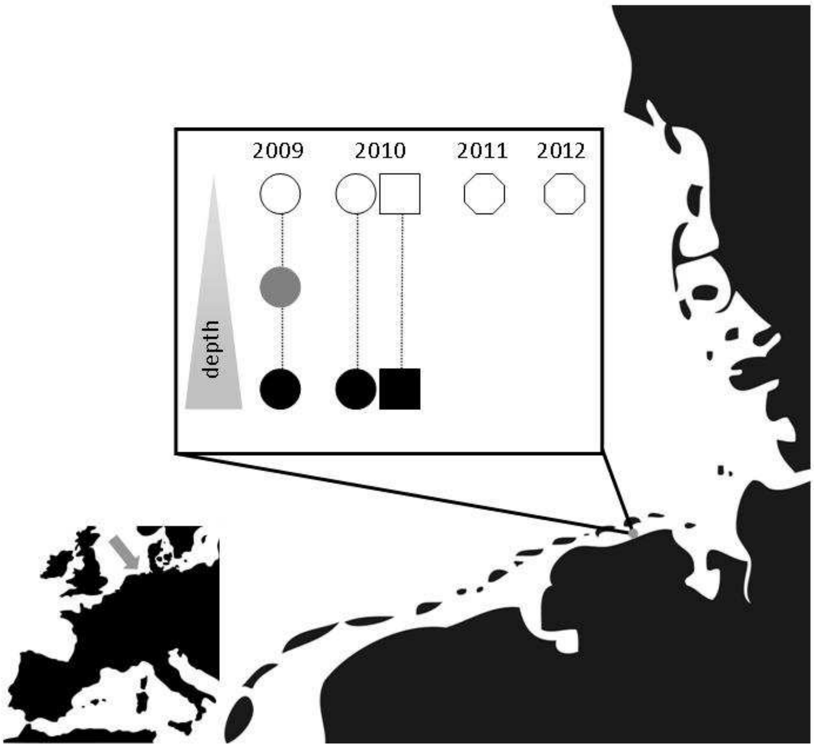


Fig. 2: Study site located in the backbarrier tidal flat of Spiekeroog Island, Wadden Sea, Germany. Sampling setup: in 2009 sampling was conducted always around half tide during incoming flood in three different depths (1 m (white circle), 6 m (grey circle) 10 m (black circle)) at the time-series station. Taxonomic resolution was highest. In 2010, only two depths (1 m and 10 m (circles)) were sampled, but additional samples were taken during slack water (squares) to account for tidal variations in the phytoplankton community. Here, and in the following years, the taxonomic resolution was less detailed. In 2011 and 2012, only surface water was sampled at different points in the tidal cycle (hexagons).

I assessed the relevance of observations and the adequacy of different spatiotemporal scales. In 2009, I started with the highest resolution, reducing sampling effort stepwise each year until 2012, thereby putting emphasis on the temporal dynamics at the seasonal time scale. Samples were always taken at half tide during incoming flood at three different depths (1 m, 6 m, 10 m, which is 1 m over ground) to assess if phytoplankton species are homogeneously distributed in the vertical direction. In 2010, I also examined shorter time scales, i.e. tidal variations in phytoplankton community structure and vertical variations in the water column - limited to a top- and a bottom-water sample (1 m and 10 m). In contrast, in 2011 I sampled only surface water at random points in the tidal cycle to test if the seasonal variation could still be related to extrinsic factors. In the third and the fourth year, resolution was further reduced to analysis of surface-water samples, which were taken at random points in the tidal cycle. The taxonomic resolution also varied among the years. In 2009, phytoplankton organisms were determined to the lowest possible level, while in the subsequent years, several phytoplankton taxa were grouped. The turnover in the phytoplankton community at different temporal scales was probably captured best in 2009, and I further assumed to find a lower biodiversity in the low resolution samples from 2011 and 2012 compared to the high resolution sampling in 2009. I also created a low resolution data set (only 1 m depth) for assessing the temporal variation in the phytoplankton community from 2009 to 2012.

2.3. MATERIAL & METHODS

I followed the phytoplankton community composition from 2009 to 2012 near the time-series station in a approximately biweekly rhythm and obtained continuous records of hydrographical, meteorological and chemical variables from the permanently installed time-series station (Grunwald et al. 2007a) to couple changes in the phytoplankton community structure to corresponding environmental variables. Water samples were collected from pre-defined depths (1, 6, 10 m in 2009, 1 and 10 m in 2010, 1 m in 2011 and 2012) using a multi water sampler (HYDRO-BIOS, Germany) with 3.5 l-Niskin bottles.

2.3.1. MICROSCOPICAL ANALYSIS

For microscopical analysis 100 ml subsamples were preserved in 1% Lugol´s iodine solution and stored in brown glass bottles. For each sample, 5 and 1 ml subsamples were counted, ensuring that rare (and mainly large) species are captured in the 5 ml subsample. Due to high amounts of detritus and sediment in most of the samples, it was necessary to count smaller species in the 1 ml subsample. Phytoplankton organisms were determined to the lowest possible taxonomic level, using an inverted microscope (ZEISS; AXIOVERT 10) (Utermoehl 1958). This microscopical analysis of > 600 cells per sample resulted in total in the determination of ~ 120 taxa according to Drebes (1974), Tomas (1997), Pankow (1999), Round (2007), Hoppenrath et al. (2009). Due to the high amount of detritus in the samples, I had to remove the Prymnesiophyceae *Phaeocystis* sp. from the data set, as proper enumeration was not possible in all samples. I also removed the dinoflagellate *Noctiluca scintillans*, because of its high error potential caused by its enormous biovolume − up to $2.7 * 10^8$ µm³ (Olenina et al. 2006). During enumeration, dimensions of simple geometrical bodies of 20 cells of each dominant taxon were measured and used for calculation of specific cellular volumes according to Hillebrand et al. (1999). Cell volumes of rare taxa were taken from the HELCOM phytoplankton check list (Olenina et al. 2006). I then defined species richness as the richness of the lowest identifiable unit.

2.4. RESULTS

2.4.1. NUTRIENT ANALYSIS

Pronounced seasonal cycling of the dissolved nutrients could be observed throughout the sampling campaign. Cycling of dissolved nutrients was strongly governed by phytoplankton primary production. Within short time spans, diatom spring blooms always led to rapid decreases in nutrient concentrations. Blooming events persisted until nutrients were depleted each April. From early summer onwards, the P-pool was replenished, when bacterial remineralisation prevails over phytoplankton activity. Consequently, P reached a peak (2 - 3 µmol / l) in each July / August (Fig. 3b). During the winter month, P values were decreasing even though phytoplankton biovolumes were lowest at this time. Si and N concentrations were low (< 10 µmol / l) during summer month until remineralisation in October (Fig. 3a). In contrast to P, highest N (30 - 40 µmol / l) and Si (20 - 30 µmol / l) concentrations were found during winter months, when phytoplankton biomass was low (Fig. 3a). A single peak was observed for Si (> 40 µmol / l) and N (> 70 µmol / l) (P values are missing) in February 2011 accompanied by a drop in salinity (~ 26 PSU), indicating possible freshwater inflow via the flood gate in Neuharlingersiel (Fig. 3a, c). Water temperature also showed a consistent seasonal pattern during the time of the study with lowest temperatures in January and February (~ 0 °C) and highest temperatures in July / August (~ 23 °C) (Fig. 3c). However, in summer 2011 water temperatures were generally lower than in 2009 and 2010 with maxima below 20 °C. Salinity did not show a pronounced seasonal trend, but fluctuated between 28 and 33 PSU (Fig. 3c).

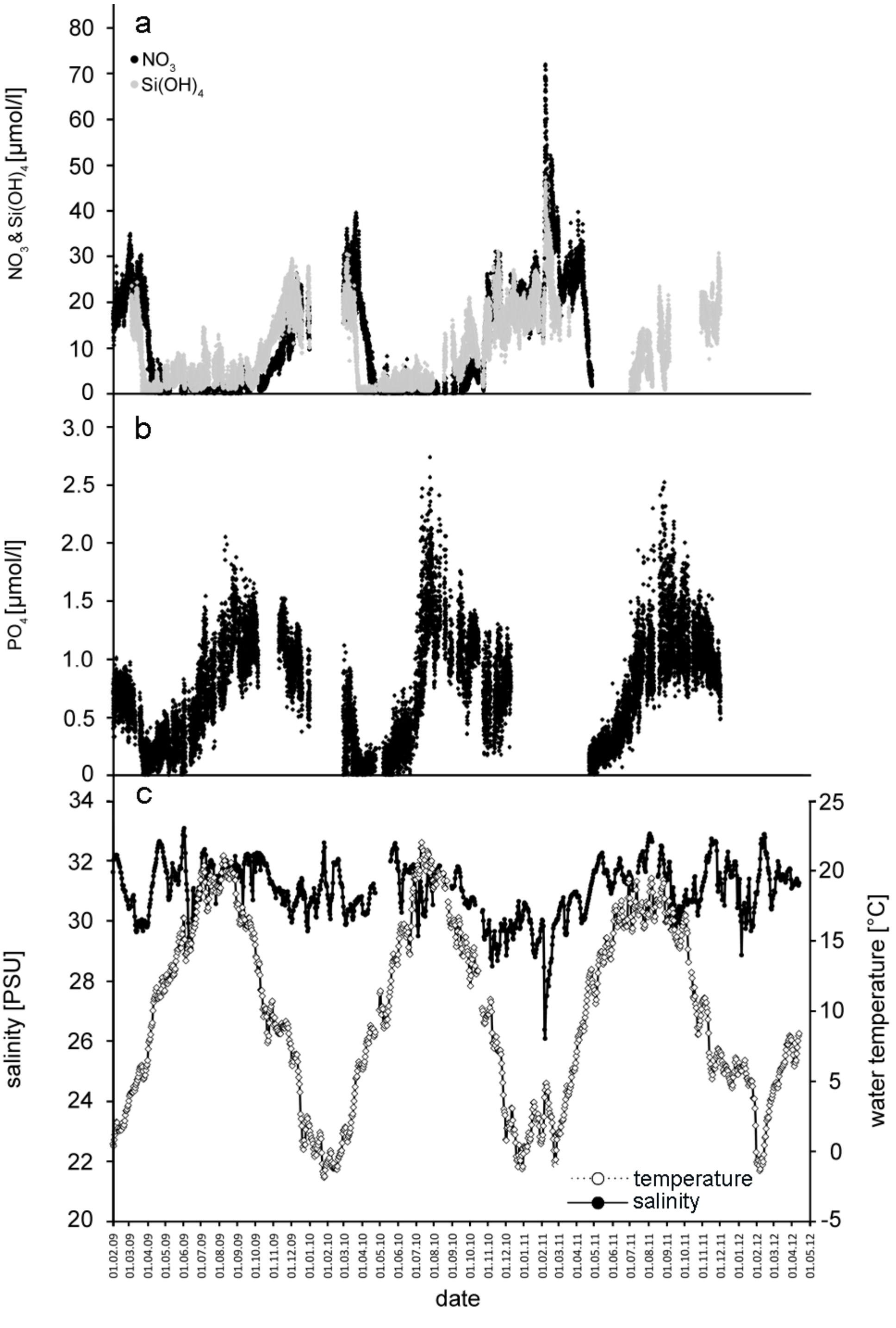


Fig. 3: Temporal variation of a) NO_3, $Si(OH)_4$, b) PO_4 [µmol / l], c) salinity [PSU] and water temperature [°C] (data obtained from the time-series station).

During the sampling campaign in 2010, tidal variations in nutrient concentrations were assessed. When compared to high tide and mid tide samples, Si and P concentrations were generally higher during low tide, when the water column was influenced by the sediments in the Wadden Sea (Fig. 4). Only few significant differences with depth were found, indicating a well-mixed water column throughout the year.

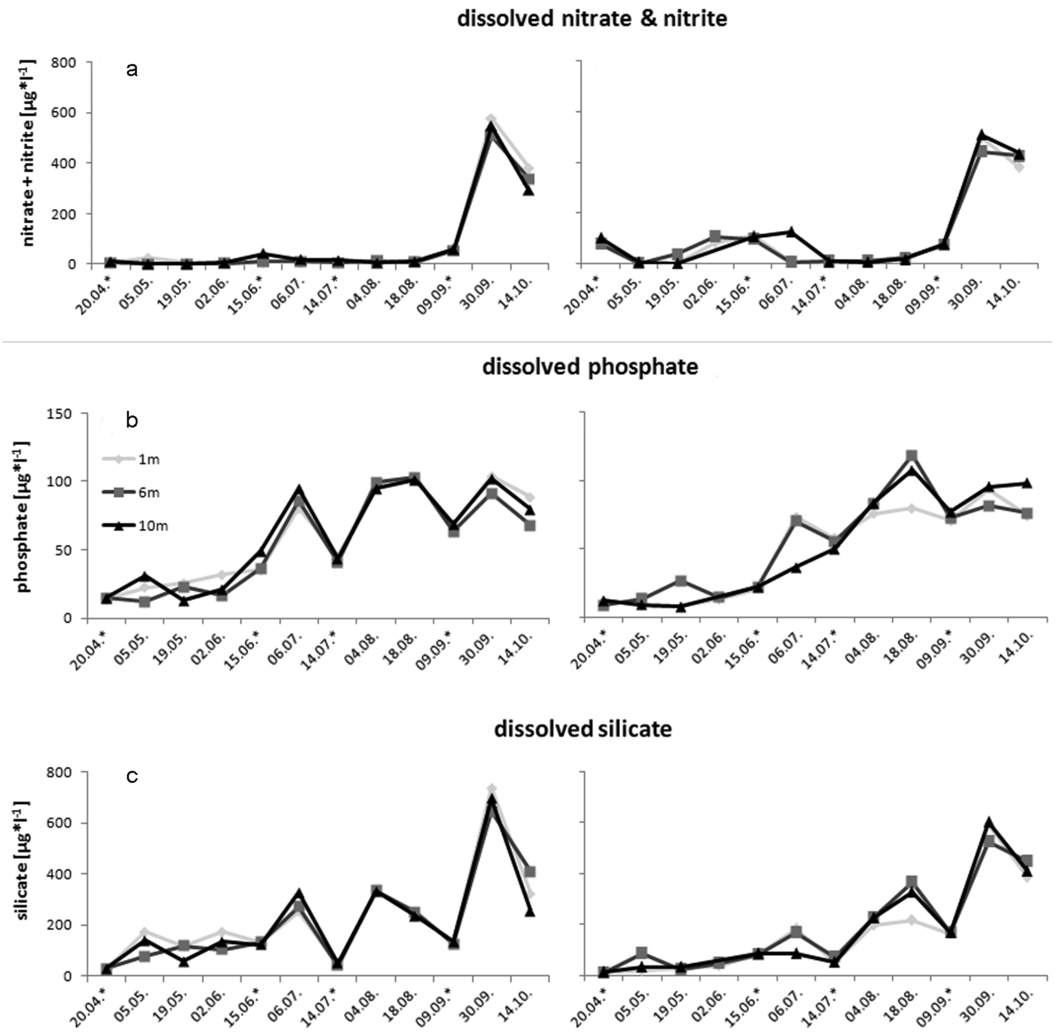


Fig. 4: Vertical variation of dissolved nutrients (total N, P, Si) during different tidal phases (left side: slack water (low tide or high tide); right side: mid tide) in 2010.

2.4.2. TEMPORAL PHYTOPLANKTON DYNAMICS

A strong seasonal turnover of the three dominant phytoplankton classes – diatoms, dinoflagellates and flagellates (Fig. 5) – was observed in all three years. Around 120 phytoplankton taxa were recorded in total. In each year, increasing water temperatures (Fig. 3c) promoted the development of diatom spring blooms in the end of March. In 2009, within only five days (March 26th – 31st 2009) a pronounced spring bloom developed with a peak at a biovolume of 6.6 * 10^7 µm³ / ml. This bloom was mainly composed of *Thalassiosira nordenskioeldii*, *Asterionellopsis glacialis* and *M. helysia*. This was the first time that a bloom of *M. helysia* was observed in this region (see Chapter 2). From this time on, the invasive diatom dominated the phytoplankton community not only during all spring bloom formations, contributing 90% to total diatom biovolume, but also during the summer month, contributing between ~ 20% and ~ 70% to the total diatom biovolume, but then dropping each autumn (< 10% of total diatom biovolume). The diatom spring bloom in 2010 occurred later than in 2009 probably caused by the strong ice winter in 2009 / 2010. It was mainly composed of *M. helysia* and *A. glacialis*, but presumably not fully captured since nutrient stocks were already depleted at the first sampling date (April 20th) (Fig. 3a, b and Fig. 4), indicating the end of the spring bloom. In 2011, a spring biomass peak was observed on April 20th with *M. helysia* peaking at 5.5 * 10^7 µm³ / ml, accounting for ~ 94% of total biovolume (Fig. 6). Consequently, total biovolume during spring bloom decreased strongly from 8.7 * 10^7 µm³ / ml in 2009 to 5.6 * 10^7 µm³ / ml in 2011. Moreover, when considering a consistent low sampling resolution from 2009 – 2012 (Fig. 2), the increasing dominance of *M. helysia* during spring blooms (from 75% of total diatom biovolume in 2009 to 94% in 2011), was accompanied by decreasing species richness − 80 taxa in 2009 to 59 taxa in 2011 (Fig. 9); cell densities of taxa, which were abundant in 2009, also declined in the subsequent years.

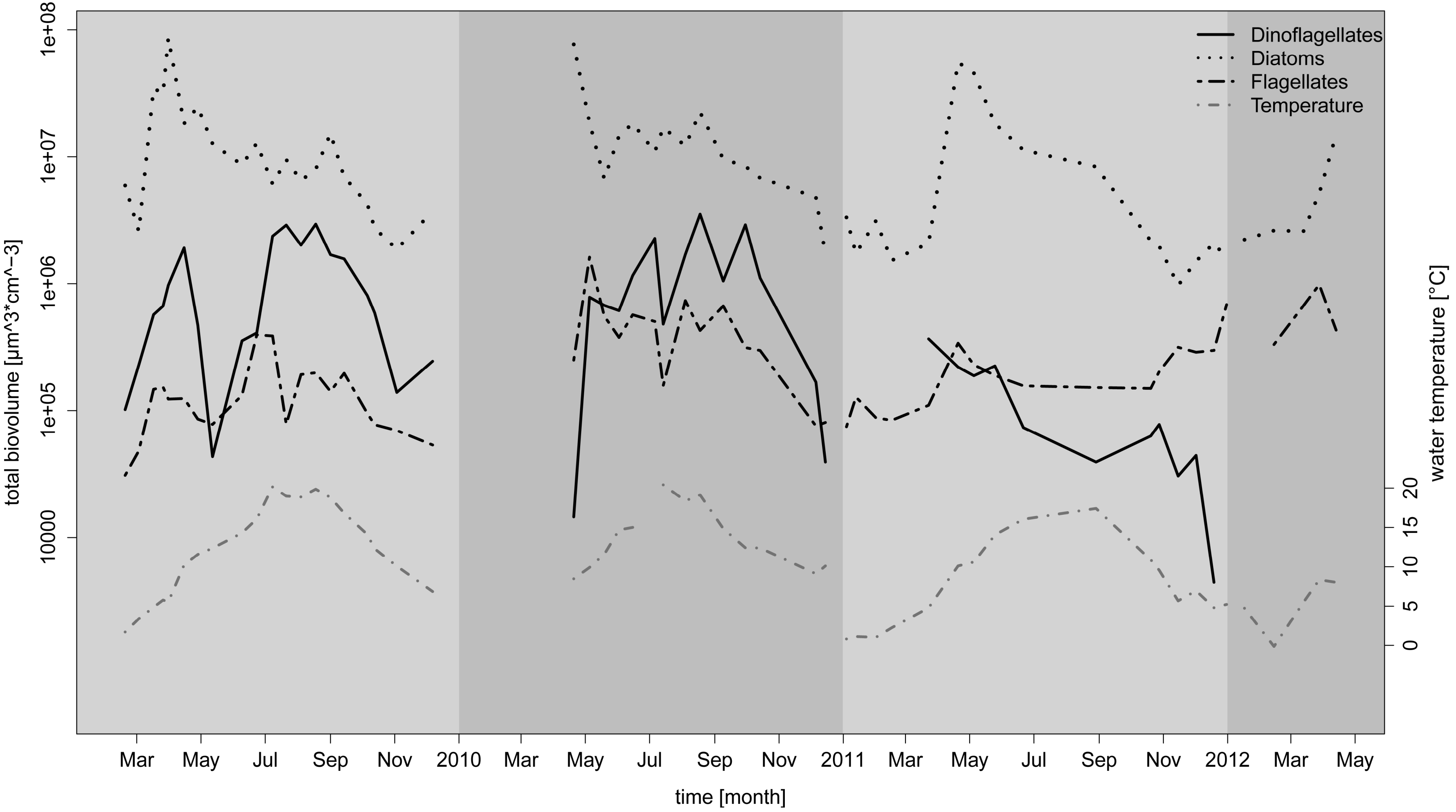


Fig. 5: Seasonal and inter-annual development of the three dominant groups (diatoms, dinoflagellates, flagellates) in relation to water temperature. Dotted line = diatoms; solid line = dinoflagellates; dashed black line = flagellates; dashed grey line: water temperature.

While I was not able to quantify the presence of *Phaeocystis* sp. after these spring blooms, I observed some smaller diatom blooms composed of species with a more restricted seasonal occurrence (e.g. *Guinardia delicatula*, *Guinardia flaccida*, *Leptocylindrus danicus*, *Rhizololenia imbricata*) around June each year. Diatoms (e.g. *Chaetoceros* spp., *Odontella* spp.) dominated the phytoplankton community throughout the year except for July and August, when dinoflagellates (especially *Gymnodinium* sp., *Gyrodinium* sp. and *Noctiluca scintillans*) prevailed. Smaller diatom autumn blooms appeared again during October each year, in which high biovolumes of benthic species (such as *Brockmanniella brockmannii* or *Gyrosigma / Pleurosigma* sp.) were recorded, which was probably due to high resuspension induced by autumn storms. Other flagellates did not show a distinct seasonal pattern and contributed only a smaller part to the total biovolume (Fig. 5).

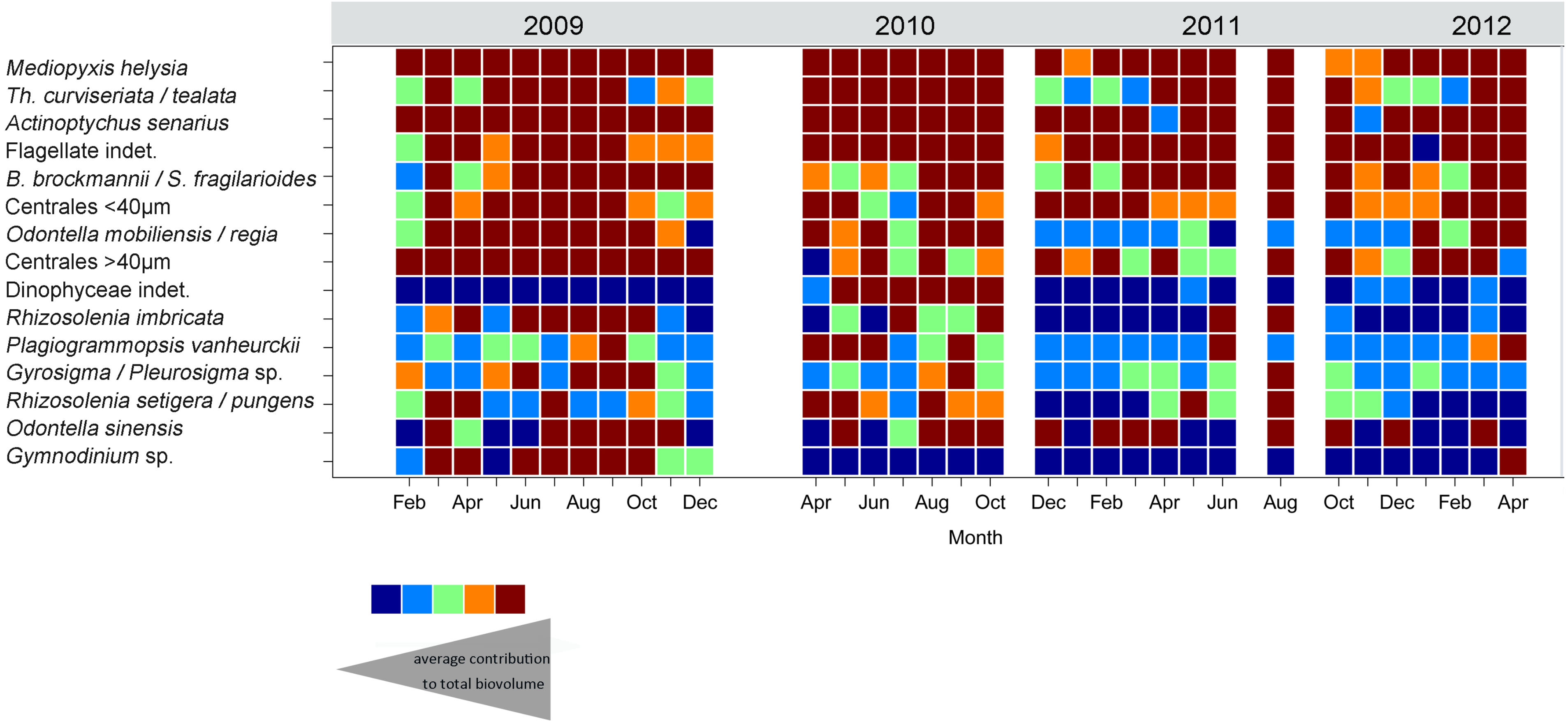


Fig. 6: Temporal sequences and the month of peak abundance for the 15 best performing taxa are displayed via a colour gradient. Data have been cubic root-transformed before analysis and arithmetic means of all data have been calculated. Dark red = above the median, bluish colours below the median.

2.4.3. TIDAL VARIATIONS IN THE PHYTOPLANKTON COMMUNITY STRUCTURE

In addition to seasonal changes in the phytoplankton community structure, species turnover was also influenced by tidal currents. I expected that low tide samples should comprise species typical for the Wadden Sea, while high tide and mid tide samples should reflect the phytoplankton community typical for the open North Sea (Niesel 1997). Analysis of Bray-Curtis taxon dissimilarities (Fig. 7) revealed that low tide and mid tide communities were more dissimilar to each other than high tide and mid tide communities. This indicates that the water body of the incoming flood and the subsequent high tide were more similar to each other than low tide and high tide water bodies. This feature was most pronounced in spring and early summer.

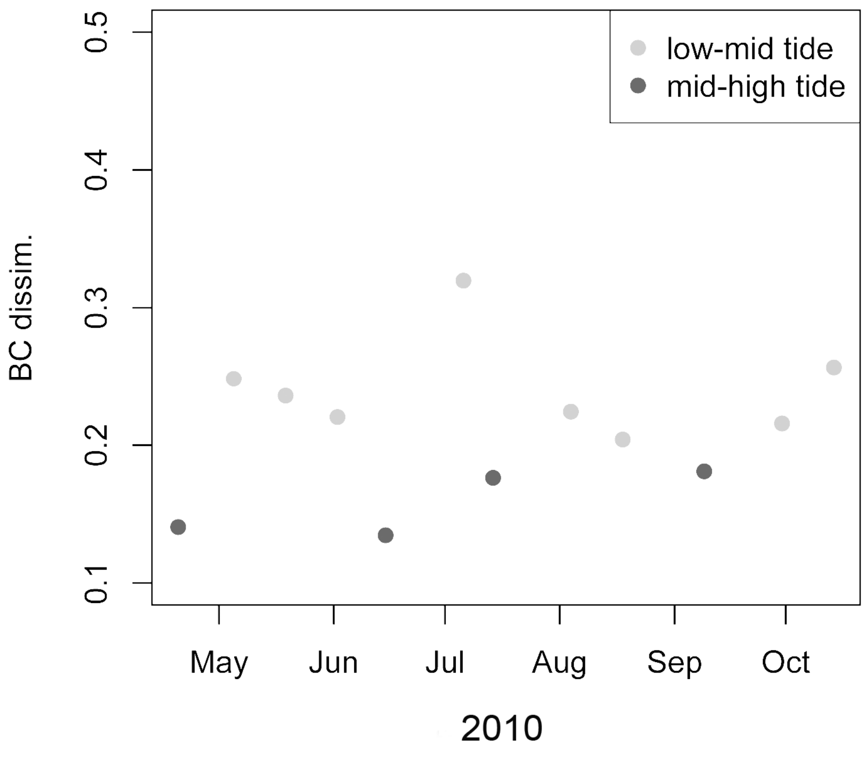


Fig. 7: Comparison of low tide - mid tide Bray-Curtis dissimilarities (light grey) and mid tide - high tide Bray-Curtis species dissimilarities (dark grey). The community composition was generally more dissimilar in mid tide - high tide samples.

2.4.4. BENTHOPELAGIC COUPLING

I classified species as benthic, benthopelagic or pelagic according to literature data, to quantify the contribution of microphytobenthic species to the pelagic phytoplankon community. A strong vertical species turnover could be assessed in 2009 (Fig. 8), which was mainly driven by resuspension of microphytobenthic species into the pelagial at both tidal and seasonal time scales. Since samples were taken during half tide in 2009, high current velocities promoted a strong benthopelagic coupling. Particularly in spring and autumn, benthic species (such as *B. brockmannii*, *P. vanheurckii*, as well as *P. sulcata* or *Gyrosigma / Pleurosigma* sp.) were suspended in the whole water column and made up more than 50% of the total biovolume in all three depths (Fig. 8), even though benthic diatoms are generally small-sized.

In contrast, benthic species contributed only a very small proportion to the total biovolume (1 - 5%) in 2010. However, benthic species were found constantly among all samples, and homogeneously distributed in the water column. Total biovolume of benthopelagic species was significantly higher than that of the benthic species. For this group, a general seasonal pattern was observed with peaks in May, June and Sept, whereas in July lowest amounts were found. Surprisingly, the benthopelagic biovolume in the surface samples were higher than in the 10 m water samples. Vertical differences in species composition were also attributable to the diatom *M. helysia*, which was found mostly in the surface water layer (up to $9.9 *\cdot 10^7$ µm³ / ml) during spring bloom.

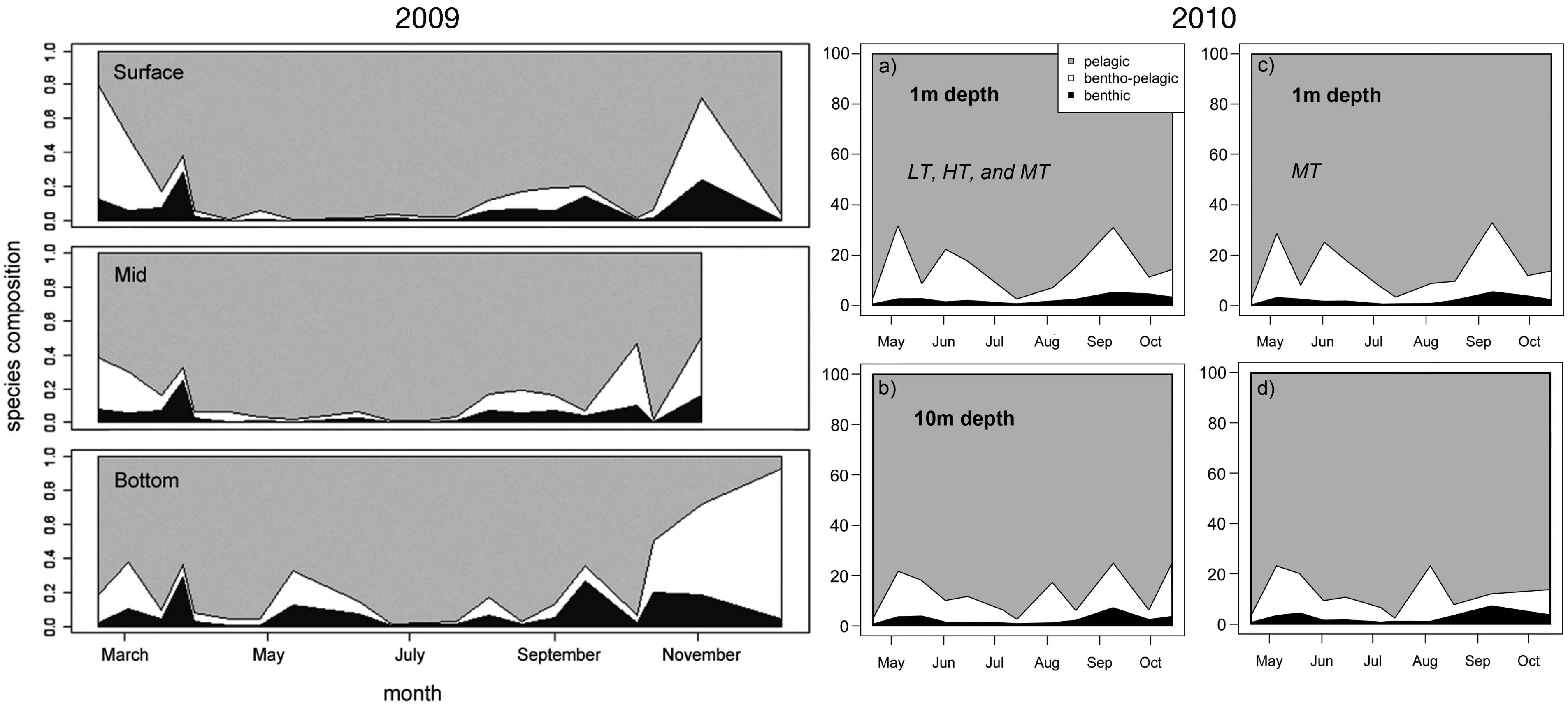


Fig. 8: This figure presents the contributions of pelagic (grey), bentho-pelagic (white) and benthic (black) species to total biovolume. In 2009, I took samples at three different depths (surface / mid / bottom − 1 m above the sea floor), while in 2010 sampling took place during different tidal phases (LT = low tide; HT = high tide and MT = mid tide at the surface and at the bottom of the water column.

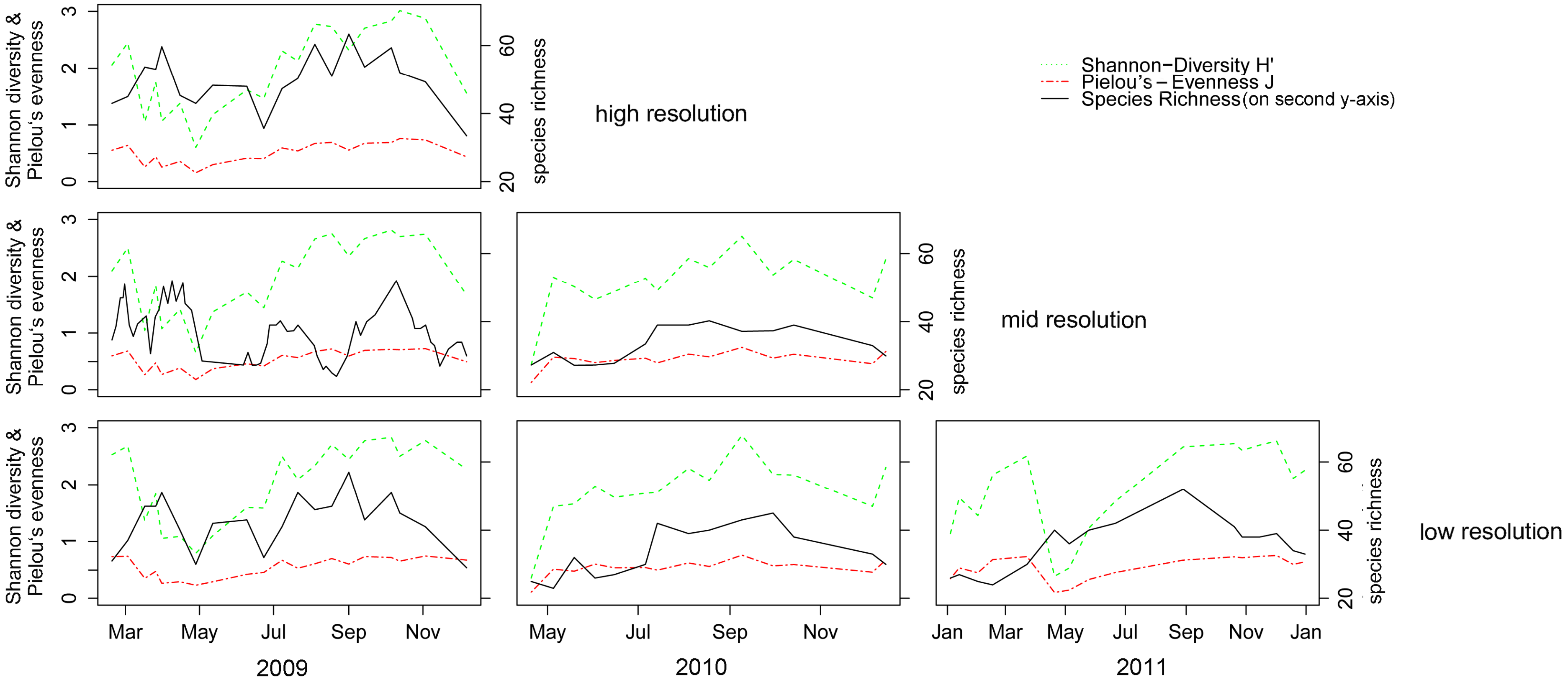


Fig. 9: Sampling effort: Shannon diversity, Pielou´s evenness and species richness are shown for all three years. See Fig. 2 for different sampling strategies. I applied the mid and the lower resolution to data from 2009 and 2010 respectively to compare the outcomes of different resolutions.

In 2009, species evenness was very high during spring (around 0.95; Fig. 9), indicating a monospecific bloom. Along with the end of the diatom spring bloom, evenness declined to levels around 0.86 in May (Fig. 9), until it peaked again in September. Species richness was low during the spring bloom, whereas it also increased in September to up to 80 species per sample (Fig. 9).

2.5. DISCUSSION

2.5.1. NUTRIENT DYNAMICS

As nutrients and organic matter are perpetually exchanged between the coupled systems of the Wadden Sea and the adjacent North Sea (Postma 1981), I observed tidally-driven variations in the nutrient concentrations during the sampling campaign in 2010. Highest nutrient concentrations were always been measured at low tide, suggesting an enhanced influence of the benthic microbial heterotrophic activity in relation to a shallower water body (Postma 1981). Thereby, nutrients are assumed to be predominantly dispensed into the water column by pore water advection, while freshwater contribution should be less relevant (Grunwald et al. 2010). The expected vertical gradient with higher nutrient concentrations towards the sea floor could not be observed, suggesting a strong vertical mixing. Regarding the horizontal dimension, N, P and Si were exported from the 'bio-reactor' Wadden Sea into the German Bight, where the newly remineralised nutrients were taken up by high phytoplankton biomass (Grunwald et al. 2010). Thus, the North Sea can be seen as a sink for nutrients and as a source for organic matter, which is transported into the Wadden Sea (Postma 1981).

Seasonal variations in nutrient concentrations overrode inter-annual and tidal differences. Thereby, the seasonal dynamics followed a typical pattern for this area (Grunwald et al. 2010) consistently throughout all years, which was controlled by phytoplankton primary production and microbial remineralisation. N and Si showed highest values during the winter month, when primary production was low and microbial remineralization processes prevailed. In contrast, P already peaked at the end of the summer. This difference is according to Grunwald et al. (2010) due to the fact that the P-cycle is not only determined by primary production and decomposition, but also by adsorption and desorption processes by ferric oxyhydroxides in the sediments. Lowest nutrient concentrations always could be found after diatom spring blooms, which persisted until nutrients, in particular Si, were depleted. It is important to notice, that the often used concept of the limiting nutrient (Liebig´s law) is not applicable to this highly dynamic, non-steady-state-system since a pulsed nutrient supply (e.g. via zooplankton excretion, bacterial degradation, pore water supply, tidal currents) may allow phytoplankton species to grow efficiently in nutrient-poor waters (Harris 1980). Even if nutrient concentrations are below the detection limit, brief phases of nutrient supply are sufficient for algal cells to cover their needs for cell division. Consequently, I still found relatively high phytoplankton cell densities after the collapse of the spring blooms. Ultimately, the spatiotemporal scales of the nutrient availability on the - for phytoplankton individuals relevant - micro-scale need further in-depth investigation (Harris 1980).

2.5.2. PHYTOPLANKTON DYNAMICS

Phytoplankton community assembly is highly complex and dynamic on a range of temporal scales. On tidal scales, spatial (vertical) differences in community composition were expected to mainly originate from different degrees of bentho-pelagic coupling caused by changing resuspension of sediments due to tidal fluctuations of the current velocities. The vertical turnover was also affected at seasonal scales, since resuspension rates were likely to be enhanced by changing weather conditions or decreased by the production of sediment-stabilizing polysaccharides and temporal stratification of the water column. In spring and autumn 2009, a strong benthopelagic coupling could be observed: severe current velocities promoted the resuspension of high abundances of living benthic microalgae into the water column. In spring 2009, the augmented suspension may be attributed to microphytobenthic blooms, which developed while the phytoplankton biomass remained low (Lucas et al. 2000). During this time more than 50% of the total biomass was built up by benthic species in all three depths (agreeing with results of Lucas et al. (2001)), indicating a well-mixed water body. These spatial and temporal variations in the suspension of benthic microalgae may impact the water column productivity as well as food quality and quantity available to deposit and suspension feeders (Lucas et al. 2000).

Along with the findings for the nutrient variability, vertical and tidal variations in the phytoplankton community composition were also surpassed by the strong seasonality, characterized by low phytoplankton biomass during wintertime and highest biomass during annually recurring diatom spring blooms. The extent, timing and species composition of the spring bloom varies from year-to-year (Dakos et al. 2009), whereby the onset of the spring bloom presumably is triggered by day length or light intensity and thus is relatively stable in time (Edwards and Richardson 2004), even though I observed short delays in the years 2010 and 2011 in comparison to 2009 (see below). While Bresnan et al. (2009) report for the east coast of Scotland that dominant spring bloom species were *Chaetoceros* spp. and *Skeletonema* complex, *M. helysia* presumably suppressed those species, which were also dominant constituents of the spring blooms before the invasion of *M. helysia* in the backbarrier tidal flats. Bresnan et al. (2009) report furthermore that the diatom spring bloom was usually followed by a *Phaeocystis* sp. bloom, which I unfortunately had to exclude from my data set. As soon as the depleted Si concentrations have been remineralized to a certain degree, few smaller peaks of diatoms with restricted seasonal occurrences could be observed usually around June. During the summer months dinoflagellates prevailed, followed by elevated diatom biomass in October. This classical pattern of succession was also found by Bresnan et al. (2009). At the end of a seasonal cycle, population densities were reset during winter. Different community assemblages enter the winter season and consequently impinge the composition of the next spring bloom (Dakos et al. 2009). Cyst-forming and large species (with a high possibility to store nutrients) are thereby more likely to survive harsh winter conditions. Both traits apply to *M. helysia*, potentially contributing to its spring bloom dominance.

The time span since the start of the monitoring is too short to identify significant long-term changes, which could be predominantly influenced by stochastic fluctuations in climatic and weather conditions as well as by anthropogenic nutrient inputs (Clark and Frid 2001; Dakos et al. 2009). For the evaluation of shifts in the community composition due to environmental change (e.g. global warming or eutrophication), longer periods in time need to be monitored. Bresnan et al. (2009) state that a decade represents the absolute minimum of data for the observation of any long term trends.

Mechanisms regulating phytoplankton dynamics

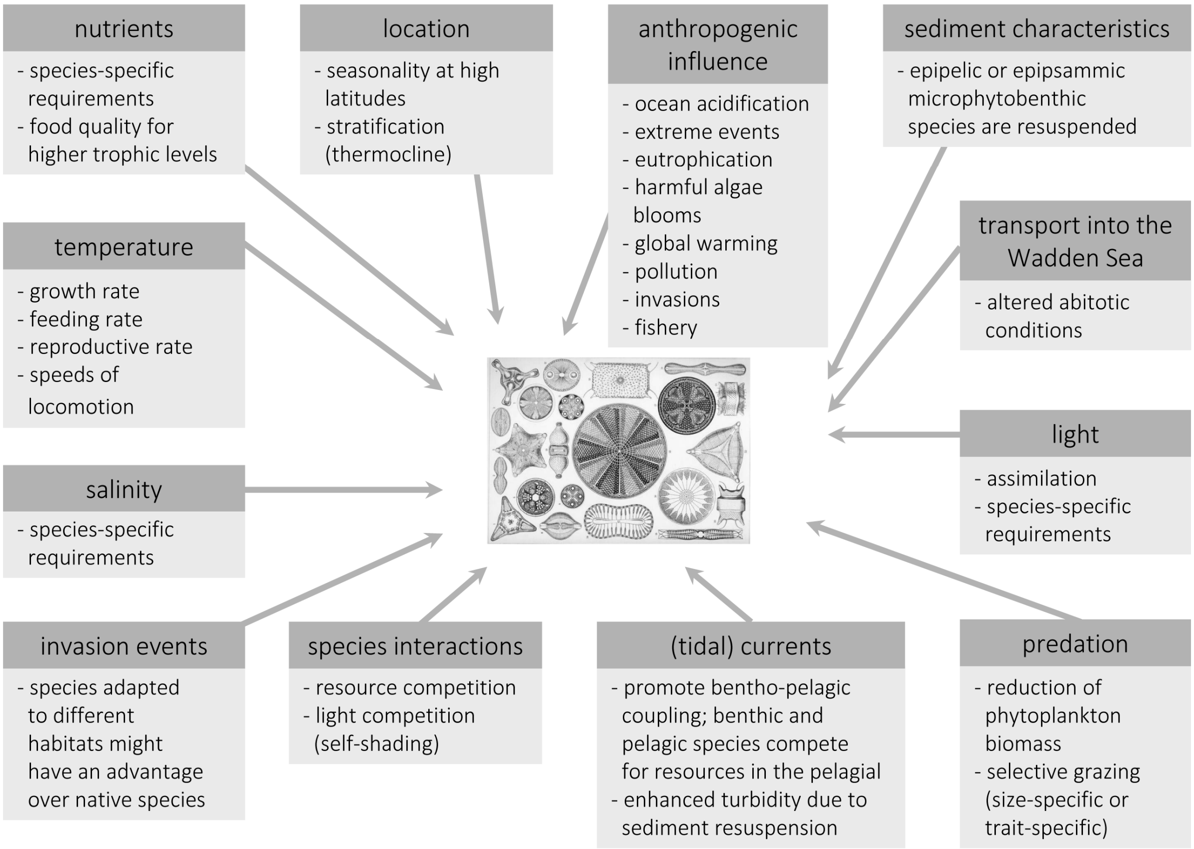


Fig. 10: Intrinsic and extrinsic mechanisms potentially regulating phytoplankton dynamics. The interplay of these biotic, abiotic and anthropogenic factors determines the community composition. Schematic diagram drawn according to statements in Clark and Frid (2001).

Phytoplankton dynamics are complex responses to fluctuations of a multitude of forcing factors (internal community dynamics and extrinsic abiotic factors operating at different spatial and temporal scales) (Clark and Frid 2001; Dakos et al. 2009). In Fig. 10, I visualized different mechanisms affecting phytoplankton dynamics based on my own observations and literature data (Clark and Frid 2001). The relative importance of single factors or the interaction of different processes is thereby difficult to detect (Clark and Frid 2001). Abiotic and biotic factors are closely tied, since abiotic changes might influence the level of competition and selection in the community.

Hence, available nutrient concentrations widely influence biodiversity, productivity and the phytoplankton community composition, scaling up to higher trophic levels. Further potential factors fuelling community turnover are salinity, water temperature and light availability. Temporal (seasonal / tidal) fluctuations in these variables, as well as spatial shifts (e.g. transport into Wadden Sea waters with different abiotic conditions) have to be tolerated by species occurring in this region.

Extrinsic factors shaping phytoplankton community assembly are hierarchically ordered. Large-scale, low frequency variations (e.g. seasonality, or zooplankton blooms) in the phytoplankton community structure can be observed, which are primarily caused by climatic fluctuations (Harris 1980) (Fig. 1). Biomass distributions appear to vary mostly at these large scales (Harris 1980). However, parallel existent small-scale, high frequency variations in the micropatchiness of abiotic and biotic components in the water column also widely affect the community assembly (Harris 1980; Clark and Frid 2001) (Fig. 1 & 10). Planktonic individuals continuously experience changing (a)biotic conditions, when they are passively dispersed through a multitude of micropatches. Individual responses (species interactions, mortality, proliferation, competition, nutrient consumption) to the surrounding conditions (resource availability) add up and result in positive or negative peaks in abundances. Thereby, environmental fluctuations at the micro-scale offer several niches, maintaining a high biodiversity of mostly rare species with slightly different optima (Caron and Countway 2009) (see Chapter 3). Since the study system is not at steady-state, rare species are not competitively excluded. If local extinction occurred in some micro-patches, they would be perpetually replenished via currents from favouring microhabitats. This rapid community re-assembly leads to continually changing combinations of rare taxa. Triggered by periodical (tidal) changes in abiotic conditions, temporarily repeating networks of species guilds with similar abiotic requirements might occur (Caron and Countway 2009). The high species richness of the phytoplankton community facilitated at the micro-scale has a tremendous importance for the maintenance of ecosystem functioning under altered environmental conditions (resilience) since the ecological redundancy serves as a buffer (Caron and Countway 2009; Lear et al. 2014).

Conventional sampling procedures need to be reflected in the light of the 'true' scale of variability in phytoplankton communities (Harris 1980; Dornelas et al. 2012), Additional sampling at the small-scale seems to be required (Harris 1980). However, as this is very cost and time intensive, the analysis of species traits might be a useful surrogate (Edwards et al. 2013). The evaluation of the sampling effort revealed that the biweekly sampling of surface water at random points in the tidal cycle might be sufficient for tracking general seasonal phytoplankton dynamics. Yet, more frequent (e.g. weekly) sampling intervals would allow the acquisition of short-term variation and smaller, temporally restricted peaks (e.g. small blooms of *Rhizosolenia imbricata* or *Guinardia delicatula*). However, when comparing high and low taxonomic and spatial resolution, around 1/3 of the total species richness, mostly rare species, remains unrecognized with the low resolution sampling procedure. The determined species richness (~ 120 species) at the highest resolution only is an approximation of the actual species richness. Particularly rare species, which form the greatest fraction, may be underestimated (Dornelas et al. 2012). Yet, the more species are differentiated, the better might be the quality of the predictability of future community assembly, as rare species potentially might become dominant under changing environmental conditions (Caron and Countway 2009). Consequently, morphological identification methods need to be extended by molecular methods to gain in-depth insights in the actual species diversity.

CHAPTER 2

3. *DOMINANCE OF THE NON-INDIGENOUS DIATOM MEDIOPYXIS HELYSIA IN WADDEN SEA PHYTOPLANKTON CAN BE LINKED TO BROAD TOLERANCE TO DIFFERENT SI AND N SUPPLY*

3.1. ABSTRACT

The non-indigenous diatom *Mediopyxis helysia* was first observed in spring 2009 in the backbarrier tidal flats of Spiekeroog, North Sea (53°45´01.00´´ N and 007°40´16.30´´ E) and has dominated spring blooms throughout the years 2009 - 2011. However, it also prevailed during the remainder of the season and – compared to 2009 – the total biovolume during spring blooms decreased by 36% and phytoplankton species richness as well as evenness declined concomitantly. *M. helysia´*s rapid establishment led to the questions: (1) Which advantageous traits does this species possess? (2) Which environmental conditions facilitate its expansion? Therefore, I here amend my field observations with an experiment with artificial communities cultured under six different combinations of Si and N concentrations in presence and absence of *M. helysia* for one month. The relative biomass of *M. helysia* with regard to different nutrient concentrations in an artificial community were examined with exclusion of grazing. In the experiments, the phytoplankton community was dominated by the only lab culture, the chlorophyte *Dunaliella salina*, but *M. helysia* was the most successful diatom. Its presence changed the community composition irrespective of the chosen Si and N concentrations. *M. helysia* significantly diminished chlorophytes and dinoflagellate percentages, whereas diatom growth was supported and total biovolume increased in the experiment. Moreover, *M. helysia* enhanced the evenness in the artificial community. The broad persistence of *M. helysia* under different resource conditions might have fostered the successful establishment of *M. helysia* in the North Sea and its dominance throughout the year.

3.2. INTRODUCTION

The North Sea phytoplankton community has frequently been subject to invasions of non-indigenous species (NIS) in the last century. Susceptibility to invasions in the marine environment increases as the number of linkages in an ecosystem decreases due to biodiversity loss as a consequence of eutrophication and climate change (Nehring 1998a; Nehring 1998b; Levine and D´Antonio 1999; Hufbauer and Torchin 2007). On the one hand, such invasions occur by range expansions: a natural mechanism mediated, e.g. by ocean currents or migrating birds (Nehring 1998b). On the other hand, anthropogenic vectors such as aquaculture (e.g. Pacific oyster) or transportation of species on ship hulls and in ballast water lead to the introduction of NIS into new habitats (Rick and Duerselen 1995; Nehring 1998b; Klein et al. 2010a). Transport of NIS in ballast water presents a particular problem, since it is a phyletically and ecologically non-selective transport vector, enhancing the number of NIS transported to new habitats (Ruiz et al. 1997; Minchin et al. 2009; Mather et al. 2010). During transportation, natural borders to dispersal such as spatial distance or currents are transgressed (Carlton and Geller 1993). However, during their introduction process, NIS need to survive a number of 'filter barriers' such as species losses during ballast uptake, transport, discharge, and establishment in the receiving habitat (Klein et al. 2010a; Villac and Kaczmarska 2011). Hence, 'filtering' leads to a relatively low infection rate with an overall increase in phytoplankton species numbers of about 1% in the North Sea in the last century, despite the enormous amounts of water that are transported into the German Bight (Nehring 1998b).

Long-term records of North Sea phytoplankton report successful colonization of several planktonic organisms introduced by diverse immigration pathways (Nehring 1998b; Gollasch 2006). The establishment of *Odontella sinensis* (Ostenfeld 1908) in the North Sea around 1903, which was introduced via ballast water from Hong Kong, led to a depression in the succession dynamics of indigenous species (Nehring 1998b; Gomez 2008; Nehring et al. 2009; Klein et al. 2010b). The large centric diatom *Coscinodiscus wailesii* (Gran and Angst 1931) was probably either introduced to northern Europe from the Indo-Pacific via ballast water or with the establishment of the oyster *Crassostrea gigas* aquacultures in France (Edwards et al. 2001). This species exhibited an unusual proliferation in 1977 (Nehring 1998b). Nowadays, both diatoms *O. sinensis* and *C. wailesii* are important constituents of the autumn and spring diatom community (Edwards et al. 2001). Other examples of NIS, which were able to establish persistent populations in the North Sea, are the diatoms *Thalassiosira tealata*, *Thalassiosira punctigera* and *Thalassiosira hendeyi* (Streftaris et al. 2005).

Recently, the centric diatom *M. helysia* has been observed in the North Sea, where it has established extensive blooms of significant biomass (Kraberg et al. 2011). Concomitant with Kraberg et al. (2011) and Loebl et al. (2012), I define *M. helysia* as non-indigenous species as it is unlikely that it was missed during prior sampling due to its conspicuous shape and large body size − length 44 - 125 µm, width 22 - 78 µm (Kuehn et al. 2006). Nevertheless, some taxonomists might have confused it with *Helicotheca tamesis* (even though this species forms twisted chains) or *Lithodesmium undulatum* (even though this species is triangular). Therefore, I cannot exclude that *M. helysia* might have been part of the hidden flora as a cryptic species before 2003, but its massive occurrence in different parts of the North Sea during a short period of time makes a recent introduction very likely. In 2003, it was first detected in the North Sea near the islands Helgoland and Sylt (Fig. 1) at very low cell densities (Kuehn et al. 2006) and in 2005 on the East Coast of Scotland (McCollin 2008). In spring 2009, *M. helysia* was first observed in my study area, the backbarrier tidal flats of Spiekeroog (Fig. 1), and has dominated the phytoplankton biomass ever since. *M. helysia* was first recorded as a new species in the Gulf of Maine and the adjacent Bay of Fundy in 1996 and in 2002 respectively (Martin and LeGresley 2008). Hence, it is likely to originate from the East Cost of the United States of America. Recent findings of *M. helysia* in the Bay of Bengal (Panda et al. 2010) further suggest its ability to overcome ballast water conditions (Liebich et al. 2012), but its concise dispersal pathways await further molecular investigations.

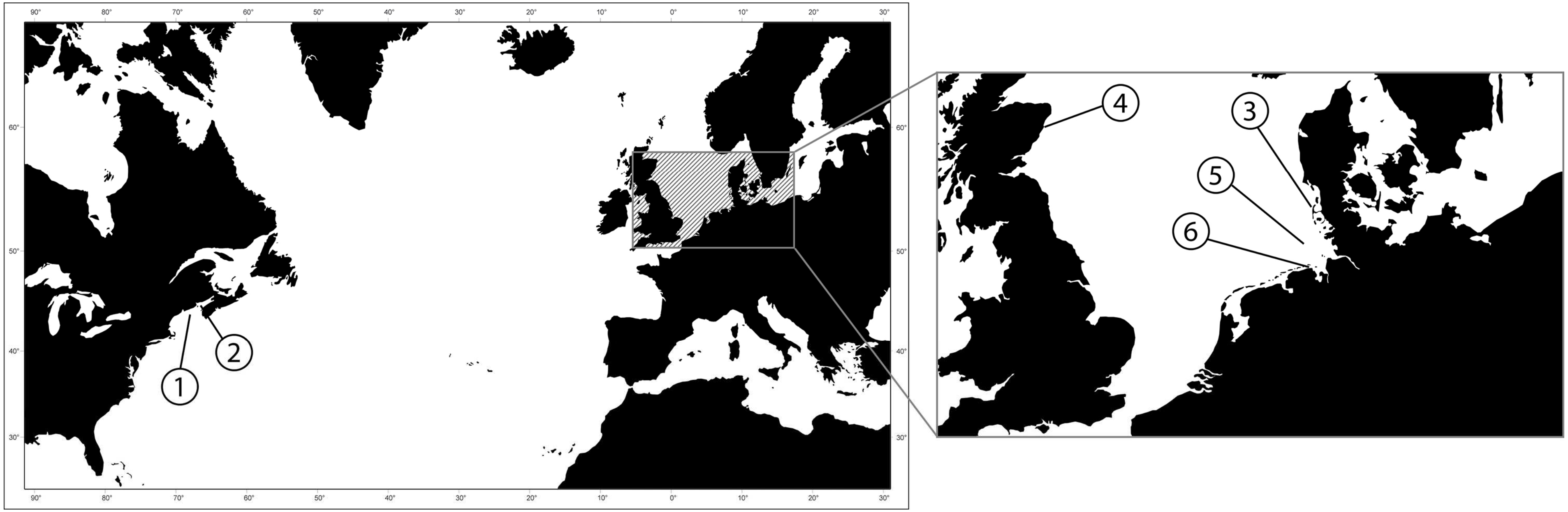


Fig. 1: *M. helysia* was first observed in the Gulf of Maine (1996; 1) and the adjacent Bay of Fundy (2002; 2). In 2003, first cells were detected at Sylt (3) and Helgoland (5) and in 2005 also at the east coast of Scotland (4). In 2009, *M. helysia* had been introduced into the German and Dutch Wadden Sea, where it established extensive blooms of significant biomass. Since then, *M. helysia* is the dominant species in the backbarrier tidal flats of Spiekeroog during spring blooms (6).

The dominance of *M. helysia* led to two important research questions (1) Which advantageous traits does this species possess? and (2) Which environmental conditions facilitate its expansion? In my study, I contribute to answering these questions in two major parts: first, I present observational data of *M. helysia*´s abundance during three consecutive years (2009 – 2011) in the backbarrier tidal flats of Spiekeroog supplemented by environmental parameters from a permanently installed time-series station (Grunwald et al. 2007a; Reuter et al. 2009). Since *M. helysia* has been described only recently (Kuehn et al. 2006), information about its autecological characteristics in its native range and its invaded habitats is scarce. To date it is only known that *M. helysia* prefers low salinities (Kraberg et al. 2011). By comparing the occurrence of *M. helysia* to the environmental parameters, I was able to characterize the realized niche of this species in the Wadden Sea. My second objective was to identify experimentally the nutrient conditions under which *M. helysia* performs best (in the absence of grazers). Therefore, I conducted an experiment, in which I analyzed the influence of nutrient supply (Si and N) on the performance of a phytoplankton community with and without *M. helysia.* The Si : N ratio is decisive for the success of diatoms (Sommer 1994) and varies strongly throughout the year as the often diatom-dominated spring bloom depletes dissolved silicate concentrations. N concentrations in the backbarrier tidal flat range from > 30 µM before the onset of the spring bloom and < 10 µM during the summer month, whereas Si concentrations in this area lie between > 20 and < 1 µM (Grunwald et al. 2010). The experiment had a 2 x 3 orthogonal design (2 Si- x 3 N-concentrations in the presence as well as absence of *M. helysia*, see Table 1) inoculated with 11 species belonging to different phytoplankton groups isolated from the tidal flats of Spiekeroog. The algae community was grown in a semi-continuous culture for four weeks. I analyzed the proportion of *M. helysia* in the different Si and N treatments and used the comparison of phytoplankton in absence and presence of *M. helysia* as an estimate for effects of this species on the phytoplankton community.

3.3. MATERIAL AND METHODS

3.3.1. I. OBSERVATIONAL STUDY

I followed the phytoplankton community composition from spring 2009 to winter 2011 in a biweekly rhythm (54 samples in total) in the tidal flats of Spiekeroog. Using a multi water sampler (HYDRO-BIOS, Germany) with 3.5 l-Niskin bottles, I collected water samples from 1m depth in close proximity to a permanently installed time-series station in the tidal inlet "Otzumer Balje" − the maximum water depth is about 13 m (Grunwald et al. 2007a; Reuter et al. 2009). The time-series station provides meteorological, hydrographical and biogeochemical data continuously throughout the year; temperature and salinity data are validated on a regular basis by reference CTD-measurements.

For microscopic analysis, 100 ml subsamples taken from the surface water sample were preserved in 1% Lugol´s iodine solution and stored in brown glass bottles. For each sample, both 5 and 1 ml subsamples were counted, ensuring the capture of rare species in the 5ml subsample; more common species were quantified in the 1ml subsample. Phytoplankton organisms were determined to the lowest possible taxonomic level using an inverted microscope (ZEISS; AXIOVERT 10) (Utermoehl 1958). This microscopic analysis of ≥ 600 cells per sample resulted in an overall determination and quantification of ~ 120 taxa based on Drebes (1974), Tomas (1997), Pankow (1999), Round (2007), Hoppenrath et al. (2009), Klein et al. (2010b). Only cells > 5µm with intact protoplasm and distinct chloroplasts were considered. During enumeration, the dimensions of simple geometric bodies of 20 cells of each dominant taxon were measured and used for calculation of specific cellular volumes

(Hillebrand et al. 1999), complemented by data from the HELCOM phytoplankton check list (Olenina et al. 2006).

3.3.2. II. EXPERIMENTAL STUDY

Artificial communities with 11 different species (excluding *M. helysia*) representing Bacillariophyceae, Chlorophyceae and Dinophyceae, were cultivated both in the presence and absence of *M. helysia* under different Si and N concentrations. The experiment started with N and Si concentrations ranging from 20 to 100 µM. Starting concentrations for both nutrients in the experiment were chosen higher than *in situ* to guarantee that a sufficient amount of Si and N is available to support algae growth from a small volume inoculum. With this, I also mimicked nutrient conditions prior phytoplankton spring blooms *in situ*, when nutrients are not limiting (Sommer 1994). This experimental set-up allows us to assess the optimal nutrient conditions under which *M. helysia* performs best in such artificial communities.

3.3.3. ORGANISMS

Mimicking phytoplankton associations from the North Sea, I used microalgae belonging to different phytoplankton groups, which were isolated from samples collected from the tidal inlet "Otzumer Balje", located in the backbarrier flats of Spiekeroog: *Stephanopyxis turris*, *Actinoptychus senarius*, *Odontella sinensis*, *Mediopyxis helysia*, *Rhizosolenia setigera*, *Lithodesmium undulatum* (closely related to *M. helysia* (Kuehn et al. 2006)), *Thalassiosira rotula*, *Gyrosigma* sp., *Chaetoceros danicus* (Bacillariophyceae); *Prorocentrum micans* and *Pentapharsodinium* sp. (Dinophyceae). The water body in the Otzumer Bale is relatively well-mixed and thus all these species coexist in space and time and are likely to find in one single sample.

The chlorophyte *D. salina* originates, however, from a lab culture. Clonal cultures were established in f / 2 medium (Guillard and Ryther 1962) before the experiment.

3.3.4. CULTURE CONDITIONS

The experiment had three fully orthogonal factors: Si, N and presence/absence of *M. helysia* (Table 1). In order to obtain different nutrient supply treatments I used a 2 x 3 factorial design of Si and N concentrations in a modified f / 2 medium (Guillard and Ryther 1962): 100 µM Si (as $Na_2 SiO_3 * 9 H_2O$) or 20 µM Si and 20, 50 or 100 µM N (as $NaNO_3$) respectively.

Table 1: Matrix of the experimental setup with a 2 x 3 combination of Si- and N-concentrations in the presence / absence of *M. helysia.*

11 species plus *M. helysia*			**11 species without *M. helysia***		
low Si (20µM) **low N** (20µM)	**low Si** (20µM) **medium N** (50µM)	**low Si** (20µM) **high N** (100µM)	**low Si** (20µM) **low N** (20µM)	**low Si** (20µM) **medium N** (50µM)	**low Si** (20µM) **high N** (100µM)
high Si (100µM) **low N** (20µM)	**high Si** (100µM) **medium N** (50µM)	**high Si** (100µM) **high N** (100µM)	**high Si** (100µM)) **low N** (20µM)	**high Si** (100µM) **medium N** (50µM)	**high Si** (100µM) **high N** (100µM)

Triplicates of each Si and N combination-treatment were inoculated with and without *M. helysia* in 36 100 ml Erlenmeyer flasks in total. Equivalent biovolumes (on average ~ 1.6 * 10^5 µm^3 / ml per species; calculated according to Hillebrand et al. (1999)) were inoculated from stock cultures to compensate for differing cell sizes of the respective species. In +*M. helysia*-treatments, this species was added at an equivalent biovolume to that of the other species, enhancing the overall biovolume, which is the same situation as after an invasion event *in situ*. The inoculum made up ~ 10% of the total volume. The experiment was carried out under defined light conditions (60 $\mu E*m^{-2} * s^{-1}$) in a 12 : 12h light : dark cycle at constant temperature (18 °C). Cultured species were adapted to the predefined temperature and light settings in the climate chamber and all showed high positive growth rates. Erlenmeyer flasks were randomly arranged on a shaker (Rüttler-Gerhardt Laboshake with 50 rpm), keeping the cultures in suspension. The duration of the experiment was 28 days, corresponding to approximately 28 - 56 generations of microalgae.

3.3.5. SAMPLING AND COUNTING

On days 3, 6, 13, and 21 of the experiment, 10 ml algal suspension in each flask was replaced with 10 ml of fresh modified medium (semi-continuous culture) to maintain the gradient in nutrient supply between the treatments. On day 28, samples were fixed with 0.1 ml of Lugol´s iodine. Depending on cell abundance, 3 to 5 ml of each sample was settled in an Utermöhl chamber. Counting procedures and biovolume estimation were performed as described above.

3.3.6. STATISTICAL ANALYSIS

All analyses were carried out using R, Version 2.10.1 (R Development Core Team 2012). The dataset of the observational study comprises salinity [PSU], water temperature [°C], PO_4, NO_3 and Si [µmol / l]. The latter three were log-transformed prior to analysis and cases with missing data were deleted to avoid variations in the number of observations used in the analyses. I conducted step-wise AIC tests (Akaike Information Criterion) (Akaike 1973) followed by linear regressions. The most parsimonious models are presented.

Experiment data were checked for normality and a Cochran´s C Test (GAD package) was performed to test the homogeneity of the variances. The effect of Si and N on the absolute and relative biomass of *M. helysia* was analyzed by a two-way ANOVA. Three-way ANOVAs were conducted to test effects of *M. helysia* presence and different Si and N concentrations on final biomass of each algae group as well as total biomass and Pielou´s evenness (i.e. Shannon-Wiener Index divided by species number). Tukey´s HSD (honestly significant difference) post-hoc tests were used in order to check for statistical significance within and among groups.

3.4. RESULTS

3.4.1. OBSERVATIONAL STUDY

During the observational study (2009 – 2011) *M. helysia* dominated the phytoplankton community during all spring bloom formations, accounting for up to 90% of the total diatom biomass. *M. helysia* was first detected in low abundance in the first sample (18.2.2009) at the time-series station installed in the backbarrier tidal flats of Spiekeroog (Fig. 2a). Within only five days (March 26th – 31st 2009) it developed a pronounced spring bloom with a peak at a biovolume of $6.6 * 10^7$ µm³ / ml, representing 75% of the total diatom biovolume. This bloom in particular was accompanied by the diatoms *Thalassiosira nordenskioeldii* and *Asterionellopsis glacialis*, which are regular members of the spring phytoplankton community in this area. On April 15th, a rapid break-down was registered ($7 * 10^6$ µm³ / ml), induced by depleted nutrient stocks (Fig. 2a, b, c). Thereafter, *M. helysia* contributed between ~ 20% and ~ 70% to the total diatom biovolume during summer, but dropped in autumn (< 10% of total diatom biovolume).

The spring bloom in 2010 occurred later than in 2009 probably due to a strong ice winter in 2009 / 2010. The peak of the *M. helysia* bloom in 2010 was presumably not captured (Fig. 2a), since nutrient stocks were already depleted at the first sampling date (April 20th) (Fig. 2b, c), signalizing the end of the spring bloom. Nevertheless, at this date, biovolumes of *M. helysia* were comparable to those measured during the peak of the spring bloom in 2009 (March 31st), indicating that the bloom in 2010 was more pronounced (before April 20th 2010) than in 2009. *M. helysia* accounted for up to 90% of total biomass in April 2010, accompanied by high cell numbers of *A. glacialis*. However, due to *M. helysia*´s large size, its biovolume surpassed the biovolume of *A. glacialis* by far. Although its relative contribution decreased over the year, *M. helysia* still contributed approximately 20 to 50% to the total diatom biovolume until October, exhibiting three smaller biomass peaks during this time (Fig. 2a). In 2011, spring bloom occurred on April 20th with *M. helysia* peaking at $5.5 * 10^7$ µm³ / ml, accounting for ~ 94% of total biovolume (Fig. 2a). Thus, total biovolume during spring bloom decreased strongly from $8.7 * 10^7$ µm³ / ml in 2009 to $5.6 * 10^7$ µm³ / ml in 2011. Moreover, with the increasing dominance of *M. helysia* during spring blooms (75% of total diatom biovolume in 2009 to 94% in 2011), species richness decreased from 80 taxa in 2009 to 59 taxa in 2011; the cell densities of taxa, which were abundant in 2009, also declined in the subsequent years.

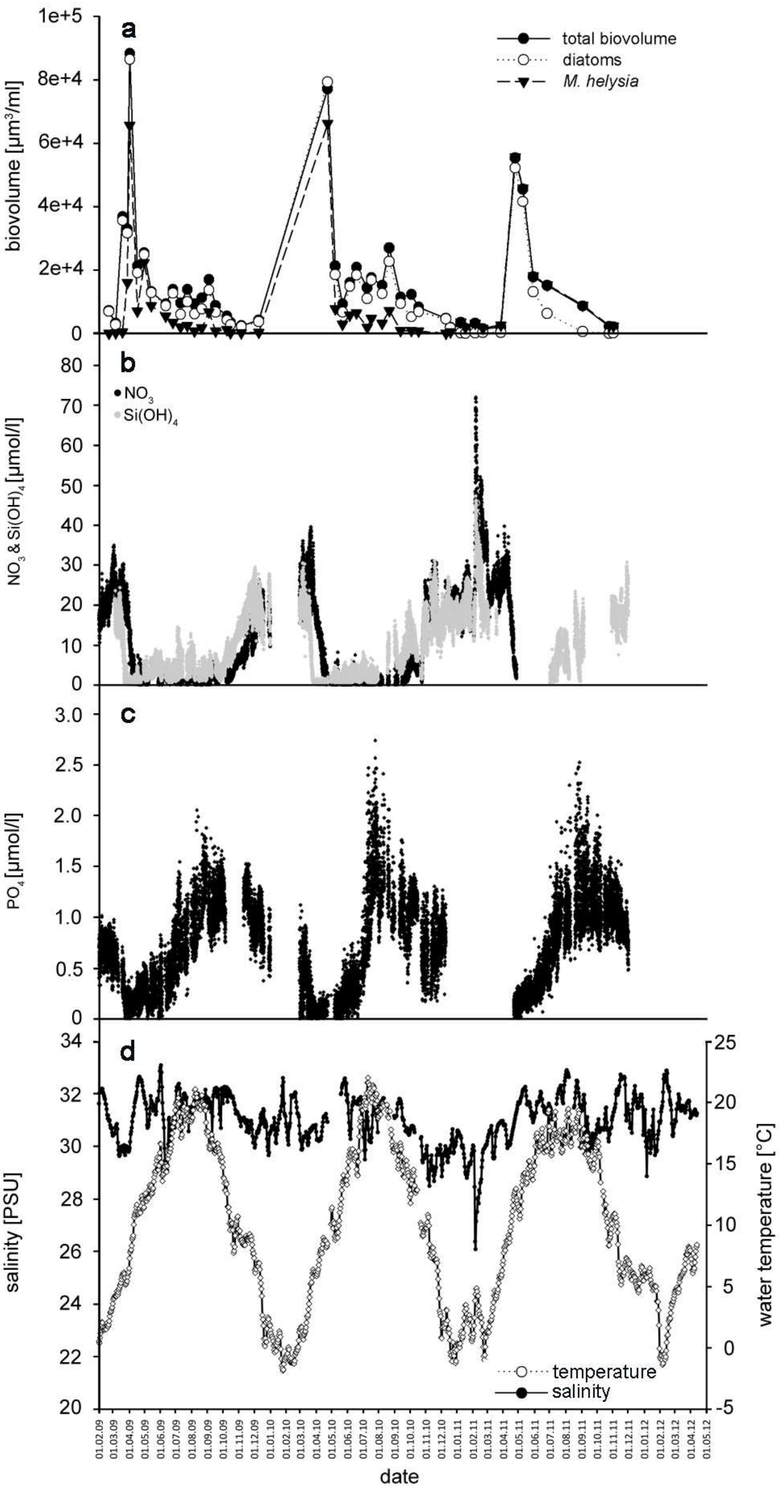


Fig. 2: Annual development from 2009 - 2012 of a) *M. helysia*, other diatoms and the total biovolume (b and c) dissolved nutrients (hourly data from the time-series station). Note that the short-term variations in the concentrations are due to tidal dynamics (Grunwald et al. 2010), and d) mean salinity and water temperature values of measurements during sampling from the time-series station of three consecutive years in the backbarrier tidal flats of Spiekeroog.

Statistical modelling (Table 2) revealed that high biovolumes of *M. helysia* were significantly ($p < 0.001$) coupled with low Si and P nutrient concentrations as well as low light availability, as it is often found during the spring season in the water column. The relative contribution of *M. helysia* to total biovolume was significantly ($p < 0.001$) higher during phases of low water temperature and low Si and N concentrations.

Table 2: Results of the most parsimonious General Linear Model for absolute biovolume (Model 1) and proportion (Model 2) of *M. helysia.* The most parsimonious model was selected by AIC, remaining factors in the model are given with parameter estimates, standard errors, and statistical significance. P-values significant at <0.05 are marked bold.

Model 1 with 107 degrees of freedom **Log biovolume *M. helysia***	**Estimate**	**Standard error**	**t-value**	**P-value**
Salinity	0.3792	0.1579	2.401	**0.0181**
log (PAR)	-0.1504	0.0666	-2.260	**0.0258**
log (PO_4)	-1.4159	0.2243	-6.312	**<0.0001**
log (Si)	-0.7916	0.1337	-5.920	**<0.0001**
Water temperature	0.0564	0.0381	1.482	0.1412
Wind velocity	0.1996	0.1082	1.845	0.0678
Model 2 with 109 degrees of freedom **% of *M. helysia* to total biovolume**				
Salinity	0.0924	0.0367	2.52	0.0132
log(NO_x)	-0.1202	0.0294	-4.086	**<0.0001**
log (Si)	-0.1188	0.0221	-5.377	**<0.0001**
Water temperature	-0.0454	0.0059	-7.662	**<0.0001**

Table 3: F- and P-values of separately conducted three-factor ANOVAs. P-values significant at <0.05 in bold and P-values <0.001 are indicated as such. Degrees of freedom for Si = 1; degrees of freedom for N = 2; degrees of freedom for Si x N = 2; degrees of freedom for Si x *M. helysia* = 2; degrees of freedom for Si x N x *M. helysia* = 2. Residual error for one-way ANOVAs = 31, residual error for three-way ANOVAs = 24.

Depending variable	**Si**		**N**		***M. helysia***		**Si x N**		**Si x *M. helysia***		**N x *M. helysia***		**Si x N x *M. helysia***	
	F-value	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**
Chlorophyte biovolume [µm³ / ml]	3.0928	0.0885	70.0489	**<0.001**	3.9280	0.0885	0.2959	0.7465	0.3516	0.5587	1.1464	0.3346	0.4446	0.6463
% of chlorophytes to total biovolume	1.1686	0.2472	9.9486	**<0.001**	53.4140	**<0.001**	0.4590	0.6374	0.1930	0.6643	3.6506	**0.0413**	2.4498	0.1076
Dinoflagellate biovolume [µm³ / ml]	0.5917	0.4476	29.6240	**<0.001**	0.2416	0.6265	0.1065	0.8994	3.3506	0.0796	0.1234	0.8845	0.3090	0.7371
% of dinoflagellates to total biovolume	0.1198	0.7315	4.9398	**0.0137**	10.0929	**0.0034**	0.1027	0.9028	1.4976	0.2329	0.3291	0.7228	0.9513	0.4003
Biovolumes of other diatoms [µm³ / ml]	9.2110	**0.0048**	1.9994	0.1525	1.7641	0.1938	0.0543	0.9473	0.0877	0.7696	1.2667	0.2999	3.1761	0.0597
% of other diatoms to total biovolume	1.1761	0.7359	13.5626	0.4026	60.2308	**0.0312**	0.1436	0.8670	0.1093	0.7438	1.6248	0.2179	2.6087	0.0944
Biovolume of *M. helysia* [µm³ / ml]	0.0720	0.8800	0.0060	0.8592	-		0.0240	0.965	-		-		-	
% *M. helysia* to total biovolume	0.2891	0.7009	0.2957	0.7443	-		0.1568	0.2848	-		-		-	
Total biovolume [µm³ / ml]	8.1182	**0.0077**	58.0508	**<0.001**	52.3024	**<0.001**	0.4049	0.6715	0.2723	0.6066	0.9506	0.4006	1.9898	0.1586
Evenness	0.0053	0.9425	10.5819	**<0.001**	7.0549	**0.0125**	0.5069	0.6089	0.9615	0.3370	5.2610	**0.0131**	0.3507	0.7079

3.4.2. EXPERIMENT

3.4.2.1. I. TREATMENT EFFECTS

At day 28, communities in all treatments showed a slightly positive growth (data not shown), indicating a transient state of the phytoplankton composition. Total biovolume was mainly dominated by the fast growing *D. salina,* which was the most successful species throughout the experiment: it contributed up to 65% to the total biovolume on day 28 (low Si | medium and high N -*M.* as well as high Si | high N -*M.*) (Fig. 3).

Total biovolume increased significantly with increasing N concentrations ($F_{2,31} = 58.05$, $P < 0.001$; Table 3) in both, -*M.* and +*M.* treatments, mainly due to significantly enhanced growth of *D. salina* ($F_{2,31} = 70.05$, $P < 0.001$) and dinoflagellates ($F_{2,31} = 29.62$, $P < 0.001$) in high N treatments. Both grew best in the high Si | high N –*M.* treatment, reaching a biovolume of $6.7 * 10^7$ µm³ / ml (65% of tot. biovol.) and $1.4 * 10^7$µm³ / ml (13% of tot. biovol.), respectively.

Likewise, total biovolume increased significantly with increasing Si concentrations ($F_{1,31} = 8.12$, $P = 0.008$) in both -*M.* and +*M* (Table 3), but to a lesser extent since only diatoms responded to silicate concentrations. Their summed biomass increased significantly with increasing Si supply ($F_{1,31} = 9.21$, $P = 0.005$), but not with different N levels ($F_{2,31} = 1.99$, $P = 0.153$; Table 3).

3.4.2.2. II. PERFORMANCE OF *M. HELYSIA*

M. helysia contributed on average 25% to the total biovolume, with a maximum of 29% (low Si | medium N, d 28). It was not significantly influenced by silicate ($F_{1,31} = 0.072$, $P = 0.88$) or nitrate ($F_{2,31} = 0.006$, $P = 0.859$; Table 3), but was able to produce comparable biovolumes in all treatments (Fig. 3). Addition of *M. helysia* led to an inherent increase in total biovolume (4.4% of total biovolume). However, *M. helysia* contributed up to 23% to total biovolume (high Si | high N) at the end of the experiment – which is almost equivalent to the summed biovolume of all other diatoms (Fig. 3), which decreased from 97% (d0) to 21% (d28, high Si | high N), emphasizing the increasing importance of *M. helysia* over time. Nonetheless, summed diatom biovolume decreased even more in the absence of *M. helysia* (Fig. 3).

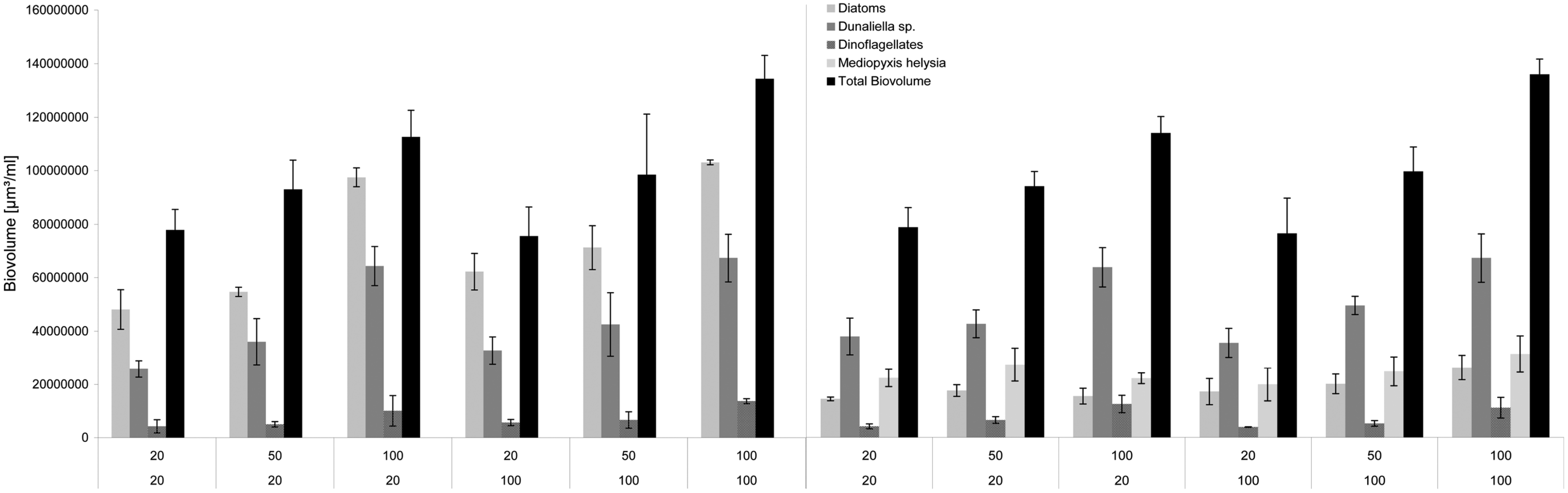


Fig.3: Biovolumes of the three different algae groups are displayed for each treatment (Si x N) in presence (a) and absence (b) of *M. helysia* at the end of the experiment (d28).

In contrast to the supported diatom growth, the presence of *M. helysia* significantly diminished percentages of dinoflagellates ($F_{1,31}$ = 10.09; P = 0.0034) and chlorophytes ($F_{1,31}$ = 53.41; P < 0.001) (Fig. 4). As the latter dominated the community in the high N treatments (Fig. 3), evenness was significantly reduced by N-supply ($F_{2,31}$ = 10.58; P < 0.001; Table 3 and Fig. 5). Here, interactive effects between presence of *M. helysia* and N treatments significantly altered percentages of *D. salina* ($F_{1,31}$ = 53.41; P < 0.001; Fig. 4). The proportion of *D. salina* increased with increasing N only in absence of *M. helysia*, whereas the presence of the NIS reduced the proportion of *D. salina* only at medium and high N (significant Tukey HSD posthoc test) (Fig. 4). Consequently, presence of *M. helysia* significantly enhanced evenness of the community structure ($F_{1,31}$ = 7.05; P = 0.013); Table 3 and Fig. 5) mainly by reducing dominance of *D. salina*. This effect was small in overall magnitude, however it is contrasting to field observations, where the dominance of *M. helysia* lowered the evenness of the phytoplankton community.

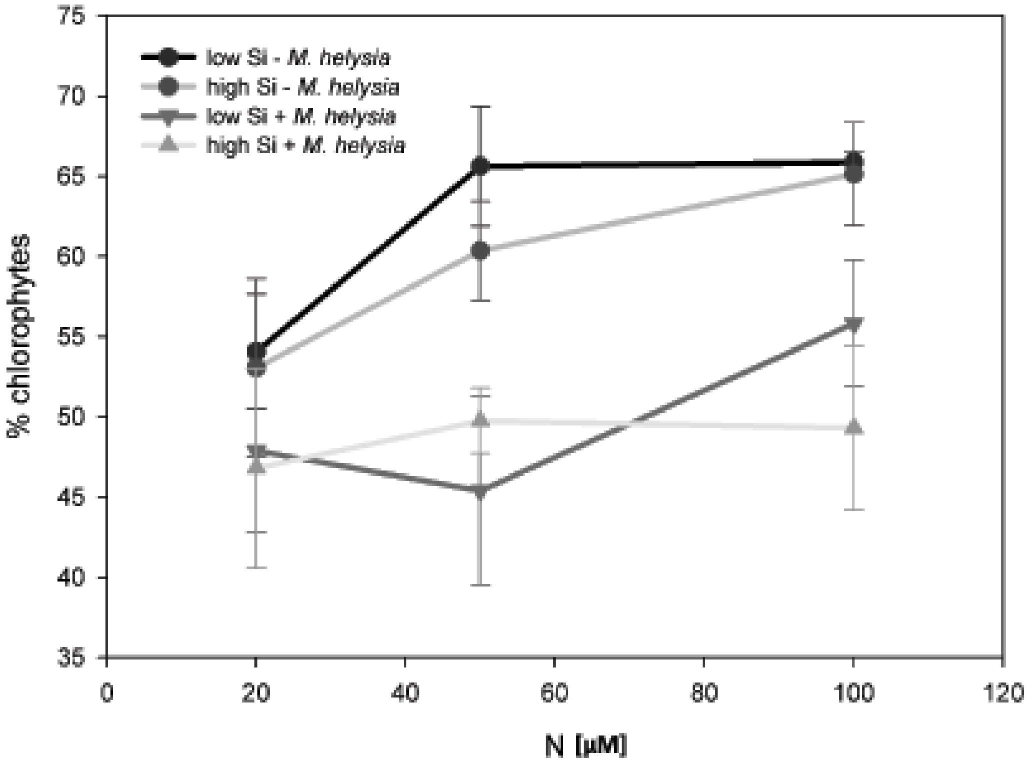


Fig. 4: Interactive effects of *M. helysia* and N concentrations on % chlorophytes.

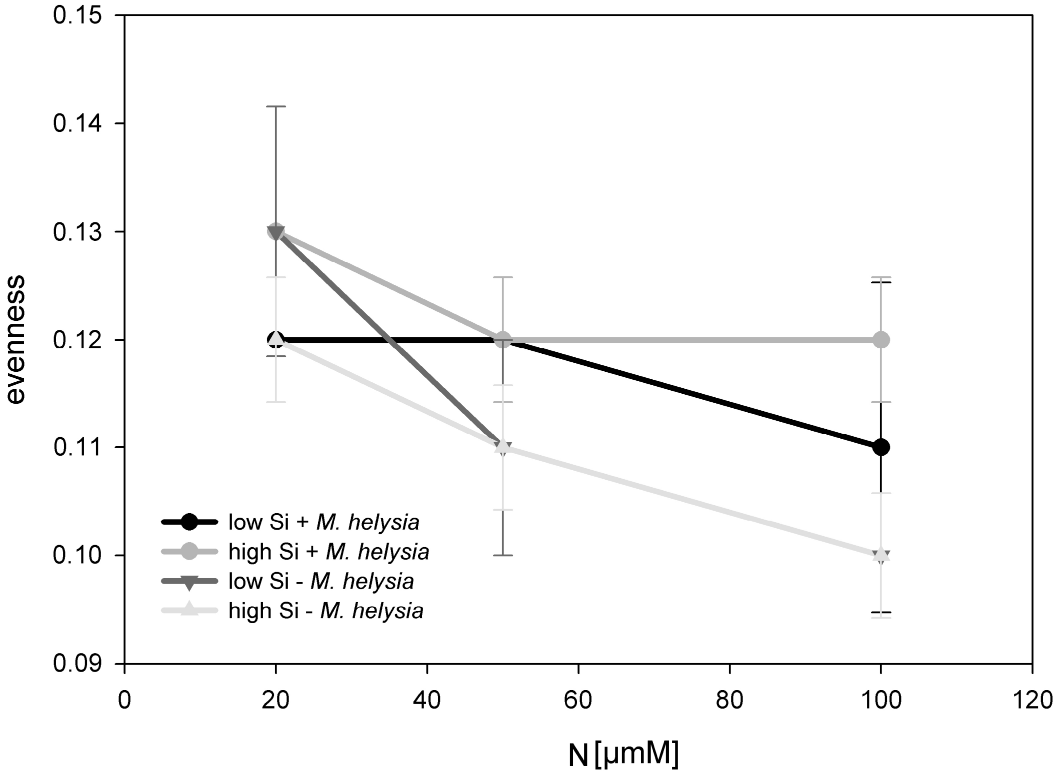


Fig. 5: Interactive effects of *M. helysia* and N concentrations on evenness.

3.5. DISCUSSION

Since its first detection in the backbarrier tidal flats of Spiekeroog in spring 2009, the non-indigenous diatom *M. helysia* bloomed each spring and was found in high abundance throughout all seasons for three consecutive years. Despite fluctuations in the environment of the Wadden Sea on temporal (tidal, seasonal, inter-annual) and spatial (horizontal and vertical) scales, this non-indigenous diatom persisted under this wide range of conditions. *M. helysia* occurred in high relative numbers (>90 − 20% of the total biovolume (Fig. 2)) throughout all seasons. At the same time, its peak abundance occurred in early spring, which was reflected by significant relations to low temperature observed in the statistical model. Contrary to the findings of Kraberg et al. (2011), who experimentally showed that *M. helysia* grows fastest at low salinities (27 PSU), my model revealed that high biovolumes of *M. helysia* significantly correlated with high salinities. Apart from temperature and salinity, the statistical modeling showed negative relationships between the abundance of *M. helysia* and measured low Si and N concentrations. Here, I assume that – rather than *M. helysia* being adapted to low nutrient concentrations – this pattern is caused by the species itself as its vast abundance depletes nutrient stocks during the spring bloom (see discussion below).

Corroborating the observed persistence under widely different environmental regimes, *M. helysia* was the only diatom in the experiment not affected by variations of Si and N concentrations. It was able to produce equal amounts of biomass in all treatments and was the dominant diatom species, although it was surpassed in the experiment by the chlorophyte *D. salina*. Whereas all other species were isolated from water samples of the backbarrier tidal flats of Spiekeroog, the fast growing *D. salina* (Sosik and Mitchell 1994) was the only species originating from a lab culture and thus well-adapted to experimental conditions. Since it was also the smallest species in my experimental assemblage, it might have rapidly taken up nutrients to their higher surface area to volume (Irwin et al. 2006), a trait often leading to dominance of certain species in culture experiments by monopolisation of available resources (Schmidtke et al. 2010). In the following discussion, I focus on the questions why *M. helysia* is successful, which effects it potentially has, and what I can learn about these effects from previous phytoplankton NIS.

3.5.1. POTENTIALLY ADVANTAGEOUS TRAITS OF *M. HELYSIA*

In the absence of species-specific trait information for all species in the experiment, my field and experimental data allow two preliminary conclusions: *M. helysia* showed high proportional representation in the natural phytoplankton in the Wadden Sea across and within years and remained the most dominant diatom under all nutrient conditions in the experiment. This suggests that this species has a broad realized niche, as it survives and dominates under varying nutrient regimes and physical conditions. Future measurements of resource requirements and growth efficiency in this and co-occurring species are needed to gain a mechanistic understanding of *M. helysia´s* success.

General traits of non-indigenous phytoplankton species facilitating the establishment in a receiving habitat are flexible resource requirements, inedibility, the ability to overcome unfavourable conditions by the formation of resting stages and a high reproduction rate (Edwards et al. 2001; Streftaris et al. 2005; Klein et al. 2010a; Klein et al. 2010b). Such traits seem to be present in *M. helysia*, which shows dominance under variable resource conditions and the formation of resting stages (Fig. 6). However, species traits, which enable an invader to overcome 'filter barriers' and establish populations, are species-specific (Nehring 1998b). My results are only indicative of such species-specific traits based on the occurrence of *M. helysia* in the field and its performance in the experiments.

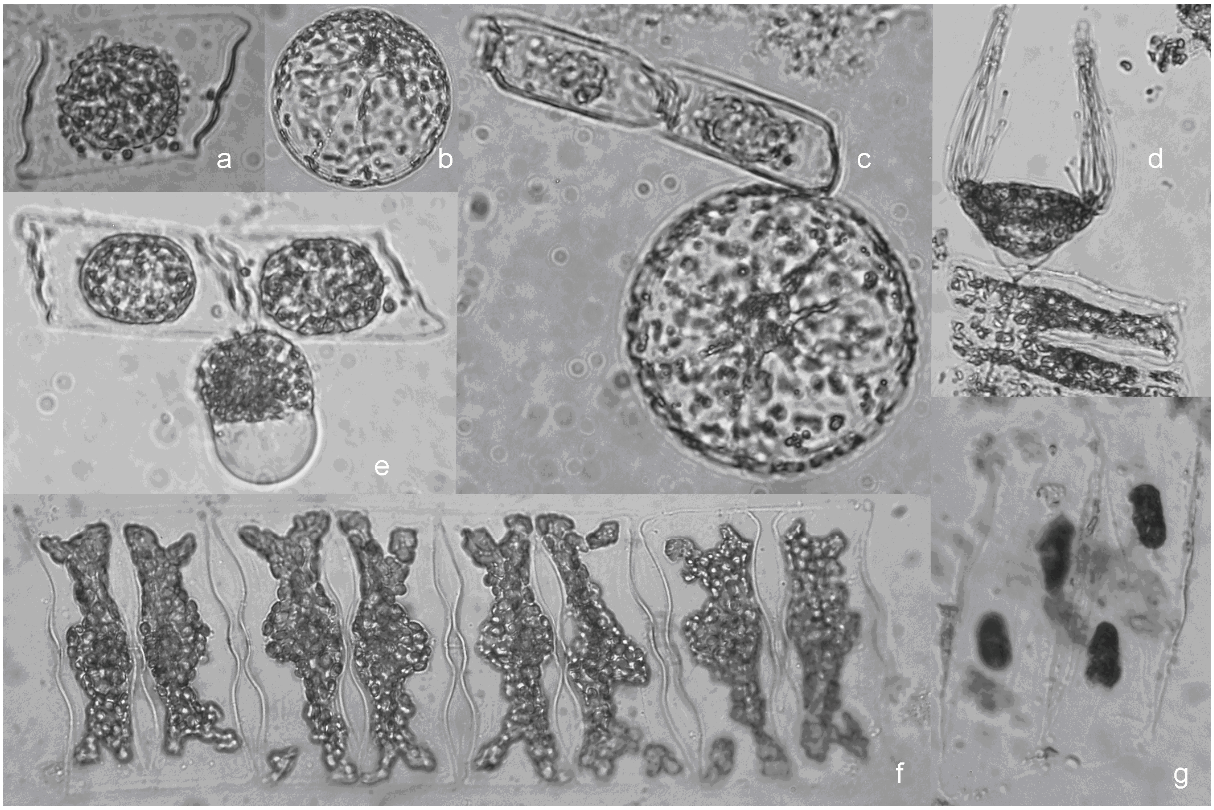


Fig. 6: Microphotographs of *M. helysia.*

a) Auxospore in *M. helysia* cell

b) Auxospore of *M. helysia*

c) Chain and auxospore of *M. helysia*

d) *Acineta* sp. attached to a *M. helysia* cell

e) Chain and auxospore of *M. helysia*

f) Chain of *M. helysia*

g) *Paulsenella vonstoschii* inside cells of *M. helysia*

In the case of *M. helysia* one advantage might be, similar to *Odontella sinensis* or *Coscinodiscus wailesii,* its large size. Species with large sizes are generally known to prevail in the variable environmental conditions of coastal ecosystems, which are characterized by nutrient pulsing (Irwin et al. 2006). Large species possess relatively high nutrient uptake capacities (Stolte and Riegman 1995) and growth rates under high nutrient, and high flow rates (Irwin et al. 2006; Key et al. 2010), enabling them to respond rapidly after periods of unfavourable conditions (Gómez and Souissi 2007; Klein et al. 2010a; Klein et al. 2010b; Villac and Kaczmarska 2011). Moreover, large species are less susceptible to ultraviolet radiation damage and suffer less photo-inhibition than small species, especially under low temperatures (Key et al. 2010). In consequence, these size-related traits may favour the establishment of *M. helysia* in the highly dynamic Wadden Sea, particularly during spring conditions. Following the results of a grazing experiment conducted by Loebl et al. (2012), a further potential advantage of the chain-forming *M. helysia* is its grazing resistance (Stolte and Riegman 1995) as it might not be ingested by important microzooplankton grazers in the Wadden Sea food web, which prefer phytoplankton cells < 100 µm (Harris 1982; Jansen 2008). However, I frequently found the ciliate *Acineta* sp. attached to *M. helysia* (Fig. 6d) as well as the parasitic dinoflagellate *Paulsenella vonstoschii* (Fig. 6g) inside cells of *M. helysia* in samples of the backbarrier tidal flats.

3.5.2. EFFECTS OF *M. HELYSIA* ON THE PHYTOPLANKTON COMMUNITY

The short time span since the first occurrence of *M. helysia* does not allow for a sufficient estimation of its potential permanent presence in the North Sea phytoplankton communities. Many invasions only have a temporal character because of irregular inoculation caused by currents and altered environmental conditions in the receiving ecosystem. Hence, non-indigenous species might exert only initial impacts on the ecosystem, before they become separated and less relevant (Nehring 1998b). Nevertheless, some potential effects can be derived from my results and from comparison to non-indigenous species such as *Odontella sinensis* or *Coscinodiscus wailesii* which were able to establish permanent populations that coexist with native phytoplankton populations (see below).

In my experiment, presence of *M. helysia* led to changes in the community structure. Independent of prevailing Si and N concentrations, growth of dinoflagellates was significantly diminished in presence of *M. helysia*. Furthermore, percentages of *D. salina* in high N treatments were decreased by the presence of *M. helysia*. *M. helysia* significantly enhanced evenness, particularly in high N treatments, where dominance of *D. salina* was most pronounced. The increase in evenness was significant despite a low overall effect size, which was due to low overall evenness in the experimental cultures. While growth of dinoflagellates and chlorophytes was diminished, growth of diatoms was supported by *M. helysia*. The nature of this beneficial effect of the invader on a related group of species is at present still elusive. One possibility is that *M. helysia* was able to use untapped resources (e.g. light via a highly adaptive photosystem (Key et al. 2010)) efficiently, as it also enhanced the overall biovolume in the experiment. The usage of resources which are not (efficiently) used by native species opens a new ecological niche for the non-indigenous species (empty niche hypothesis (Hufbauer and Torchin 2007)). Alternatively, the presence of *M. helysia* might inhibit underyielding in the phytoplankton community, as it decreases the dominance of the species monopolizing resources (Schmidtke et al. 2010), allowing sustained growth of other species.

It is difficult to determine whether the strong decline of the total biovolume, species richness and evenness during spring bloom events between 2009 and 2011 has been caused by the invasion of *M. helysia* or whether it has happened coincidentally (e.g. due to inter-annual variability in environmental conditions), as I do not have a baseline against which changes associated with invasion can be judged (Hufbauer and Torchin 2007; Villac and Kaczmarska 2011). However, it is likely that especially during spring blooms, *M. helysia´s* massive abundance depleted nutrient resources for other species, directly impacting the phytoplankton community in the Wadden Sea.

Further consequences depend on the response of key components in the food web. The potentially limited feeding of copepods on *M. helysia* could have important implications for the structure of pelagic food webs. If microzooplankter are excluded from the food web, it can become unstable and the food quality for higher trophic levels is likely to change (Rick and Duerselen 1995; Jansen 2008; Loebl et al. 2012). Moreover, with increasing size of prey, 'sloppy feeding' increases. In consequence, large debris particles sink to the ground, altering microbial decomposition (Jansen 2008). A shift towards large-sized phytoplankton species thus does not only affect higher trophic levels, but also carbon biogeochemistry and the biogenic carbon pump (Key et al. 2010). The validity of these predictions have to be tested in further observational and experimental studies.

3.5.3. WHAT CAN BE LEARNED FROM PREVIOUS INVASION EVENTS?

In 1889, *O. sinensis* (Ostenfeld 1908) was the first recorded non-indigenous diatom in the North Sea, which presumably was transported in ship ballast water from warm Chinese waters (Edwards et al. 2001; Gollasch 2006). *O. sinensis* has the ability to survive adverse conditions during ship transfer by the formation of resting stages when exposed to darkness, anoxia, fluctuating salinity, temperature and nutrient availability (Klein et al. 2010a; Villac and Kaczmarska 2011). Even though *O.*

sinensis is a very large diatom, which forms dense blooms with potentially adverse effects on higher trophic levels due to their inedibility for most copepods, no negative effects were identified. In contrast, the large centric diatom *C. wailesii* (Gran and Angst 1931), which was introduced in 1977, presumably also via ship ballast water or by oyster aquaculture from Japan and North America, caused sharp decreases of phyto- and zooplankton populations (Gollasch et al. 1999; Edwards et al. 2001). In the subsequent years it formed extensive blooms and was able to dominate phytoplankton biomass by outperforming indigenous species for resources (such as inorganic nitrogen), thus reducing phytoplankton biodiversity. In addition, it produced excess mucilage that even clogged fish nets (Boalch 1987; Rick and Duerselen 1995; Edwards et al. 2001; Villac and Kaczmarska 2011). Beneficial species traits for a successful invasion of *C. wailesii* are its ability to cover a larger ecological range than other diatoms with regard to physical and chemical environmental parameters such as temperature, salinity and nutrient regimes (Rick and Duerselen 1995) as well as its large body size of up to 175 – 500 µm in diameter (Edwards et al. 2001). All of these traits are shared by *M. helysia*. Nowadays, both *O. sinensis* and *C. wailesii* are, besides other non-indigenous phytoplankton species such as *A. glacialis*, *Rhizosolenia indica*, *Thalassiosira nordenskioeldii* and *Thalassiosira tealata,* frequent members of the phytoplankton community. Thus, *M. helysia* could become a permanent member of the phytoplankton community like *O. sinensis* without any known adverse effects. Alternatively, results of the experiment indicate that progressive dominance of *M. helysia* might result in quantitative changes of phytoplankton biomass and biodiversity (Rick and Duerselen 1995; Nehring 1998a; Nehring 1998b). Biological homogenization of habitats concomitant with loss of genetic diversity (Klein et al. 2010b) may be further effects of the establishment of *M. helysia* in the Wadden Sea. If *M. helysia* causes alternations in nutrient cycling, its presence might result in widely unknown ecological and economic consequences. Therefore, future investigation of population dynamics of *M. helysia* with focus on its temporal dynamics and underlying (a)biotic conditions is required. This should be amended by lab experiments concerning *M. helysia*´s development under different light and temperature conditions and its potential interactive effects with consumers and microbes.

The thorough study of invasion events and their effects on the recipient community is of great importance, as in the course of climate change the number of invasion events is likely to increase (Vitousek et al. 1997; Ward and Masters 2007). Warm-water species expand their spatial ranges towards higher latitudes at the expense of native species (Nehring 1998a; Stachowicz et al. 2002). Moreover, temporal shifts in species ranges, such as seasonal changes in life cycle events (e.g. earlier timing of phytoplankton blooms) may lead to a decoupling of predator-prey dynamics (trophic mismatch) (Chapin III et al. 2000; Daufresne et al. 2009; Hallegraeff 2010), opening up potential niches for non-indigenous species, thereby enhancing the invasion probability.

3.6. CONCLUSION

Since its first detection in the backbarrier tidal flats of Spiekeroog in spring 2009, the non-indigenous diatom *M. helysia* has bloomed every spring and was found in substantial abundance throughout three consecutive years. As it reached dominance in the natural and artificial community, it led to changes in the community composition irrespective of the chosen Si and N concentrations. *M. helysia* significantly diminished chlorophyte and dinoflagellate percentages, especially in high N treatments, in which the green algae dominated. In contrast, *M. helysia* supported diatom growth and total biovolume increased in this experiment. Moreover, *M. helysia* affected the evenness of the community: it enhanced evenness in the artificial community, while it lowered the evenness in the natural community. Thus, cautious extrapolation of the results indicates that a successful establishment of *M. helysia* might have far-reaching consequences not only for the phytoplankton community of the Wadden Sea, but also for higher trophic levels.

CHAPTER 3

4. *SPATIOTEMPORAL TURNOVER OF A PHYTOPLANKTON COMMUNITY IN A NATURAL COASTAL SYSTEM*

4.1. ABSTRACT

Metacommunity studies have mainly addressed the regional influence on local patterns and processes in enclosed systems. However, in open environments such as the marine realm, this research question has to be reversed, as there the role of local dynamics in ephemeral patch formations within the apparent homogeneous pelagic zone needs to be investigated. Phytoplankton community dynamics were monitored in a biweekly rhythm in the backbarrier tidal flats of Spiekeroog island (North Sea) over three consecutive years. I analyzed the relative fractions of temporal (tidal, seasonal, inter-annual) and spatial (horizontal and vertical) turnover of the phytoplankton community by assessing species-time-relationships versus species-area-relationships as well as beta diversity (Bray-Curtis dissimilarity). Changes in the phytoplankton community structure were further coupled to corresponding environmental variables gained from a nearby time-series station using non-metric multidimensional scaling and permutational multivariate analysis of variance. I observed significant temporal patchiness (different from random) and a weaker, non-significant spatial imprint on the distribution of phytoplankton taxa. Moreover, the compositional turnover in time was significantly associated with seasonal changes in environmental conditions, reflecting especially trends in P, N, photosynthetically active radiation and temperature. Combined, the results suggest that ephemeral patch formation and species sorting mechanisms play an important role for community assembly in this highly dynamic system.

4.2. INTRODUCTION

Phytoplankton communities drive ecosystem processes such as primary production and biogeochemical cycling as well as ecosystem services such as fisheries and the biological carbon pump (Falkowski et al. 1998). Phytoplankton communities exhibit a vast biodiversity, even though they compete for only a restricted number of limiting resources, including light and mineral nutrients. The high planktonic biodiversity in natural systems in the face of a limited number of potential competitive niches has been described as 'paradox of the plankton' (Hutchinson 1961; Sommer et al. 1993; Scheffer et al. 2003). This apparent paradox requires that the establishment of an equilibrium and thus competitive exclusion is prevented, despite the aquatic habitat being perceived as dynamic, well-mixed and open. Instead, elevated levels of biodiversity have to be sustained by various non-equilibrium mechanisms of coexistence (Watson et al. 2012).

On one hand, complex intrinsic phytoplankton community dynamics (e.g. resource partitioning, priority effects or among-species differences) may prevent competitive exclusion (Huisman and Weissing 1999; Scheffer et al. 2003). In models, many species can coexist if they show cyclic competition advantages for three or more limiting resources, resulting in chaotic population dynamics (Huisman and Weissing 1999). Recently, Feudel et al. (unpublished) showed that such supersaturation can be found across a broad variable space, including realistic abiotic conditions.

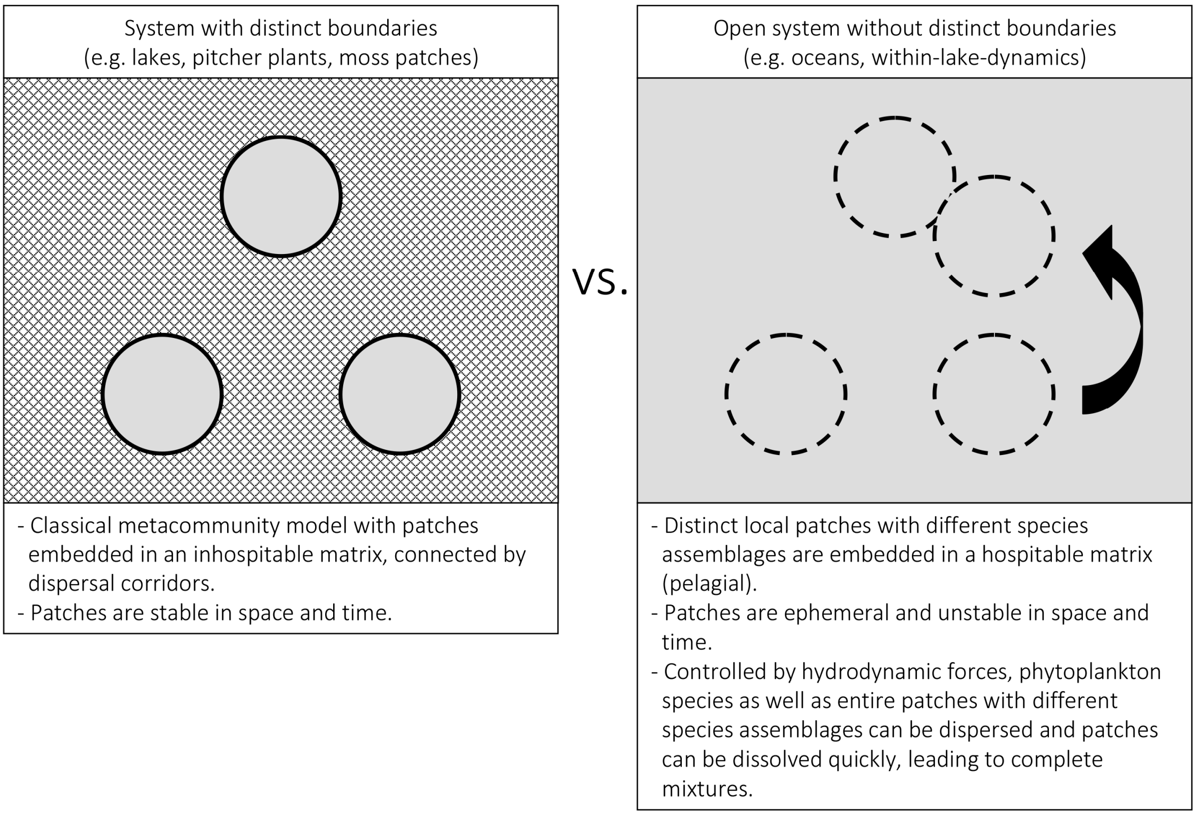
On the other hand, extrinsic factors may provide temporal, spatial or environmental heterogeneity, i.e., allowing the formation of distinct, but transient local patches. The idea that phytoplankton assemblages are not homogeneously distributed, but occur in ephemeral spatial patches shaped by imperfect mixing, dispersal and local dynamics is not new (Allen et al. 1977; Harris 1980; Ptacnik et al. 2010). Patch formation may be induced by turbulent flow (stochastic dispersal) caused by meso-scale eddies and fronts, which provide structure and often generate dispersal barriers in the apparent homogeneous pelagic zone (Scheffer et al. 2003; Berkley et al. 2010). The resulting spatiotemporally complex micropatches can contain distinct species assemblages of different biodiversity, as the relatively short generation times and high population growth rates allow small species to quickly track changes in the local environment (Allen et al. 1977; Korhonen et al. 2010; DeBie et al. 2012). Recent models have analyzed the occurrence of such patches at the mesoscale, their effects on plankton bloom formation and distinct species composition (Sandulescu et al. 2007; Bastine and Feudel 2010).

The question whether such fine spatial structures can contribute to the biodiversity of phytoplankton hinges upon the ratio of exchange between the patches and their longevity. If patches exist long enough to allow differential temporal development of the assemblage, but short enough to prevent competitive exclusion, then mixing between these patches potentially contributes to maintaining coexistence at larger temporal or spatial scales that encompass these heterogeneous patches (‘temporal and spatial storage effects’) (Chesson 2000; Davies et al. 2005; Gilbert et al. 2008; Berkley et al. 2010). The level of exchange between patches has to be limited at least partially as coexistence is more likely in ecosystems, where different patches are sparsely connected, than in systems with highly interconnected patches, which behave like a single homogenized patch (Scheffer et al. 2003; Van der Gucht et al. 2007; Berkley et al. 2010; Watson et al. 2012).

Thereby, the idea of fine-scale heterogeneity driving phytoplankton biodiversity and functioning becomes analogous to questions analyzed in the field of metacommunity ecology (Leibold et al. 2004). A metacommunity is generally defined as a set of local communities (patches) that are linked by dispersal. Metacommunity theory strives to understand how local within-patch interactions between species (e.g. competition) and dispersal between patches shape community composition and biodiversity at local and regional (metacommunity) scales. Although the question for phytoplankton in coastal seas is the same, there is an important discrepancy.

The metacommunity concept has triggered numerous empirical analyses, recently reviewed by Logue et al. (2011). Metacommunity dynamics have mainly been studied in ecosystems with patches showing distinct and permanent boundaries (Logue et al. 2011), such as rock pools (Vanschoenwinkel et al. 2007), lakes (Cottenie et al. 2003), pitcher plants (Kneitel and Miller 2003), moss patches (Gilbert et al. 1998) as well as in various microcosm experiments (Cadotte 2006). All these patchy habitats are embedded in a more or less inhabitable matrix (Fig. 1). Consequently, dispersal to adjacent patches is limited as organisms have to cross the inhabitable matrix by active movement or passive dispersal, sometimes enhanced by dispersal pathways (corridors, vectors). Metacommunity studies of enclosed systems have provided insight into the role of regional dynamics for local patterns and processes by explaining when and how local processes affecting community dynamics are overridden by regional processes linked to dispersal.

In contrast, the research question for open, marine environments has to be reversed to ask how important local (i.e. within-patch) dynamics, which potentially are highly ephemeral, may be in a larger context. In the apparent homogeneous marine pelagic zone, the whole matrix (water column) is habitable and the assumed distinct (but transient) micropatches are potentially well connected by hydrological processes, which induce great energy flow as well as material and propagule exchange (Fig. 1). Controlled by hydrodynamic forces, phytoplankton species as well as entire patches with different species assemblages can be dispersed and patches can be dissolved quickly, leading to complete mixtures. Hence, in open systems, we need to reverse the classic metacommunity view and look for local species pools inside a habitable region, which potentially maintain the high phytoplankton diversity (‘ephemeral islands within the ocean’). Watson et al. (2012) were among the first to realize this analogy and modelled the metapopulation dynamics of marine organisms under constant and stochastic connectivity, the latter reflecting the ephemeral nature of boundaries in the ocean.



System with distinct boundaries
(e.g. lakes, pitcher plants, moss patches)

- Classical metacommunity model with patches embedded in an inhospitable matrix, connected by dispersal corridors.
- Patches are stable in space and time.

Open system without distinct boundaries
(e.g. oceans, within-lake-dynamics)

- Distinct local patches with different species assemblages are embedded in a hospitable matrix (pelagial).
- Patches are ephemeral and unstable in space and time.
- Controlled by hydrodynamic forces, phytoplankton species as well as entire patches with different species assemblages can be dispersed and patches can be dissolved quickly, leading to complete mixtures.

Fig. 1: Metacommunity dynamics in a system with distinct boundaries vs. a open (marine) system

In order to detect such ‘ephemeral islands in the sea’, I analyzed the spatial and temporal changes of the phytoplankton community at relatively small spatial scales in the horizontal (< 10 km) and vertical (10 m depth) dimension in the backbarrier tidal flats of Spiekeroog island, North Sea. This area belongs to the Wadden Sea, an UNESCO world heritage site, and is strongly influenced by tides and tidal currents. The strong mixing and hydrodynamic displacement of organisms raises the question, how much spatial and temporal variation in species composition can occur. In order to answer this question, I investigated the tidal, seasonal and inter-annual turnover of phytoplankton species in the vertical and horizontal direction in a biweekly rhythm over three consecutive years. Thereby, I tested whether distinct local patch formations with specific community assemblages can be found in this open system, referring to two fundamental questions on spatially structured communities derived from Legendre *et al.* (2005): 1) Is there significant spatial and temporal patchiness (different from random) in the distribution of species? 2) To what extent does environmental variability explain the variation seen in the community composition? The latter point refers to the idea that rapid and divergent shifts in the community composition of dynamic multispecies assemblages can only be expected if the temporal turnover reflects the abiotic heterogeneity of the environment (Harris 1980; Pinckney et al. 1998; Ryabov et al. 2010).

Using three years of phytoplankton observations, I analyzed the temporal and spatial turnover in species composition of phytoplankton with three consecutive analyses. First, I characterized the absolute and relative extent of compositional turnover in time and space calculating the cumulative species richness found when increasing the temporal extent (increasing the number of samples at one station over time) and the spatial extent (increasing the number of sampling points during one sampling day) (only for data of 2009; see Table 1 for sampling setup). This approach is related to constructing species-time-area-relationships, STAR (Adler et al. 2005). Analyses of STAR integrate species-time-relationships (STR) and species-area-relationships (SAR). Comparing slopes of the STR and SAR allows detecting relative roles of temporal and spatial turnover in species composition STAR (Adler et al. 2005), informing us about the first question posed by Legendre et al. (2005). Comparing the turnover in the community composition in the vertical and horizontal dimension to the turnover in time allows characterizing the degree of patch formation and persistence. I used Permutational Multivariate Analysis of Variance Using Distance Matrices (*adonis* function in R) to assess horizontal and vertical differences in the community composition (only data of 2009).

In order to answer the second question posed by Legendre et al. (2005), I coupled changes in the phytoplankton community structure to corresponding environmental variables gained from a nearby monitoring station gathering time series data (Grunwald et al. 2007b). Comparing dissimilarity in environmental conditions and dissimilarity in species composition over time provides insight into seasonality-driven changes in the phytoplankton. Moreover, I used non-metric multidimensional scaling (NMDS) as a robust, unconstrained community ordination method to characterize species responses to changes in abiotic variables. I tested for significance of the environmental influence on the community composition using the *adonis* function in R.

4.3. MATERIAL & METHODS

Examining phytoplankton community composition from 2 **|** 2009 to 4 **|** 2012 (Table 1), I collected water samples in an approximately biweekly rhythm from pre-defined depths using a multi-water-sampler (HYDRO-BIOS, Germany) with 3.5 l-Niskin bottles, which then represented the scale of a local patch.

Time series data were compiled of single studies addressing different research questions in 2009 and 2010, while in 2011 and 2012 sampling was combined with routine maintenance work at the time-series station. Thus sampling regimes changed over time (Chapter 1; Table 1).

Table 1: Sampling strategy over three consecutive years (see Chapter 1). As my sampling design varied over the years, I subset the phytoplankton data, addressing different research questions. For example, in 2009 I focussed on the spatial and temporal community variation at three stations (high resolution), while in 2010, I addressed the tidal variation at one station (mid resolution). In 2011 and 2012, samples were collected independently from the tidal phase and only from surface water (low resolution), complementing information on the inter-annual variation.

Year	**Station 1**	**Station 2**	**Station 3**	**Tidal Phase**	**LOW**	**MID**	**HIGH**
					RESOLUTION		
2009	3 depths: surface, mid & bottom water	3 depths: surface, mid & bottom water	3 depths: surface, mid & bottom water	half tide, incoming flood	St. 1 surface water (2009-2012)	St. 1 3 depths in 2009 and 2 depths in 2010	St. 1 - 3 3 depths (2009)
2010	2 depths: surface & bottom water	-	-	half tide, incoming flood & slack water			
2011	surface water	-	-	different tidal phases			
2012	surface water	-	-	different tidal phases			

In 2009, I took samples always during half tide with incoming flood at three different points with three different depths each (station 1: 53°45’01.00’’ N, 007°40’16.30’’ E; station 2: 53°43’75.70’’ N, 007°42’42.80’’E; station 3: 53°43’38.90’’ N, 007°43’58.40’’ E) and vertically (1, 6, 9 resp. 10 m, which is approximately 1 m over ground) (Table 1). The stations were located along a current velocity gradient, where station 1 had highest current velocities. The mean current velocities were exemplarily measured over a 20 min period with an ADCP (RD Instruments Workhorse Sentinel 1200 kHz) at 7.7.2009: station 1: 0.969 m / s; station 2: 0.636 m / s; station 3: 0.660 m / s. In 2010, sampling effort was reduced only to the first station, but besides the sampling during half tide, samples were also taken during slack water to analyze the variation in the phytoplankton community originating from changing current velocities during different tidal phases. In contrast, in 2011 and 2012 surface water samples were collected at random points in the tidal cycle only at the first station (Table 1). I obtained corresponding hydrographical, meteorological and chemical variables from a permanently installed time-series station (Grunwald et al. 2007b) (Chapter 1).

4.3.1. STATISTICAL ANALYSES

All multivariate statistics were performed using the vegan package (Oksanen et al. 2012) in R (version 3.0.2) (R Development Core Team 2012). Species abundances were cubic-root-transformed and nutrient data were log-transformed prior to analysis. *Noctiluca scintillans* was excluded from the data due to the high error probability caused by the large cellular biovolume (~ $2.7 * 10^8$ µm^3) of this species.

Investigating the spatial variation in the phytoplankton community, I performed the *adonis* function, which is a permutational multivariate analysis of variance using distance matrices. For each month in 2009, the percentages of variance explained (R^2) were calculated for the vertical and horizontal direction. Only time points with samples from 3 stations with 3 depths each were included in the analyses (May - November) (Table 2).

The cumulative number of taxa was plotted against the number of samples taken spatially and temporally. For data of 2009, for the STR curve samples of the 1st station (1 m depth) were randomly accumulated over time (100 permutations) (Fig. 2a). For the SAR curve, samples were randomly accumulated from station 1 - 3 over all three depths for each sampling in 2009, where 3 x 3 samples were available (11 times). I only present the SAR curves with the lowest and highest slopes z, which were calculated with an Arrhenius model ($k * area^z$; *fitspecaccum* function). Thereby, k is the expected number of species in a unit area. For the STR curve from 2009 to 2012, samples of the 1st station (1 m depth) were randomly accumulated over time (100 permutations) (Fig. 2b).

I visualized the relationship between environmental factors and the phytoplankton community variation by calculating Bray-Curtis species dissimilarities together with Euclidean distances of environmental variables for each sampling point in relation to temporal distance. A proxy for environmental distance was calculated as smooth fit (GAM; $R^2 = 0.23$, $p < 0.001$) for the Euclidean distances of the environmental factors (photosynthetically active radiation, wind velocity, water temperature, and concentrations of dissolved N, Si and P) (only for 2009 (Fig.3a)). The environmental distances are dimensionless, since the square-roots of the sum of distances of various variables are all having different units. In addition, I addressed inter-annual variations in the community turnover by visualizing the Bray-Curtis dissimilarity from 2009 - 2012, complemented by a GAM-model for the temporal variation (Fig. 3b). Furthermore, the influence of the environmental variation on the phytoplankton community of surface water samples of three different stations in 2009 was assessed using NMDS (Fig. 4a). In a second NMDS analysis, I addressed the inter-annual variation in the phytoplankton community in relation to environmental variation. Therefore, I used data only from the first sampling station (1 m depth) and mid taxonomic resolution (from 2009 to 2012). I included dissolved P, Si and N [µmol / l] as well as wind speed [m / s], photosynthetically active radiation (PAR [W / m^2]) and water temperature [°C]. I fitted the environmental variables using the *envfit* function (Fig. 4b).

4.4. RESULTS

The monthly *adonis* analysis for the horizontal and vertical dimension of the community variation in 2009 did only reveal a significant horizontal structure at a single sampling day (May 2009) (Table 2). As the *adonis* analysis is based on transformed biovolume data, it is mainly affected by dominant species, indicating that the dominance structure of the phytoplankton community was not spatially segregated during the most of the year.

Table 2: Results of the monthly *adonis* analysis for the horizontal and vertical dimension.

Month	**Spatial direction**	**R^2**	**P**
May	vertical	0.14	0.24
	horizontal	0.19	**0.04***
June	vertical	0.02	0.98
	horizontal	0.05	0.60
July	vertical	0.04	0.81
	horizontal	0.03	0.88
August	vertical	0.07	0.31
	horizontal	0.04	0.73
September	vertical	0.03	0.98
	horizontal	0.06	0.50
October	vertical	0.04	0.92
	horizontal	0.07	0.31
November	vertical	0.11	0.58
	horizontal	0.15	0.26

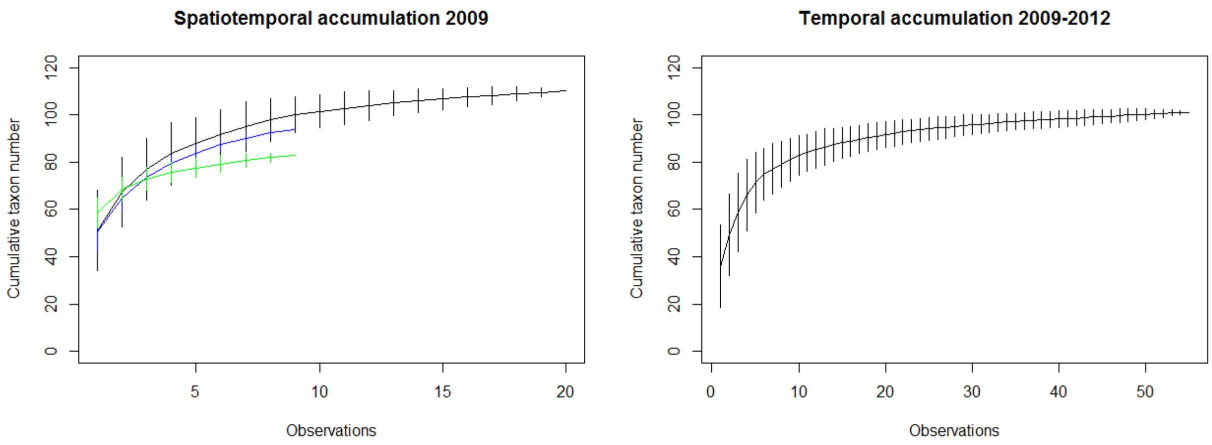


Fig. 2a: Cumulative species numbers 2009 (blue line = SAR curve of the sampling date with the highest slope z; green line = SAR curve of the sampling date with the lowest slope z, black line = STR curve). Fig. 2b: STR curve (2009 - 2012), low taxonomic resolution.

While the *adonis* analysis is mainly affected by the dominance structure of the community, the SAR curves reflect the number of species found at different stations. The addition of samples in space increased the number of species observed. On average, 50 species were found per sample. The mean (100 permutations) slopes z of the SAR curves ranged from 0.15 to 0.27, the slope of the STR was 0.16 ± 0.025, and the expected number of species k was 54.24 ± 5.26 (high resolution data of 2009; Fig. 2a). While the SAR curves were steeper at the very beginning, the temporal turnover ultimately exceeded the spatial turnover. The inter-annual turnover was reflected by slope z of the STR for 2009 - 2012 (low resolution) was 0.16 ± 0.026 and the expected number of species k was 54.24 ± 5.26 (Fig. 2b).

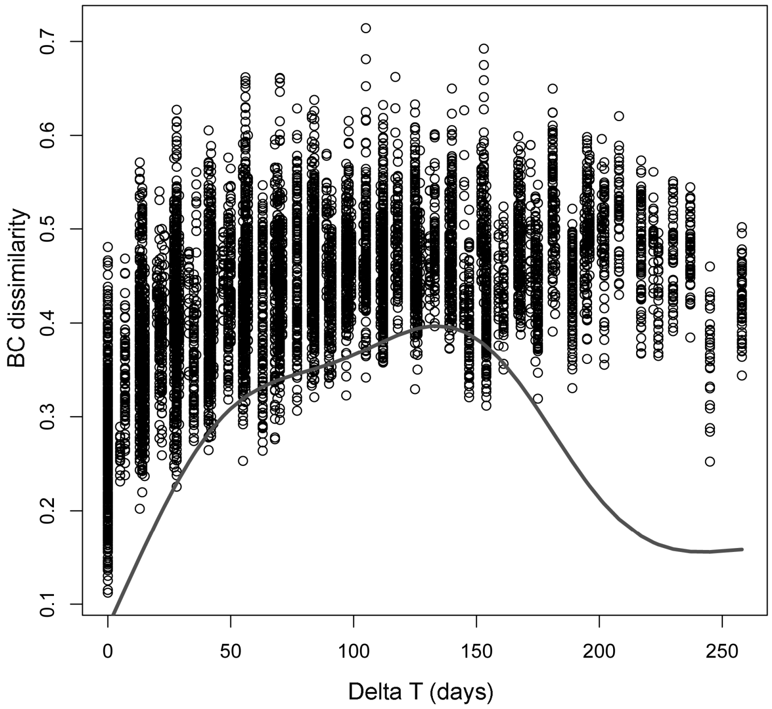


Fig. 3a: Bray-Curtis dissimilarities in relation to environmental distances over time (2009).

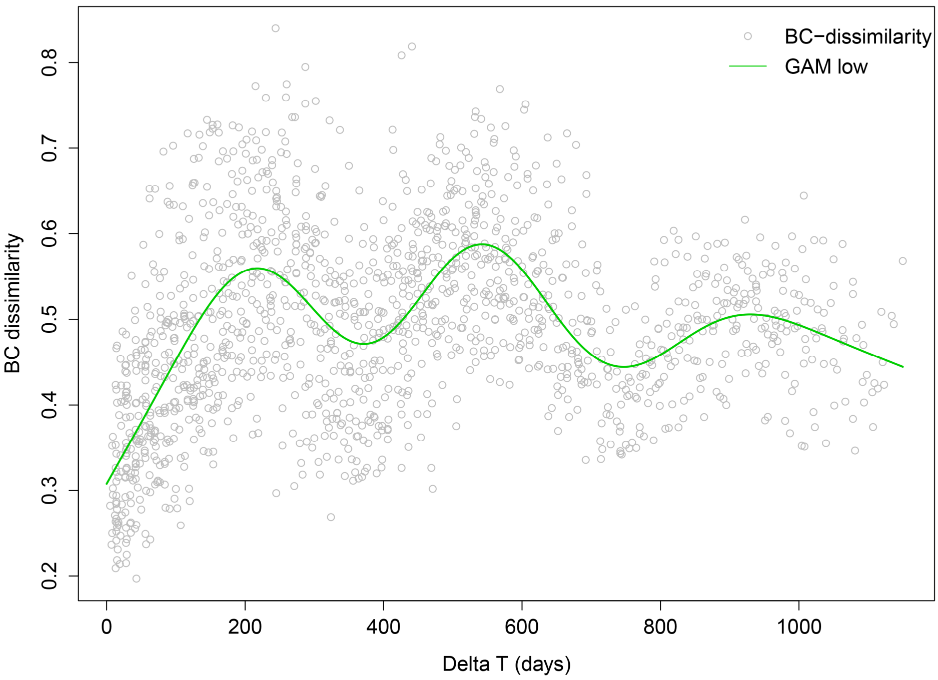


Fig. 3b: Inter-annual species turnover (low resolution 2009 - 2012): Bray-Curtis dissimilarities over the time course of three consecutive years. The GAM model represents the temporal turnover. Note the declining slope towards 2012 despite the adjusted sampling and taxonomic resolution.

The strong temporal turnover (Fig. 3a) became visible in the hump-shaped curve of dissimilarity on temporal distance with a peak at around 150 days (Fig. 3a). This pattern showed that species composition diverged with distance in time, but then converged again later in the year. However, for samples > 150 days apart, abiotic conditions converged more rapidly than the species composition, indicating that similar abiotic environments in spring and autumn do not result in similar phytoplankton communities. In each year, communities were thus more similar to each other in spring and autumn than to the community in summer. The seasonal development of the plankton community was reflected by the almost parallel curve of environmental distance between the samples: dissimilarity in species composition peaked at maximum dissimilarity in environmental conditions. Average Bray-Curtis dissimilarity within a single day among different depths was 0.34, indicating that patches showed a 66% overlap in species composition only.

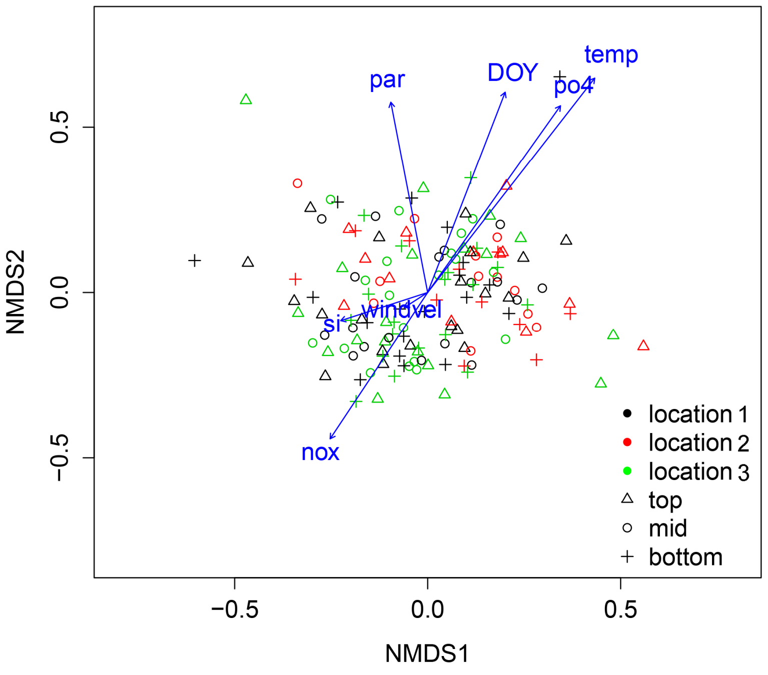


Fig. 4a: NMDS showing the phytoplankton community in relation to environmental variation. Temperature, P, PAR, and N largely influenced the community, while a clear spatial trend is not detectable (2009).

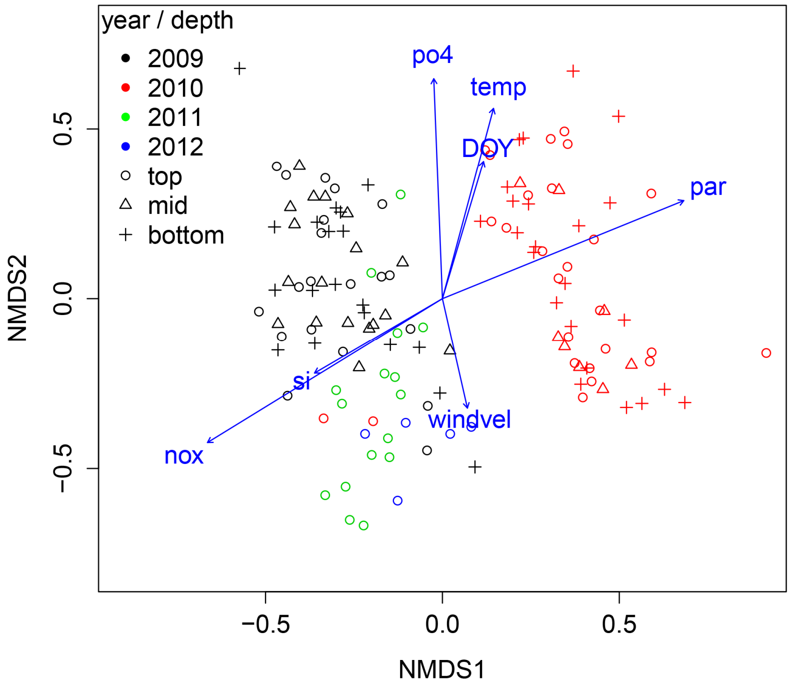


Fig. 4b: Inter-annual phytoplankton community composition in relation to environmental variation. Temperature, P, PAR, and N were also the most influential variables in 2010 - 2012.

The NMDS of high resolution data from 2009 (Fig. 4a) revealed that most influential environmental variables were temperature, P, N, and PAR. Species belonging to different stations or depths did not show a distinct pattern, suggesting that the seasonal variation of environmental factors overrode the spatial influence on the phytoplankton community structure. The NMDS of mid resolution data from 2009 - 2012 indicated that the same environmental variables as in 2009 were most influential also over the period of three years (Fig. 4b). The communities of 2009, 2011 and 2012 were relatively separated, while strong differences between 2010 and the other years became visible, which can probably be attributed to changes in the personnel in 2010. The permutational multivariate analysis of variance showed that the phytoplankton community was significantly ($P < 0.05$) influenced by P, Si, N and water temperature, while wind velocity was not significant, confirming the results of the NMDS (Fig. 4b; Table 3).

Table 3: Results of permutational multivariate analysis of variance showing significant influences of environmental variation on the phytoplankton community (2009 - 2012). In total, 45 % of the community variation can be attributed to the environmental variation. Most influential variables were P, N, Si, and temperature.

Environmental variable	R^2	P
PO_4	0.11	< 0.001***
SiO_2	0.16	< 0.001***
NO_3	0.13	< 0.001***
temperature	0.06	0.04*
wind velocity	0.02	< 0.78

4.5. DISCUSSION

My analyses shed light on two fundamental questions of the spatiotemporal turnover of the phytoplankton community derived from Legendre et al. (2005): first, I observed significant temporal patchiness (different from random) and an evident spatial separation in species number, while the dominance structure of phytoplankton showed a non-significant spatial imprint during the most of the year. Second, environmental variability explained a significant part of the variation seen in the community composition. These observations represent a first step towards a more process-oriented understanding of ephemeral patch dynamics in such a highly turbulent system as the Wadden Sea. Before discussing the future directions based on these results, I first discuss the context of these results in more detail.

4.5.1. EXISTENCE OF SPATIAL AND TEMPORAL TURNOVER

Our sampling was designed in a way that each additional sample accumulated in time or space added the same volume (3.5 l) - thus, the sampling effort increased on the same scale. Yet, I expected a higher STR than SAR slope, since tidal and other currents move water quickly in this system (average current velocity 0.7 - 1.3 m * s^{-1} at station 1 (Bartholomä and Flemming 1993)), whereas the temporal distance reflected multiple generations in phytoplankton development. While the SAR curves were steeper at the very beginning, the temporal turnover ultimately exceeded the spatial turnover, confirming my expectation. The slopes of the SAR varied among sampling dates, indicating temporal fluctuations in the degree of spatial patch formation.

Although significant horizontal or vertical differences in the dominance structure in the phytoplankton community could not be found during the most of the year, Bray-Curtis taxon dissimilarities showed that communities of the nine stations sampled in 2009 were spatially structured to a certain degree. As samples were accumulated randomly in the SAR, it can be assumed that horizontal changes in the species number of the phytoplankton community at the range of kilometers equalled those found over few meters in the vertical dimension, which coincides with results of Seliger *et al.* (1981), Hugueny (2007), Harris & Smith (1977).

The comparably steep STR reflects the short generation times of the phytoplankton species: the time range of this study corresponds to a few hundred phytoplankton generations. It is important to note this when comparing the STR slope with those of e.g. terrestrial plants, which only have one generation a year (Adler and Lauenroth 2003).

The phytoplankton community was temporally structured at different scales. First, differences in the inter-annual turnover of the phytoplankton community could be detected. The slope of the general additive model representing the temporal turnover declined towards 2012, indicating that the community was less dissimilar in 2011 and 2012. The reason for this result remains elusive at present but future monitoring may show if this decline was associated with the recent establishment of the invasive diatom *M. helysia*, which is known to reduce the evenness of the phytoplankton community in this region (Chapter 2). Moreover, the community composition in 2010 differed from the other years. This can probably be attributed to a change in the analyst, rather than to actual changes in the community − unfortunately, the community variation due to the between-analyst differences in the identification of species could not be eliminated completely (Wiltshire and Dürselen 2004a). Second, a strong intra-annual turnover became visible in the hump-shaped curve of Bray-Curtis dissimilarity on temporal distance with a peak at around 150 days. This pattern showed that species composition diverged with temporal distance but then converged again later in the year.

4.5.2. ENVIRONMENTAL DRIVERS OF VARIABILITY

The compositional turnover in time was significantly associated with seasonal changes in environmental conditions, reflecting particularly trends in P, N, PAR and temperature. Phytoplankton quickly tracks changing environmental conditions and can therefore be seen as a 'living transfer function' of the environment (Allen 1977): the diatom spring bloom, for example, was triggered by day length or light intensity (Edwards and Richardson 2004) and it ended when nutrients were depleted. During the summer months, when water temperature is highest, dinoflagellates prevail, while in autumn usually smaller diatom blooms occured (Chapter 1). N and Si showed highest values during the winter month, when primary production was low and microbial remineralization processes prevailed (Grunwald et al. 2010).

4.5.3. PATCH DYNAMICS AND PHYTOPLANKTON COEXISTENCE IN COASTAL SYSTEMS

I found indications for spatial and temporal patchiness in the distribution of the coastal phytoplankton community. Micropatchiness in phytoplankton has already been documented in an observational study by Owen (1989), in which he reported the occurrence of compositional differences on small spatial scales and within a few days to weeks. Plankton patchiness may be created, sustained or destroyed by mechanical processes (such as heterogeneity, asymmetry and temporal variability in connectivity patterns), biological processes and the interactions of both (Owen 1989; Watson et al. 2011a; Watson et al. 2011b). Community characteristics are altered significantly where the living space fluctuates or becomes partitioned: tidal currents provide structure and often create barriers to dispersal (Owen 1989; Watson et al. 2010). This may lead species to transiently partition a spatial niche, creating a spatial storage effect, i.e. organisms may coexist in elevated numbers and diversity (Chesson 2000; Berkley et al. 2010). Further, such hydrographic structures may create ephemeral patches of different environmental conditions, which lead to divergent trajectories of community development (Bastine and Feudel 2010). The fundamental question for coexistence then is: does the formation of micropatches allow ephemeral species sorting or patch dynamics and result in lower rates of competitive exclusions? Patch dynamics and species sorting are two major concepts of metacommunity organisation, which differ in prevalence of (passive) dispersal rates (Holyoak 2005). Species sorting is driven by the strong seasonal environmental variation and ultimately leads to a conformance between environmental gradients and community composition, if species are able to disperse to the most suitable patch. Patch dynamics describes colonization-competition trade-offs when dispersal is limited among the patches.

Thus, if patches differ in their conditions (promoted by the mixing of different water masses) and phytoplankton composition quickly tracks these differences (species sorting), the competitive replacement of species differs between patches and more species coexist if the patches mix again. In this case, merging a hydrodynamic model predicting patch formation with a metacommunity approach predicting species coexistence via species sorting offers the potential to understand the spatial and temporal dynamics of phytoplankton community composition and thus biodiversity in this open system.

CHAPTER 4

5. *REMOVING BENTHIC DIATOMS FROM SEDIMENT PARTICLES FOR SPECIES COMPOSITION ANALYSIS*

5.1. ABSTRACT

Microphytobenthos (MPB) is dominated by diatoms, which act as ecosystem engineers and form as important primary producers the basis of the benthic food web. Understanding and predicting ecosystem processes accomplished by MPB requires an exact quantitative analysis of both epipelic and epipsammic diatoms. For microscopic analyses, the separation of diatoms from sediment particles is necessary. Various methods have been established in the past, mostly generating incomparable results, as they usually gain only selective subsets of the benthic diatoms, whereas the percentage of diatoms that could not be detached from the sediment or was destroyed during preparation remained unexamined. Additionally, most detachment methods work better for epipelic than for epipsammic diatoms, leading to biased quantification results.

In my study I evaluated three different treatments for the detachment of benthic diatoms from sediment particles. I quantitatively controlled treatment effects on the number of detached diatoms in the supernatant to develop a standardized method. I applied an ultrasonication probe at six defined duration times (ranging from 15 to 90s) with and without the addition of different concentrations of the surface-active detergent Polysorbat 20. Ultrasonciation for 60s significantly increased the dislodgement rate: 60% more detached diatoms were found in the supernatant of the sonicated sample than in the control. The detergent did not improve the dislodgement rates significantly, but reduced the ultrasonication time to 45 s Thus, I

recommend a detachment using ultrasonication for 45 s with addition of the detergend (0.025% final).

5.2. INTRODUCTION

Benthic diatoms are important primary producers and form the basis of benthic food webs in marine and freshwater habitats. The so-called microphytobenthos (MPB) is distributed with abundances between 10^5 cells / cm^3 to 10^7 cells / cm^3 within the upper 5 to 10 mm of the sediment in the euphotic zones, depending on location, season, and sediment properties (MacIntyre et al. 1996). In temperate regions it consists mainly of pennate benthic diatoms (Baillie 1987; MacIntyre et al. 1996; Mitbavkar and Anil 2002; Herlory et al. 2004). Diatoms are subdivided in motile, non-attached species and immobile, attached species, which are characterized with respect to the substrate they are attached on, into epipsammic (sand), epipelic (silt), epilithic (stones) and epiphytic (plants) species.

MPB is subjected to a perpetual resuspension into the water column and subsequent deposition driven by winds, tides and anthropogenic activity (Baillie and Welsh 1980; MacIntyre et al. 1996; Mitbavkar and Anil 2002). During suspension of the sediment, the associated MPB contributes a considerable fraction to the pelagic primary production, even though light penetration is reduced with increasing turbidity caused by stirred-up sediment particles (DeJonge and Vanbeusekom 1995; MacIntyre et al. 1996; Lucas et al. 2001), see Chapter 2).

Conversely, pelagic diatoms or their resting stages can also be a part of the MPB, when deposited in the benthos (Marcus and Boero 1998; Mitbavkar and Anil 2002).

Benthic diatoms also modulate nutrient fluxes across the sediment-water interface, playing a significant role in biogeochemical coupling (Nilsson and Sundback 1991; Marcus and Boero 1998). Through exuding extracellular polymeric substances (EPS), biofilms are built up, which buffer the cells during frequent physical stresses and additionally enhance the sediment stability, prevent erosion and alter the morphodynamics in tidal areas (Decho 2000). For the understanding of ecosystem functions provided by benthic diatoms, an exact quantitative analysis of the species composition is required.

MPB is often examined indirectly by using chlorophyll a as a proxy (Cahoon 1990; Delgado et al. 1991b; Cahoon et al. 1994; Yallop et al. 1994; MacIntyre et al. 1996; Nelson et al. 1999; Reiss et al. 2007). The usage of high-performance liquid chromatography (HPLC) provides additional analysis of other phytopigments such as fucoxanthin or phaeophorbides which can be used as taxonomic markers, attaining more detailed information of the MPB´s species composition (Lucas and Holligan 1999; Reiss et al. 2007). Nevertheless, the origin (MPB, sedimented phyto- and tychoplankton or macroalgae detritus) of these phytopigments remains unclear until the species composition is microscopically analyzed (Lucas and Holligan 1999). Scanning electron microscopy (SEM) provides pictures of the biofilm on the sediment surface, but is inappropriate for routine quantitative analysis as it is time-consuming and expensive. Further, obscuring effects of the sediment particles may occur (Delgado et al. 1991a; Herlory et al. 2004).

When quantitatively examining MPB with a light microscope, such obscuring effects of sediment particles need to be avoided. Thus, before the quantitative enumeration of benthic diatoms, the sample has to be gently (preventing cell breakage) mixed in order to detach cells entirely from the sediment particles. Therefore, various methods have been established, all based on different mechanisms: in some studies, no distinction was made between epipelic and epipsammic species (Uhlig 1968; DeJonge 1985; Delgado et al. 1991b; Saburova and Polikarpov 2003) and only suspended cells were examined, thus potentially biasing results compared to the entire community structure.

5.2.1. REMOVAL OF NON-ATTACHED DIATOMS

Many methods focus only on the separation of motile, non-attached (epipelic) species or already detached epipsammic species from sediment particles (Round 1965). The lens tissue technique, for example, is based on the active migration of positively phototactic, epipelic algae into lens tissue lying on the sediment (Eaton and Moss 1966; Moss 1977; Baillie and Welsh 1980; Colijn and Dijkema 1981; Delgado et al. 1991a; Yallop et al. 1994; MacIntyre et al. 1996). The living flora investigated by this method includes only part of the living species present, whereby positive phototactic, motile species are favored, which are likely to dominate biomass and productivity on intertidal sediments (MacIntyre et al. 1996). This technique provides no information on the subsurface distribution, as the exact three-dimensional volume of the sediment sampled is not known. Further, cells, which could not be suspended (mainly epipsammic diatoms) or broke during preparation were not counted. Beyond that, this procedure requires a long time (> one day), while the outcome depends on the size and quality of the lens tissue as well as on the harvesting time due to the diurnal migration of the diatoms.

In a further method, samples are shaken repeatedly in distilled or filtered seawater followed by the subsequent removal of the supernatant which then contains (a fraction of) epipelic algae (Yallop et al. 1994; Agatz et al. 1999). The easily detached epipelic cells can be counted directly in the supernatant (Eaton and Moss 1966; Hickman 1969; DeJonge 1985), whereas epipsammic diatoms remain unexamined. Thus, this method is inappropriate for studies dealing with the entire MPB community. Additionally, DeJonge (1985) and Delgado and DeJonge (1991a) used a cell homogenizer respectively a laboratory mixer to destroy sediment aggregates and to remove diatoms from their substratum. Determination of abundance, morphology, and taxonomy has been improved by the usage of epifluorescence microscopy and centrifugation ensuring samples to obtain appropriate cell densities (Delgado et al. 1991a; MacIntyre et al. 1996). However, total abundances cannot be estimated, since obscuring effects of sediment particles prevent the counting of diatoms attached to the reverse side or in depressions of sand grains (Eaton and Moss 1966).

5.2.2. REMOVAL OF ATTACHED DIATOMS

Epipsammic algae, mostly diatoms, produce more than half of the total chlorophyll a in some habitats (Nelson et al. 1999; Reiss et al. 2007), making their investigation very important. They usually occupy depressions in the grain surfaces since abrasion probably removes those attached to more prominent places (Moss 1977). Therefore, removing epipsammic diatoms from sediment particles requires a higher effort due to their severe adhesion (Round 1965). Epipsammic diatoms can be removed by boiling the sediment with attached diatoms in either hydrogen peroxide or acid (Round 1965). However, the amount of residual diatoms or broken frustules cannot be quantified.

Moreover, no distinction can be made between living cells with an intact protoplasm and distinct chloroplasts, and dead cells.

In another method, removal of epipsammic diatoms is mediated by ultrasonication (Hickman 1969; Voltolina 1991; Agatz et al. 1999). Ultrasonic waves generate cavitation in liquids (www.hielscher.com; date of access: 10. 3. 2014), leading to high shear forces. Bindings of epipsammic diatoms are thereby broken up by mechanical stress as well as by the liquid, which is pressed between the particles. Ultrasound may, however, also cause cell disruption (lysis) or disintegration (www.hielscher.com; date of access: 10. 3. 2014). The yield of removed epipsammic diatoms as well as the degree of damage of diatom frustules thereby strongly depends on the frequencies, time and temperature of ultrasonication with an ultrasonic bath or sonotrode. The output frequency should be in the 75 to 100 KHz range, since below the lower value the effectiveness of the instrument is poor, while higher frequencies have proven to damage the cells (Voltolina 1991). However, controls of the residual fraction of diatoms in the sediment as well as of damaged cells are often missing. The often incomplete information about used ultrasonic devices and treatment times is problematic, but see Voltolina (1991). If treatment times are given, they vary widely between 2 min (Agatz et al. 1999), 3 min (Voltolina 1991) and up to 10 min (Hickman 1969). The optimum period of sonication must be determined for each sediment type and for the population in question, as some algae may adhere to sediment particles more strongly than others or may be ruptured, leading to biased quantification results (Hickman 1969; Mitbavkar and Anil 2002).

In the present study, I quantitatively examined the application of an ultrasonic homogenizer for the dislodgement of the entire benthic microalgae community. Ultrasonication was conducted at six different duration levels (ranging from 15 to 90 s), and diatoms in the supernatant and the sediment were quantitatively controlled after each time, using an epifluorescence microscope. Moreover, I aimed to enhance dislodgement rates by adding the surface-active detergent Polysorbat 20 (Tween 20) in two different concentrations (0.1% and 0.025% final) (Fig. 1), which already has been successfully used for the removal of particle-associated bacteria (Velji and Albright 1993; Epstein and Rossel 1995; Mermillod ‐ Blondin et al. 2001; Amalfitano and Fazi 2008). I assessed significant effects of the different treatments using two-factorial ANOVAs with total cell number and relative removal rates as dependent factors and ultrasonication, Polysorbat 20 [0.1%] and their interactive effects as independent variables.

5.3. MATERIALS AND PROCEDURES

5.3.1. SAMPLING PROCEDURE

Sampling of sediment was conducted on July, 15th, 2009 during low tide in the eulitoral zone of the Jade Bay, Germany (53°30'39,2'' N, 8°08'01,3'' E). 15 sediment cores were taken from the upper first centimeter of the sediment surface with a cut-off syringe (∅ 3 cm) and stored in plastic tubes in darkness at 4°C. All cores were gently manually homogenized. For further procedures, a subsample (0.5 g) of the mixed sediment was filled in 2 ml PCR micro-tubes (Eppendorf) and 1.175 ml sterile filtered sea water and formaldehyde (total concentration 2%) were added, resulting in 96 replicates.

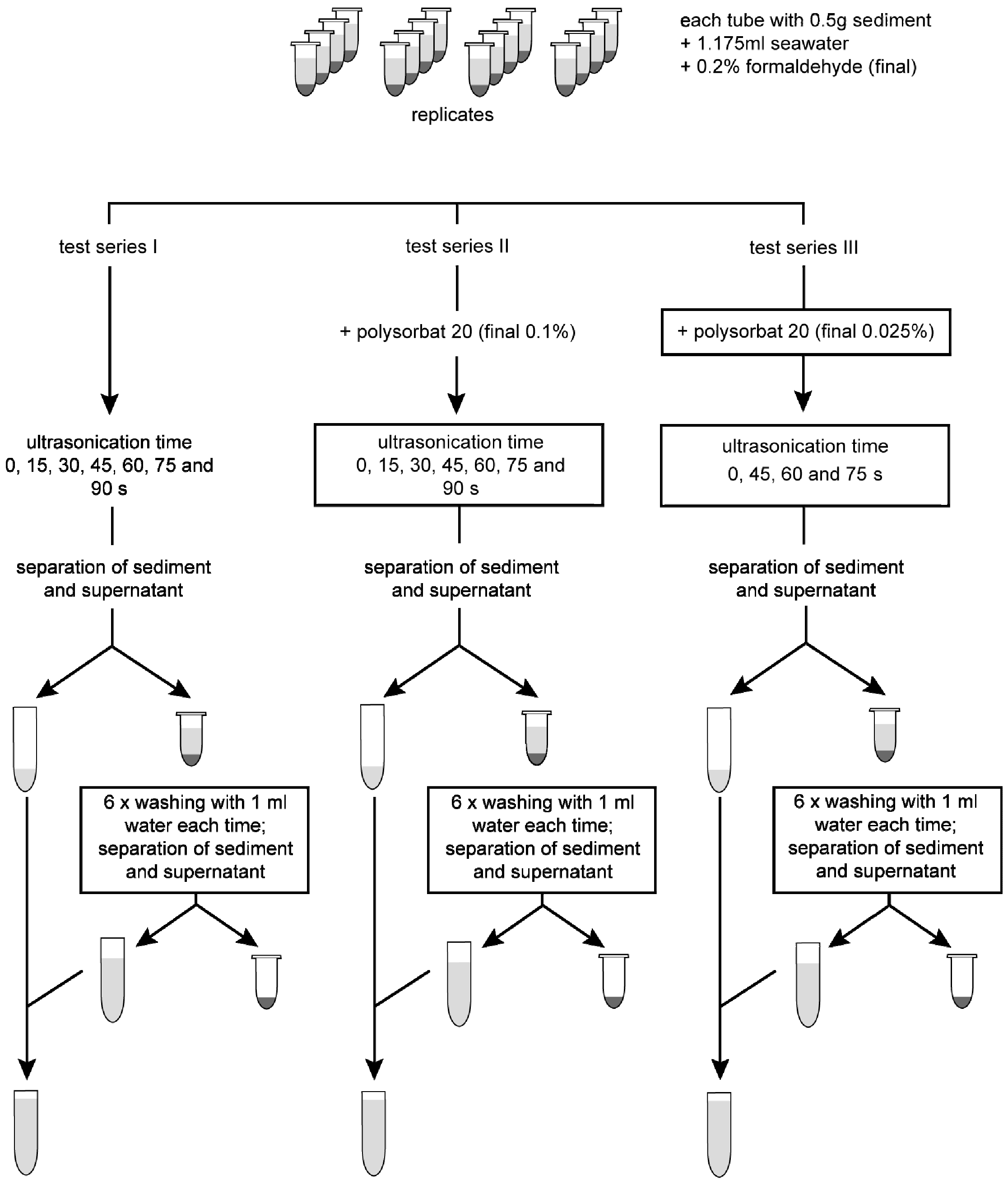


Fig. 1: Flow chart of the three different procedures tested.

5.3.2. ULTRASONICATION & POLYSORBAT 20

Ultrasonication was conducted in an ice bath to prevent heating and cell disruption. I used a sonopuls-ultrasonic homogenizer (HD 200, Bandelin, Berlin, Germany) with a MS 73 sonotrode, a horn (SH 213), an ultrasonic transducer (UW 200) and a HF generator (GM 200). The lowest possible amplitude was used (70 – 78 µm). The tip of the sonotrode was adjusted in a way that it touched the surface of the sample. Different sonication times were chosen: 0 (control), 15, 30, 45, 60, 75 and 90 s. Due to foam formation (when adding Polysorbat 20) and to prevent heating, samples were treated in 10 s intervals, with 15 s breaks in between. Polysorbat 20 was added in two different total concentrations: 0.1% and 0.025%.

5.3.3. SEPARATION INTO SOLID AND LIQUID PHASES

After sonication, samples were stirred up for 5 – 10 s (~ 1900 rpm) with a reagent shaker (Reax top, Heidolph, Schwabach, Germany). The supernatant was transferred into test tubes (Eppendorf, Hamburg, Germany). This procedure was repeated six times, each time with 1ml sterile filtered sea water and formaldehyde (2% final), enhancing the number of suspended diatoms in the supernatant.

5.3.4. PREPARATION OF MICROSCOPIC SLIDES

A defined volume of each homogenous supernatant sample was filtered over a 2 µm Isopore^{TM} membrane filter which was placed on a slide, mounted with Citifluor Ltd. AF 1, covered with a cover slip and was then frozen until further examination.

Sediment particles were also placed on slides and mounted with Citifluor Ltd. AF 1 and counted on the same day.

5.3.5. CELL COUNTINGS

Benthic diatoms of the supernatant were counted with 200-fold magnification using an epifluorescence microscope (Zeiss, Axioskop 2, Jena, Germany). Cells of 20 randomly chosen grids were evaluated on the filter; in total ± 500 cells were counted on each filter. Moreover, 20 sediment particles >90µm, covered with Citifluor Ltd. AF1, were measured with 200-fold magnification using bright field microscopy. Diatoms attached to the upper side of sediment particles were enumerated using epifluorescence. Due to obscuring effects of the sediment particles, countings for the upper side were doubled. Thus, only an approximation for the total cell number can be given:

$\textit{total cell number} = 2 * \textit{cell number} * (\textit{volume} * 2.65\ g\ cm^{-3})^{-1}$

cell number = mean cell number on 20 sediment particles

volume = mean volume of 20 sediment particles

5.3.6. STATISTICAL ANALYSIS

Effects of the different treatments (ultrasonication and Polysorbat 20 [0.1%]) were analyzed with a two-factorial ANOVA in Statistica (Statsoft, 8.0, Tulsa, Oklahoma, USA). Response variables included the number of suspended diatoms, residual diatoms in the sediment, total cell number as well as the dislodgement rate [%]. Prior to analysis, cell numbers were log-transformed and the dislodgement rate was angular-transformed.

5.3.7. PHOTOGRAPHY

Microphotographs of diatoms attached to sediment particles were taken with a digital camera (Canon Powershot A 620, Krefeld, Germany) mounted on the epifluorescence microscope. Photos were edited using the program Picolay (version 2009-09-08, www.picolay.de): different focal planes were merged to a three-dimensional picture.

5.4. ASSESSMENT

5.4.1. I. ULTRASONICATION

Ultrasonication increased the number of dislodged diatoms. Highest numbers of diatoms in the supernatant were found after 60 s (1.2 * 10^7 compared to 7.4 * 10^6 cells / g sediment in the control (Fig. 2)), which is an increase of 61%. After 75s a slight decrease in the number of dislodged cells could be noted (1.1 * 10^7 cells / g sediment), which can probably be attributed to progressing cell breakage. Thus, I recommend applying ultrasonication for 60 s. In this treatment the dislodgement rate was highest, while cell breakage remained low. However, if ultrasonic devices are used other than the one described in my study, sonication time, amplitude and frequency have to be adjusted.

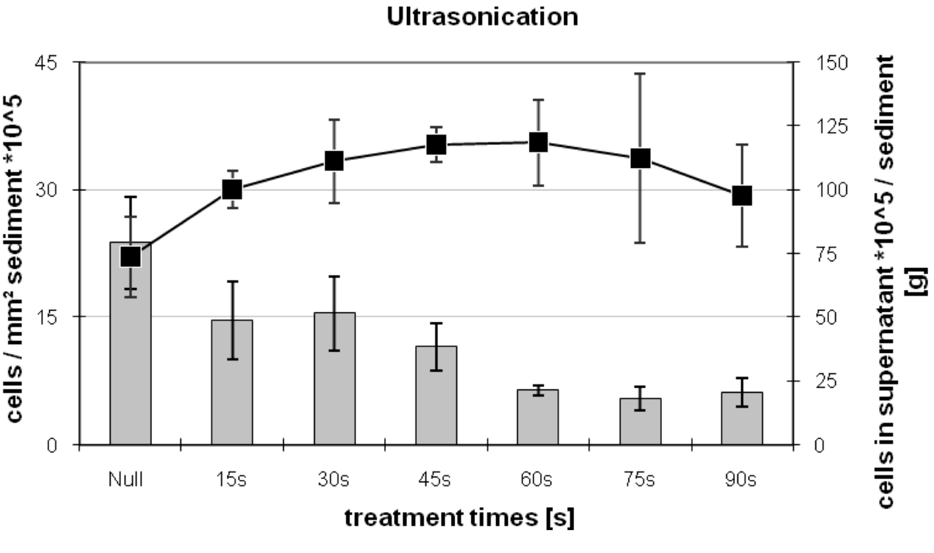


Fig. 2: Different ultrasonication times. Grey bars: remaining diatom cells in the sediment; squares: dislodged cells in the supernatant are displayed on the 2nd y-axis.

5.4.2. II. ULTRASONICATION WITH POLYSORBAT 20

Adding different concentrations (0.1% and 0.025% final) of Polysorbat 20 to the sample before ultrasonication did not significantly increase the number of dislodged diatoms in the supernatant (Fig. 3, 4 and Table 1). The dislodgement rates after 60 s ultrasonication with added Polysorbat 20 (0.1%) were comparable (both 1.2 * 10^7 cells / g sediment (Fig. 3)). However, after 75 s a strong decline in the dislodgement rate (8.8 * 10^6 cells / g sediment) was notable in this treatment, indicating enhanced cell breakages. When Polysorbat 20 was added in a lower concentration (0.025%), highest dislodgement rates were reached already after 45 s (1.19 * 10^7 cells / g sediment (Fig. 4)), thus potentially preventing cell breakages due to the shortened ultrasonication time. Yet, Polysorbat 20 caused foam formation, which could not only hamper the spread of ultrasonic waves, but also the correct adjustment of the sonotrode.

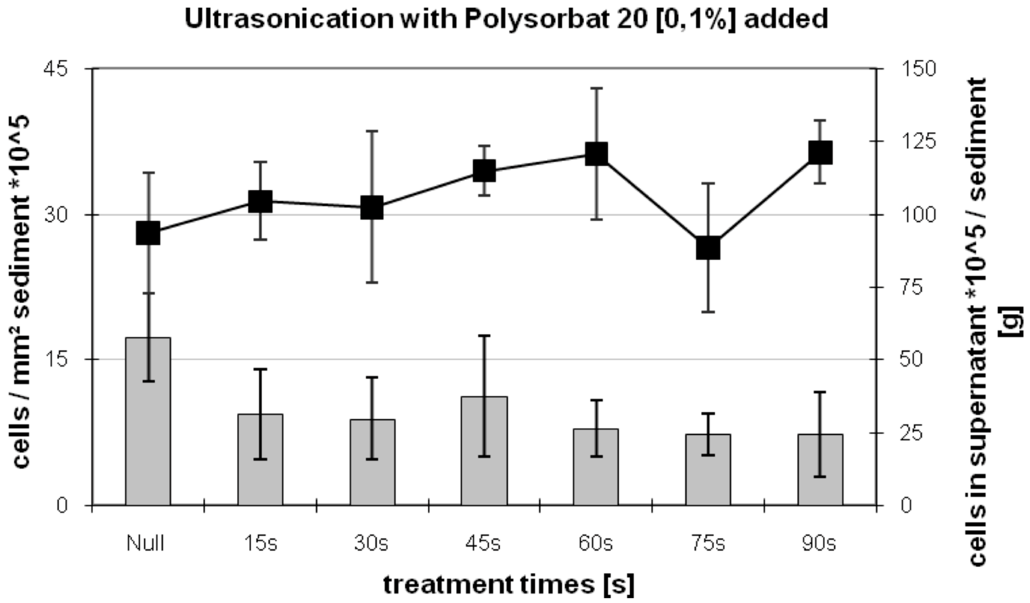


Fig. 3: Ultrasonication with Polysorbat 20 [0.1%] added. Grey bars: remaining diatoms in the sediment; squares: dislodged cells in the supernatant. Highest dislodgement rates were reached after 60 s.

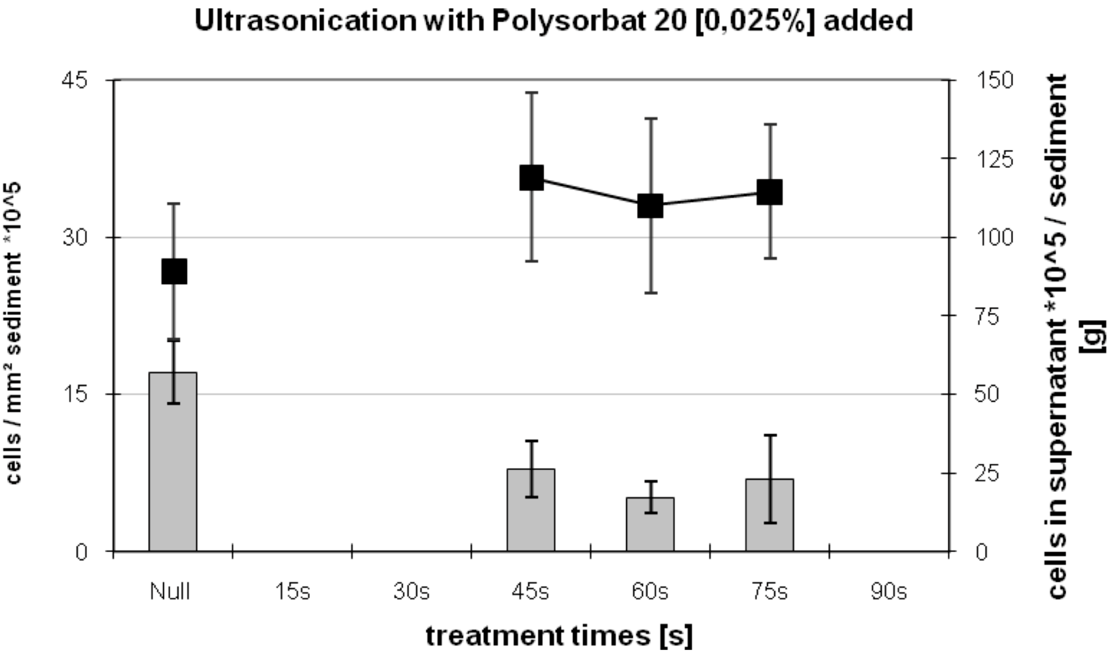


Fig. 4: Ultrasonication with Polysorbat 20 [0.025%] added. Grey bars: remaining diatoms in the sediment; squares: dislodged cells in the supernatant. Highest dislodgement rates were reached after 45 s.

5.4.3. III. COMPARISON OF DISLODGEMENT RATES

Ultrasonication significantly influenced the relative removal rates, while the total cell number was not significantly affected (Fig. 5, Table 1). Tukey´s HSD post-hoc test showed significant ($p < 0.05$) differences in the amount of dislodged diatoms for the following ultrasonication times: 45, 60 and 90 s for the supernatant as well as 30, 60, 75 and 90 s for the sediment fraction (data not shown). Effects of Polysorbat 20 and the interactive effects of both ultrasonication and Polysorbat 20 were insignificant (Table 1).

Table 1: Effects of ultrasonication and Polysorbat 20 as well as the interactive effects of both on the total cell number and the relative removal rates were tested with a two-factorial ANOVA. The table gives degrees of freedom (Df), its F-ratio and significance level (p).

FACTOR	**TOTAL CELL NUMBER**			**RELATIVE REMOVAL RATES**		
	df	F	p	df	F	p
Ultrasonication	6	1.5	0.201	6	19.9	< 0.001
Polysorbat 20 [final 0.1%]	1	0.0	0.974	1	4.3	0.045
Interactive effects of ultrasonication + Polysorbat 20 [final 0.1%]	6	1.2	0.311	6	3.0	0.179

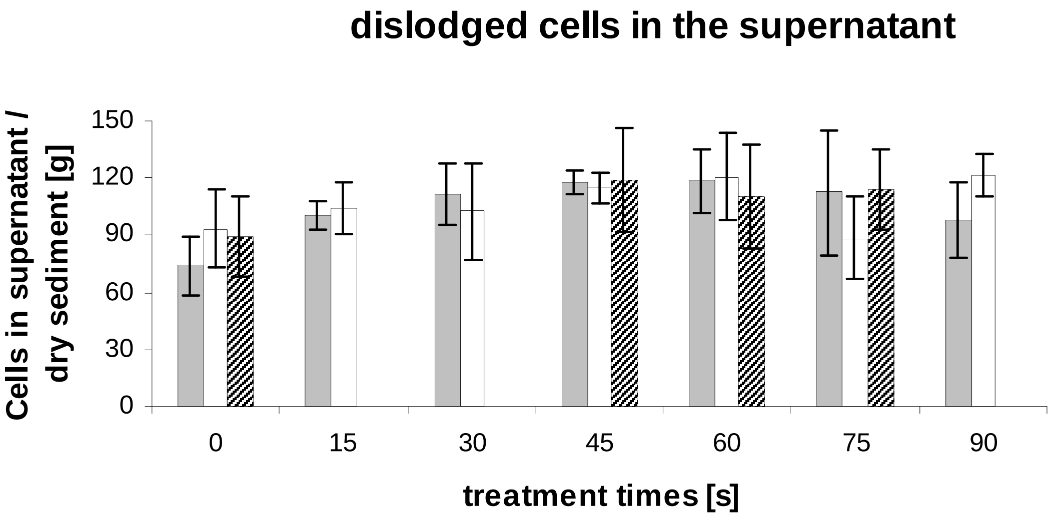


Fig. 5: Dislodged cells in the supernatant after treatment with ultrasonication (grey bars), Polysorbat 20 [0.1%] (white bars), Polysorbat 20 [0.025%] (shaded bars).

5.4.4. IV. SEDIMENT WASHING

The six-fold washing procedure caused an average increase of 14% more dislodged diatoms in the supernatant (Fig. 6).

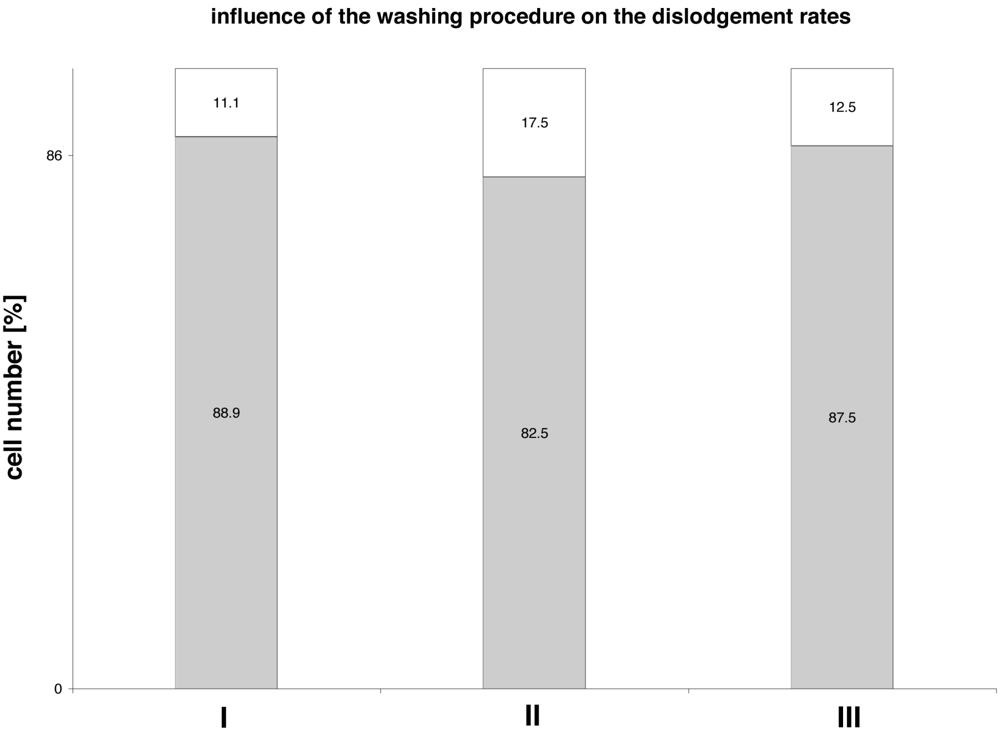


Fig. 6: Influence of the washing procedure on the dislodgement rates. Grey bars: dislodged cells in supernatant before washing [%], white bars: additional dislodged cells in supernatant after 6x washing [%]. I - III = different treatments: I = ultrasonication; II = ultrasonication + Polysorbat 20 [0.1%]; III = ultrasonication + Polysorbat 20 [0.025%].

5.4.5. V. RESIDUAL DIATOMS IN THE SEDIMENT

Lowest residual diatom numbers in the sediment were found after ultrasonication for 60 s ($5.4 * 10^5$ cells / g sediment). However, it is important to note that direct cell counting in the sediment may lead to an underestimation of the total cell number, since only the upper side of each sediment particle can be regarded (Eaton and Moss 1966). Therefore, I doubled the cell numbers, gaining an approximation. The accuracy of the countings furthermore was influenced by the heterogeneous distribution of the species on the sediment particles, with highest amounts of cells in depressions of the particles (DeJonge 1985) (Fig. 7). Moreover, the distribution of diatoms on the filter often was irregular with higher cell numbers on the edge of the filter. Chain-forming species and other cell aggregates may also lead to a heterogeneous distribution, which biases cell countings. Additionally, since the composition of the sediment was not known, the specific density of quarz sand (2.65 g / cm^3) was assumed.

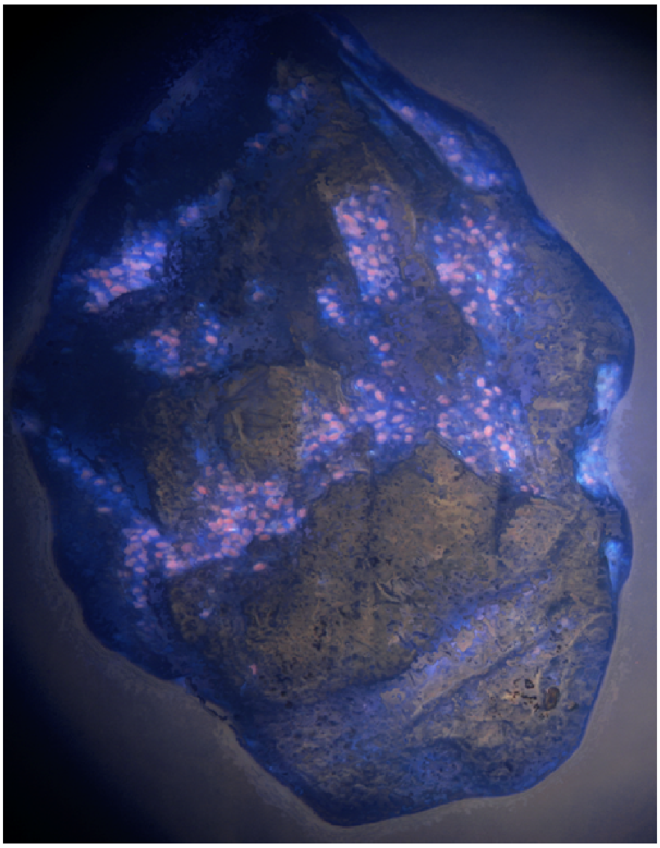


Fig. 7: Epifluorescence microscopy photo of a single sand grain with attached diatoms (light violet dots) in the depressions.

5.5. DISCUSSION

Various methods have been developed regarding the separation of MPB cells from sediment particles. Yet, some of those methods either capture only fractions (e.g. only epipelic diatoms) of the total community (Eaton and Moss 1966; Delgado et al. 1991b), or do not differentiate between living and dead cells in the sediment (Round 1965). Further, success rate and the residual fraction of diatoms in the sediment are usually not controlled and used instruments, such as ultrasonication devices, are often described incompletely (Agatz et al. 1999). Thus, comparability between different methods is almost not given, making their interpretation difficult.

I highlight the need for a standardized method for removing benthic diatoms from sediment particles, ensuring the comparability of studies on the biodiversity of MPB. The MPB has important ecological functions (e.g. enhancing sediment stability), requiring investigation on a standardized basis. My results indicate that ultrasonication for 60 s significantly increased the rate of diatoms in the supernatant by more than 60%. Ultrasonication with Polysorbat 20 [0.025% final concentration] revealed comparable results already after 45 s. The shortened ultrasonication time in this treatment may prevent cell breakages. Ultrasonication times should generally be kept short, as diatom frustules are known to break under high mechanical stress exerted at the microscale. Thereby, large epipelic cells are more prone to cell breakage than small (< 20 µm), strongly silicified epipsammic cells, potentially resulting in a distorted ratio of epipelic to epipsammic cells in the samples (Round 1965; Hickman 1969; Delgado et al. 1991a; Reiss et al. 2007).

If other ultrasonic devices are used as described in my study, sonication time, amplitude and frequency have to be adjusted, as they may affect the outcome of the treatment. Voltolina (1991), for example, proposed the usage of ultrasonic baths with frequencies between 75 and 100 KHz, as high frequencies guarantee a gentle treatment.

Additional washing procedures enhanced the overall diatom number in the supernatant by 14%. Since this practice is relatively time-consuming, I only recommend it for research questions, which focus on the exact biodiversity of the MPB community. An important part of my method is the evaluation of the number of living and dead cells remaining in the sediment after the treatment using an epifluorescence microscope (MacIntyre et al. 1996).

The method I propose here has strong advantages over previous approaches as it i) allows quantification of epipelic and epipsammic diatoms simultaneously, ii) minimizes cell loss with maximized detachment of cells, and iii) is rather cost- and time-effective.

CHAPTER 5

6. *GENERAL DISCUSSION*

6.1. *I. LONG-TERM MONITORING*

I established a long-term monitoring of the phytoplankton community in the backbarrier tidal flats of Spiekeroog, complemented by continuously recorded environmental variables from the time-series station. I followed the phytoplankton turnover over 3.5 consecutive years from 2009 to 2012 at various scales in space (horizontal and vertical) and time (tidal, seasonal, inter-annual).

Seasonal variations in nutrient concentrations, controlled by phytoplankton primary production and microbial remineralisation, overrode inter-annual and tidal differences. Lowest nutrient concentrations always could be found after diatom spring blooms, which persisted until nutrients, in particular Si, were depleted. Nevertheless, tidally-driven variations in nutrient concentrations were observed. Highest nutrient concentrations were always measured at low tide, indicating an enhanced influence of the benthic microbial heterotrophic activity in relation to a shallower water body (Postma 1981). I did not observe a vertical gradient with higher nutrient concentrations towards the sea floor, suggesting a strong vertical mixing.

Phytoplankton community turnover was observed on a range of temporal scales. Seasonal variations in the phytoplankton community composition surpassed tidal and inter-annual differences. The intra-annual turnover was characterized by low phytoplankton biomass during wintertime and highest biomass during diatom spring blooms dominated by *M. helysia*. Dinoflagellates prevailed during the summer months, followed by elevated diatom biomass in October. Further, In spring and autumn 2009, a strong benthopelagic coupling, promoted by severe current velocities, could be observed: occasionally, benthic species contributed > 50% to total biomass, indicating that the water body was temporarily well-mixed with upper sediment layers.

6.1.1. EVALUATION

Based on the evaluation of different biodiversity measures, I analyzed at which spatiotemporal resolution long-term monitoring should be conducted to gain sufficient insight into phytoplankton dynamics: a biweekly sampling of surface water at random points in the tidal cycle is sufficient for tracking general seasonal phytoplankton dynamics. Yet, more frequent (e.g. weekly) sampling intervals would allow the analysis of short-term variation and smaller short-term peaks.

The comparison of high and low taxonomic and spatial resolution indicated that around 1/3 of the total species richness, mostly rare species, remained unrecognized with the low resolution sampling procedure. However, the more species are differentiated, the better might be the quality of the predictability of future community assembly, as rare species potentially might become dominant under changing environmental conditions (Caron and Countway 2009). Morphological identification methods should therefore be extended by molecular genetic approaches to gain in-depth insights in the actual species diversity.

6.1.2. OUTLOOK

RECOMMENDATIONS FOR THE CONTINUATION OF THIS LONG-TERM MONITORING

I highly recommend continuing and also expanding this long-term monitoring. First, long-term monitoring allows tracking of eutrophication-induced changes in phytoplankton community structure. Eutrophication is known to cause increased phytoplankton productivity (Vitousek et al. 1997; Dippner 1998; Paerl et al. 2006), while reducing biodiversity, resulting in marked changes in the community composition and nutrient cycling.

Second, intensive monitoring focussing on different biological and physicochemical aspects of climate change is mandatory, as the marine ecosystem is affected by climate change in a multitude of ways. The predicted increased number of storm events, for example, may result in an intensified benthopelagic coupling. Further, climate is fundamentally important for the stability and maintenance of populations and food chains (Boelens et al. 2005): additional sampling of zooplankton species would provide information about changes in the timing, intensity and synchronization of spring algal blooms and zooplankton communities with potentially substantive consequences for the marine food web (Edwards and Richardson 2004; Boelens et al. 2005). A continuation of the long-term monitoring will moreover show, whether the observed invasion of *M. helysia* will permanently affect not only the phytoplankton community but potentially also higher trophic levels. Such long-term trends may first become apparent after decades of monitoring.

Further, species losses and invasions due to shifts in biogeographical range are expected (Boelens et al. 2005) and the number of harmful algal blooms is likely to increase (Hallegraeff 2010; Lürling and De Senerpont Domis 2013). The detection of future invasions by non-indigenous phytoplankton species should be based on intensified monitoring, preferably supplemented by molecular analyses to investigate invasion pathways and the mechanisms of the establishment in the receiving habitat (Olenina et al. 2010). Thereby, long-term monitoring data are important as a baseline against which changes associated with invasion can be judged (Nentwig 2007).

For future investigations, it would be helpful to agree on a standardized sampling strategy for different monitoring sites over wide geographical scales, assuring comparable results (Zingone et al. 2010). Synchronized sampling at different locations would provide deeper insights in the mechanisms of phytoplankton bloom formations, for example. Monitoring data combined with results of laboratory experiments might be integrated into higher frameworks such as ecosystem modelling; resulting in predictions of future ecosystem functioning, which then form the basis of management plans (McQuatters-Gollop et al. 2007; Bresnan et al. 2009; Rullkötter 2009; Zingone et al. 2010; Edwards et al. 2013; Lürling and De Senerpont Domis 2013).

6.2. *II: THE INVASION OF THE DIATOM MEDIOPYXIS HELYSIA INTO THE BACKBARRIER TIDAL FLATS OF SPIEKEROOG ISLAND AND ITS POTENTIAL FUTURE IMPLICATIONS*

A major outcome of the long-term monitoring started within the scope of this thesis was the first finding of the non-indigenous diatom *M. helysia* in the backbarrier tidal flats of Spiekeroog island in spring 2009 (Chapter 2). I followed its establishment process in this area over 3.5 consecutive years. *M. helysia* dominated the phytoplankton community during all spring bloom formations, accounting for up to 90% of the total diatom biomass. Thereafter, *M. helysia* contributed between ~ 20% and ~ 70% to the total diatom biovolume during summer times, but dropped in autumn (< 10% of total diatom biovolume). According to Olenina et al. (2010), such a level of dominance may be regarded as an essential change to the structure of the phytoplankton community. Moreover, species richness decreased from 80 taxa in 2009 to 59 taxa in 2011; the cell densities of taxa, which were abundant in 2009, also declined in the subsequent years. These results concur with findings of Vilà et al. (2011) for terrestrial ecosystems, where abundances and diversity of the resident species decreased in invaded sites. Yet, Vilà et al. (2011) also report enhanced primary production and ecosystems processes in invaded regions, which could not be confirmed in my study: compared to 2009, the total biovolume during spring blooms decreased by 36% in the following years.

To date, only little is known about this species, its seasonality and physiological requirements (Kraberg et al. 2011). My laboratory experiment (Chapter 2) showed that *M. helysia* is able to persist under a broad range of Si and N conditions, which might have fostered the successful establishment of *M. helysia* in the North Sea and its dominance throughout the year (Olenina et al. 2010). This can probably be attributed to the relative high nutrient uptake capacities and growth rates of large diatom species under high nutrient conditions (Stolte and Riegman 1995; Irwin et al. 2006; Key et al. 2010).

At present, it cannot be said, how *M. helysia* will affect the phytoplankton community in the future. In general, invasions of NIS may alter the dominance structure of natural assemblages, as NIS often show an initial dominance (Seabloom et al. 2003; Hillebrand et al. 2008). However, many NIS become rather localized or disappear after such an initial dominance and can thus be classified as temporary immigrants (Nehring 1998b). Yet, this is not the ecological fate for all invasive phytoplankton species, with some species becoming permanently established immigrants (e.g. *Coscinodiscus wailesii* and *Odontella sinensis*) (Nehring 1998b). Therefore, the monitoring programme needs to be continued to investigate *M. helysia*´s future role in the Wadden Sea.

6.2.1. POTENTIAL FUTURE IMPLICATIONS OF A SUCCESSFUL ESTABLISHMENT OF *M. HELYSIA*

In general, NIS may cause a decline in ecological quality due to changes in biological, chemical and physical properties of aquatic ecosystems (Olenina et al. 2010). Nevertheless, in many coastal areas there are indications that introduced species have become integrated into local communities without adverse effects (Puth and Post 2005). A successful establishment of *M. helysia* might have far-reaching consequences not only for the phytoplankton community of the Wadden Sea, but also for higher trophic levels. First, it is likely that species will be outcompeted, especially when nutrients are depleted by massive *M. helysia* blooms lowering the species richness. Second, there are indications, that *M. helysia* is grazing resistant

(Stolte and Riegman 1995): it may not be ingested by microzooplankton grazers, which prefer phytoplankton cells < 100 µm (Harris 1982; Jansen 2008; Loebl et al. 2012). The potentially limited feeding of copepods on *M. helysia* could have important implications for the structure of pelagic food webs (Rick and Duerselen 1995; Jansen 2008; Loebl et al. 2012).

OUTLOOK

6.2.2. JADE WESER PORT

In 2012, Germany´s sole deep-water harbor, the Jade Weser Port, was commissioned. It is the third harbor in the German Bight besides Bremerhaven and Hamburg and located at the deep fairway of the outer Jade in Wilhelmshaven in relative proximity (~ 45 km) to my study area, the backbarrier tidal flats of Spiekeroog island. With the establishment of the harbor, international shipping traffic in this region is expected to strongly increase (until 2020, up to 4.2 million shipping containers are expected to arrive each year). Thereby, the propagule pressure (inoculum size and number of events) of NIS mediated by ship hull fouling and particularly by ballast water exchange is likely to increase (Ruiz et al. 1997; Klein et al. 2010a). With increasing propagule pressure, the colonization pressure is enhanced, meaning that with higher diversity of propagules, the presence of species that possess attributes required for colonization (selection effect) becomes more likely as well (Lockwood et al. 2009). Yet, the invasion process is considerably complex, as the probability of establishment not only depends on similar environmental conditions in the donor and recipient habitat, but also on trophic levels, niche occupancy, population dynamics as well as biotic resistance (Seebens et al. 2013). Consequently, the likelihood of introductions of NIS is not only determined by traffic volume, but also by community mismatch (biogeographical distance) and by environmental similarity between the donor and the recipient habitat (Seebens et al. 2013). Thus, increased shipping traffic does not necessarily lead to invasion, so that North Sea ports currently do not have high invasion risks despite their enormous traffic (Seebens et al. 2013).

6.2.3. CLIMATE CHANGE

Due to global warming, surface water temperature in the German Bight has risen by 1.1 °C since 1962 (Wiltshire and Manly 2004a). Global warming may contribute to a successful invasion or range expansion of warm-water, potentially toxin-producing dinoflagellates and cyanobacteria. Consequently, the number of harmful algal blooms is expected to accelerate (Boelens et al. 2005; Hallegraeff 2010; Lürling and De Senerpont Domis 2013). As toxic dinoflagellates may cause paralytic shellfish poisoning, they could have severe economical consequences for the fishery industry.

6.2.4. BALLAST WATER MANAGEMENT: OPTIONS TO REDUCE MARINE SPECIES TRANSFER

Phytoplankton species richness in ship ballast water is demonstrably very high: McCarthy and Crowder (2000), for example, found 342 morphospecies in the ballast water of only 9 ships. Rigby et al. reported in 1995 that ten billion tons of shipping ballast water are transported each year, with an increasing tendacy. In 2007, for example, 16363 ships were on 490517 non-stop journeys linking 36351 distinct pairs of arrival and departure ports (Kaluza et al. 2010). Hence, facing this enormous shipping traffic, the development of effective, targeted invasion management strategies needs to be enforced rigorously (McCarthy and Crowder 2000; Seebens et al. 2013). Possible treatment methods comprise filtration, cyclone, UV-irradiation, ultrasonication, heat, ozone, electric pulse, hydrogen peroxide and biocides (Kuzirian et al. 2001). The usage of biocides is thereby limited by monetary costs, biological effectiveness and possible residual toxicity of discharged ballast water (Gregg and Hallegraeff 2007). Further, it has not yet fully been assessed, how these diverse treatments affect cysts of different diatom species, which are known to survive adverse conditions (Villac and Kaczmarska 2011). Nevertheless, if successful, on board ballast water treatment may be disproportionately effective (Seebens et al. 2013). Mid-ocean ballast water exchange is also discussed, but – while the cheapest – a less efficient method (Zhang and Dickman 1999). Another possibility presents the sterilization of ballast water in land-based treatment systems. The potentially huge economic costs of ballast water management have to be politically accepted at international level and are thus difficult to put into practice.

6.3. *III. SPATIOTEMPORAL TURNOVER OF A PHYTOPLANKTON COMMUNITY IN A NATURAL COASTAL SYSTEM*

- A META-ECOSYSTEM PERSPECTIVE

The Wadden Sea forms a transition zone between the land and the ocean and is thus highly influenced by terrestrial nutrient run-off and benthic re-mineralization processes due to the shallow water column (Fig.1). The phytoplankton community in the backbarrier tidal flats of Spiekeroog is subject to the interplay of constantly varying abiotic conditions in this highly dynamic system, grazing pressure and competition for resources within a water column that is characterized by inverse nutrient and light gradients (Ryabov et al. 2010) (Fig.1). The phytoplankton community was temporally structured at different scales. First, differences in the inter-annual turnover of the phytoplankton community could be detected. The slope of the general additive model representing the temporal turnover declined towards 2012, indicating that the community was less dissimilar in 2011 and 2012 - the reason for this remains elusive at present. Second, a strong intra-annual turnover became visible in the hump-shaped Bray-Curtis dissimilarity curve with a peak at around 150 days (2009). The temporal turnover in phytoplankton composition was significantly associated with seasonal changes in environmental conditions, particularly reflecting trends in P, N, PAR and temperature (Chapter 3).

Hydrodynamic processes produced by tides, currents, waves and wind induce interlinked fluxes of suspended particulate matter (SPM), dissolved organic carbon (DOC), nutrients and planktonic organisms (Augustinus 2003). Horizontally, water masses are exchanged between the North Sea and the Wadden Sea. Vertically, a benthopelagic coupling takes place, i.e. the re-suspension of microphytopbenthic species into the water column promoted by tidal currents (Chapter 1 and 4). During the long-term monitoring I observed significant temporal patchiness and an evident spatial separation in species number, while the dominance structure of the phytoplankton showed a non-significant spatial imprint during the most of the year. While the SAR curves were initially steeper than STR curves, temporal turnover ultimately exceeded the spatial turnover. The slopes of the SAR varied among sampling dates, indicating temporal fluctuations in the degree of spatial patch formation. Although significant horizontal or vertical differences in the dominance structure of the phytoplankton community could only be found for a single sampling day, Bray-Curtis taxon dissimilarities showed that communities of the nine stations sampled in 2009 were spatially structured to a certain degree (Chapter 3).

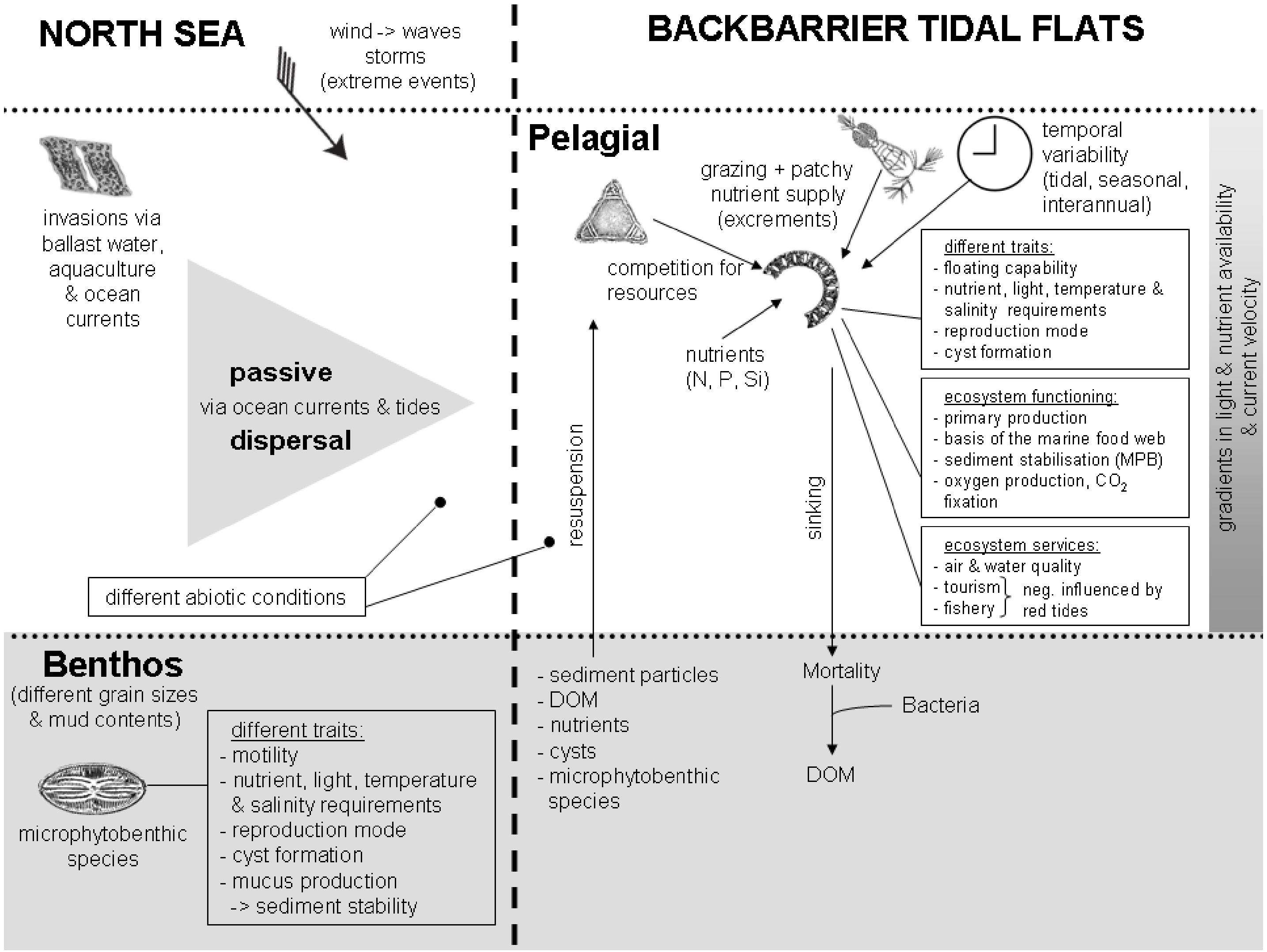


Fig. 1: Exchange of nutrients, SPM, DOM, planktonic and benthic microalgae between the North Sea, the adjacent Wadden Sea, the benthos and terrestrial river discharge.

Biogeochemical processes have been investigated by a number of studies in the Wadden Sea area (Gätje et al. 1998), and in particular in the backbarrier tidal flats of Spiekeroog (Dittmann 1999; Rullkötter 2009). These studies illustrate how hydrodynamic forces drive fluxes of nutrients, SPM and DOC between the North Sea and the Wadden Sea at different temporal scales (Stanev et al. 2008; Badewien et al. 2009; Lemke et al. 2009; Lettmann et al. 2009).

Consequently, to gain a comprehensive view of the phytoplankton community turnover, phytoplankton fluxes cannot be considered alone – rather coupled fluxes of dissolved and particulate matter and energy have to be taken into account as well (Massol et al. 2011) (Fig.1). For this, meta-ecosystem theory offers an unifying framework by linking community and ecosystem dynamics through spatial ecology (Loreau et al. 2003a; Holyoak 2005; Massol et al. 2011). Meta-ecosystems are defined as a set of ecosystems connected by spatial flows of energy, materials and organisms (Loreau et al. 2003b). Deterministic coexistence in meta-ecosystems occurs due to species sorting, mass effects and patch dynamics (Chapter 3) − yet, spatial flows of resources may considerably change the outcome of species interactions (Mouquet et al. 2013).

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Meso-scale eddies and fronts induce transient spatial structures by creating connectivity as well as barriers to dispersal (Bracco et al. 2000; Watson et al. 2010), thereby generating ephemeral patches differing in their abiotic conditions and species assemblages (Bastine and Feudel 2010) (Fig. 2). Alternatively, spatial heterogeneity can be self-generated by colonization-competition trade-offs (patch dynamics) of organisms (Hassell et al. 1994), or by nutrient dynamics within an uniform environment (Massol et al. 2011). The interaction between organisms and abiotic factors is strong and perpetual, as species act as ecosystem-engineers by modifying the abiotic environment through consuming and releasing nutrients, thereby producing a spatial dimension in the competition between the algae (Ryabov et al. 2010). Such spatial dynamics allow many species to coexist in a uniform landscape (Massol et al. 2011) (Fig. 2).

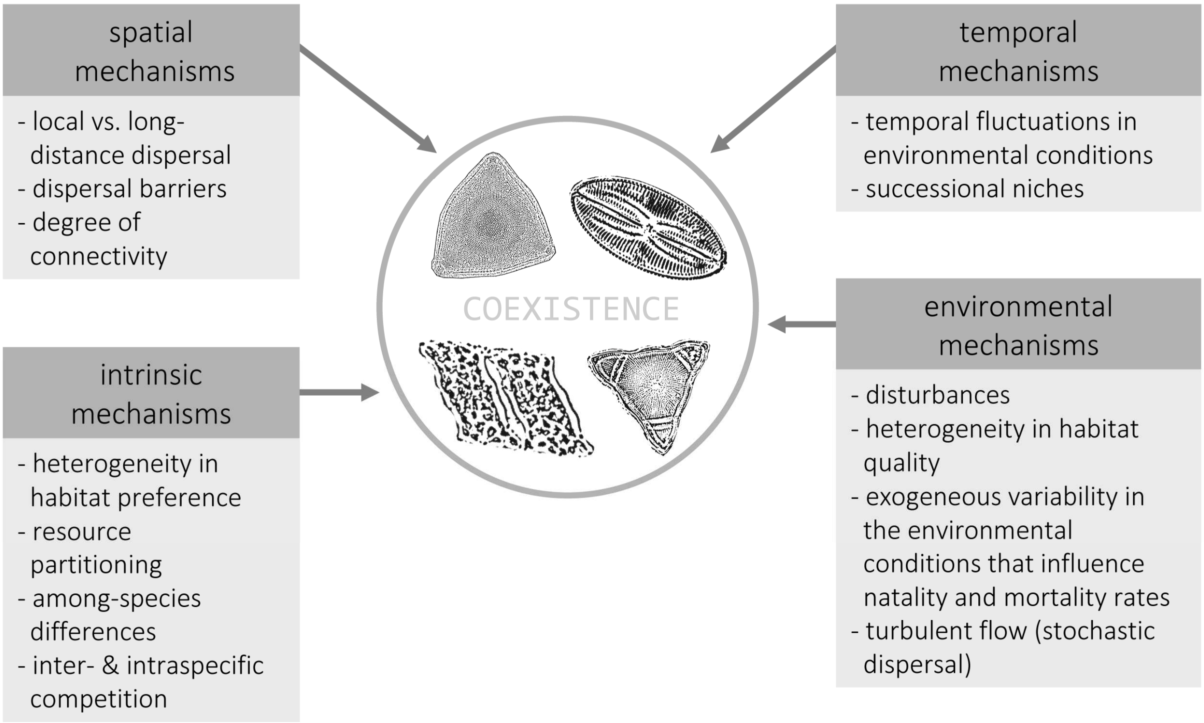

Fig. 2: The 'paradox of the plankton' may be solved by considering a number of intrinsic and extrinsic mechanisms promoting phytoplankton coexistence in the marine realm (Chapter 3).

I found indications for ephemeral phytoplankton patch formation in time and space, potentially shaped by species sorting and patch dynamics mechanisms (Chapter 3). Yet, the current patch dynamics model does not account for concomitant fluxes of resources occurring in the marine system. Recently, Gravel et al. (2010b) included the patch dynamics perspective in the meta-ecosystem framework by considering resource fluxes. However, their model is not fully applicable to my study system, as it is based on a terrestrial plant meta-ecosystem, in which only resources and propagules disperse − not the patches themselves. Consequently, patch dynamics theory needs to be further developed by integrating mutual feedbacks between ecosystem and species-level processes in dynamic open meta-ecosystems.

My study forms a starting point for further holistic investigations of the spatiotemporal phytoplankton turnover in open systems based on meta-ecosystem theory. At present, meta-ecosystem theory has mainly been approached from a modeller´s perspective (e.g. Gravel et al. 2010a, Gravel et al. 2010b, Massol et al. 2011). Hence, combining datasets of biogeochemical and phytoplankton dynamics collected in the backbarrier tidal flats of Spiekeroog could be valuable for validation purposes in meta-ecosystem modelling. For example, the transferability of terrestrial meta-ecosystem models to marine systems could be tested this way. Alternatively, existing models such as the European Regional Seas Ecosystem Model (ERSEM) and the Ecological Tidal Model (EcoTiM), which describe biogeochemical processes and dynamics of four phytoplankton groups (Kohlmeier 2004; Kotzur 2009), could be amended to address spatiotemporal coexistence mechanisms arising from the ephemeral nature of patch formation within the meta-ecosystem context. Highly resolved phytoplankton data or other biodiversity measures (beta diversity / species richness / evenness) as those assembled here would be necessary to test model predictions. Another possible application of the ERSEM could be the further investigation of marine food web functioning within the meta-ecosystem context (Massol et al. 2011) at temporal scales from hours to years, as the model contains three trophic levels (primary and secondary producers and destruents) and different material cycles (Kohlmeier 2004; Kotzur 2009).

7. *SUMMARY*

Given the central role of phytoplankton in global biogeochemistry, it is mandatory to understand the complex spatiotemporal processes regulating the coexistence and ultimately the biodiversity of phytoplankton communities. Aim of this thesis was to gather data on phytoplankton diversity in the backbarrier tidal flats of Spiekeroog at different spatial (horizontal and vertical) and temporal (tidal, seasonal, inter-annual) scales and to interpret underlying mechanisms using metacommunity theory. Therefore, I established a long-term phytoplankton monitoring in a coastal system, the backbarrier tidal flats of Spiekeroog (Chapter 1). I followed the phytoplankton turnover in a biweekly rhythm over 3.5 consecutive years from 2009 to 2012 and also evaluated different sampling strategies. Seasonal variations in nutrient concentrations overrode inter-annual and tidal differences. Thereby, lowest nutrient concentrations were always found after diatom spring blooms, which persisted until nutrients, in particular Si, were depleted. Less pronounced, tidally-driven variations in nutrient concentrations were observed with highest concentrations at low tide (enhanced benthic influence). Further, seasonal variations in the phytoplankton community composition surpassed tidal and inter-annual differences. Intra-annual turnover was characterized by low phytoplankton biomass during wintertime and highest biomass during diatom spring blooms dominated by *Mediopyxis helysia*. In spring and autumn 2009, a strong benthopelagic coupling was promoted by severe current velocities. The comparison of high and low taxonomic and spatial resolution indicated that around 1/3 of the total species richness, mostly rare species, remained unrecognized with the low resolution sampling procedure.

During the monitoring, the non-indigenous diatom *M. helysia* was first observed in this region (Chapter 2), where it essentially changed the structure of the native phytoplankton community, as it accounted for up to 90% of the total diatom biomass during spring blooms, while it contributed during summer times between ~ 20% and ~ 70% to the total diatom biovolume (Chapter 2). A laboratory experiment showed that *M. helysia* was the most successful diatom in the artificial community as it was able to persist under a broad range of Si and N conditions. This might, among others, contribute to its invasion success in the backbarrier tidal flats.

In Chapter 3 I interpreted the first results of the long-term monitoring within the metacommunity concept. During the long-term monitoring I observed significant temporal patchiness and an evident spatial separation in species number, while the dominance structure of phytoplankton showed a weaker and non-significant spatial imprint during the most of the year. While the species-area-relationships (SAR) curves were initially steeper than the species-time-relationships (STR) curves, temporal turnover ultimately exceeded the spatial turnover. The slopes of the SAR varied among sampling dates, indicating temporal fluctuations in the degree of spatial patch formation. Further, the phytoplankton community was temporally structured at different scales. First, differences in the inter-annual turnover of the phytoplankton community could be detected. Second, a strong intra-annual turnover became visible in the hump-shaped Bray-Curtis dissimilarity curve with a peak at around 150 days (2009). The temporal turnover in phytoplankton composition was significantly associated with seasonal changes in environmental conditions, particularly reflecting trends in P, N, photosynthetically active radiation (PAR) and temperature. The ephemeral phytoplankton patch formation in time and space is potentially governed by species sorting and patch dynamics mechanisms.

I further developed a standardized method for the detachment of benthic diatoms from sediment particles using ultrasonication and a surface-active detergent (Chapter 4). Understanding and predicting ecosystem processes accomplished by microphytobenthos requires an exact quantitative analysis of both epipelic and epipsammic diatoms. For microscopic analyses, the separation of diatoms from sediment particles is necessary. Established methods mostly generate incomparable results, as they usually gain only selective subsets of the benthic diatoms. I developed a standardized method by evaluating three different treatments for the detachment of benthic diatoms from sediment particles: ultrasonication at six defined duration times (ranging from 15 - 90 s) with and without the addition of different concentrations of the surface-active detergent. Ultrasonication for 60 s significantly increased the dislodgement rate: 60% more detached diatoms were found in the supernatant of the sonicated sample than in the control. I recommend using ultrasonication for 45 s with addition of a detergent (0.025% final concentration), since the same amount of diatoms is detached in this treatment, while cell breakage is reduced with reduction of the ultrasonication time.

7.1. *ZUSAMMENFASSUNG*

Angesichts der zentralen Bedeutung des Phytoplanktons in globalen Stoffkreisläufen ist es bedeutsam, die komplexen räumlichen und zeitlichen Prozesse zur verstehen, die die Koexistenz und damit die Biodiversität der marinen Phytoplankton-gemeinschaft regulieren. Ziel dieser Arbeit war es, die Diversität des Phytoplanktons im Spiekerooger Rückseitenwatt auf verschiedenen räumlichen (horizontal und vertikal) sowie zeitlichen (tidal, saisonal, inter-annual) Skalen zu erfassen und zugrundeliegende Mechanismen mit Hilfe der Metagemeinschaftstheorie zu interpretieren. Dazu wurde ein Phytoplankton-Langzeitmonitoring im Spiekerooger Rückseitenwatt etabliert und um kontinuierlich aufgezeichnete Umweltvariablen einer permanenten Station in der Otzumer Balje ergänzt (Kapitel 1). Die Probenahme erfolgte in einem zweiwöchigen Abstand über 3,5 Jahre von 2009 bis 2012. Es wurden verschiedene Probenahmeverfahren und taxonomische Auflösungen evaluiert.

Die saisonale Variation in den Nährsalzkonzentrationen (N, P, Si) übertraf inter-annuale und tidale Unterschiede. Die Nährsalzkonzentrationen waren stets nach den Frühjahresblüten am niedrigsten und am höchsten im Winter, wenn mikrobielle Remineralisierungsprozesse die Primärproduktion überstiegen. Weiterhin konnte eine tidale Variation in den Nährsalzkonzentrationen beobachtet werden: aufgrund eines erhöhten benthischen Einflusses wurden höchste Konzentrationen während des Niedrigwassers gemessen. Die saisonale Variation in der Phytoplanktongemeinschaft übertraf tidale und inter-annuale Unterschiede. Innerhalb eines Jahresganges war das Gesamtbiovolumen des Phytoplanktons während des Winters stets am niedrigsten und am höchsten während der Frühjahresblüte. Im Frühling und Herbst 2009 konnte zudem eine starke benthopelagische Kopplung beobachtet werden, die vermutlich auf erhöhte Strömungsgeschwindigkeiten zurückzuführen ist. Der Vergleich verschiedener taxonomischer und räumlicher Auflösungen zeigte, dass bei geringerer Auflösung rund ein Drittel der gesamten Artenvielfalt, insbesondere seltene Arten, nicht erfasst werden.

Zu Beginn des Monitorings wurde die nicht indigene Diatomee *M. helysia* erstmalig im Rückseitenwatt gefunden (Kapitel 2). *M. helysia* dominierte rasch die indigene Phytoplanktongemeinschaft: während der Frühjahresblüten machte sie bis zu 90% des gesamten Diatomeenbiovolumen aus und trug während der Sommer 20 - 70% zum gesamten Diatomeenbiovolumen bei. Die Dominanz von *M. helysia* resultierte in einer Senkung der Evenness und einem Verlust der Biodiversität. Da diese Art erst 2006 beschrieben wurde, ist bislang wenig über ihre Nährstoffanforderungen bekannt. Ich konnte experimentell darlegen, dass *M. helysia* ungeachtet des vorherrschenden N : Si Verhältnisses in einer artifiziellen Gemeinschaft die erfolgreichste Diatomee war. *M. helysia* weist demnach eine hohe Toleranz hinsichtlich unterschiedlicher N und Si Konzentrationen auf, welches − unter anderem − zu ihrer erfolgreichen Etablierung im Rückseitenwatt beitragen könnte.

In Kapitel 3 wurde die beobachtete zeitliche und räumliche Phytoplanktondynamik im Metagemeinschaftskontext betrachtet. Ich habe eine signifikante zeitliche und räumliche Heterogenität in der Anzahl der Arten gefunden. Da dies vor allem durch seltene Arten angetrieben wurde, zeigte die Dominanzstruktur des Phytoplanktons während des Großteil des Jahres keine signifikante räumliche Struktur. Die Variabilität in den Umweltfaktoren erklärte einen signifikanten Teil der Artzusammensetzung. Der zeitliche Artenumsatz war generell höher als der räumliche, wobei der Grad des räumlichen Artenumsatzes während des Jahres schwankte. Weiterhin war ein stark ausgeprägter saisonaler Artenumsatz erkennbar, der signifikant mit saisonalen Änderungen der Umweltbedingungen (PAR, P, N, Temperatur) verknüpft war. Die räumliche und zeitliche Heterogenität in der Phytoplanktongemeinschaft, die durch intrinsische wie extrinsische Faktoren bedingt sein kann und koexistenzfördernd ist, scheint durch *patch dynamics* und *species sorting* Mechanismen geprägt zu sein.

Desweiteren entwickelte ich eine standardisierte Methode für die Ablösung benthischer Diatomeen von Sedimentpartikeln unter Anwendung von Ultraschall und eines oberflächen-aktiven Tensids (Kapitel 4), um eine exakte quantitative Analyse von epipelischen und epipsammischen Diatomeen zu gewährleisten. Für eine mikroskopische Untersuchung müssen die benthischen Diatomeen von den Sedimentkörnern abgelöst werden. Dazu habe ich drei verschiedene Behandlungsmethoden evaluiert und daraus eine standardisierte Methode entwickelt. Eine Ultraschallsonde wurde für sechs definierte Behandlungsdauern (15 s bis 90 s) mit oder ohne Zugabe verschiedener Konzentrationen des oberflächen-aktiven Tensids appliziert. Eine Ultraschallbehandlung von 60 s erhöhte die Ablöserate signifikant um 60%. Eine Zugabe des Tensids (0.025% finale Konzentration) reduzierte bei gleichem Erfolg die Behandlungsdauer auf 45 s und damit die Wahrscheinlichkeit des Zellbruchs durch die Ultraschallbehandlung, weshalb diese Methode zu empfehlen ist.

LITERATURE

8. *LITERATURE*

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PHYTOPLANKTON MICROPHOTOGRAPHS

9. *PHYTOPLANKTON MICROPHOTOGRAPHS*

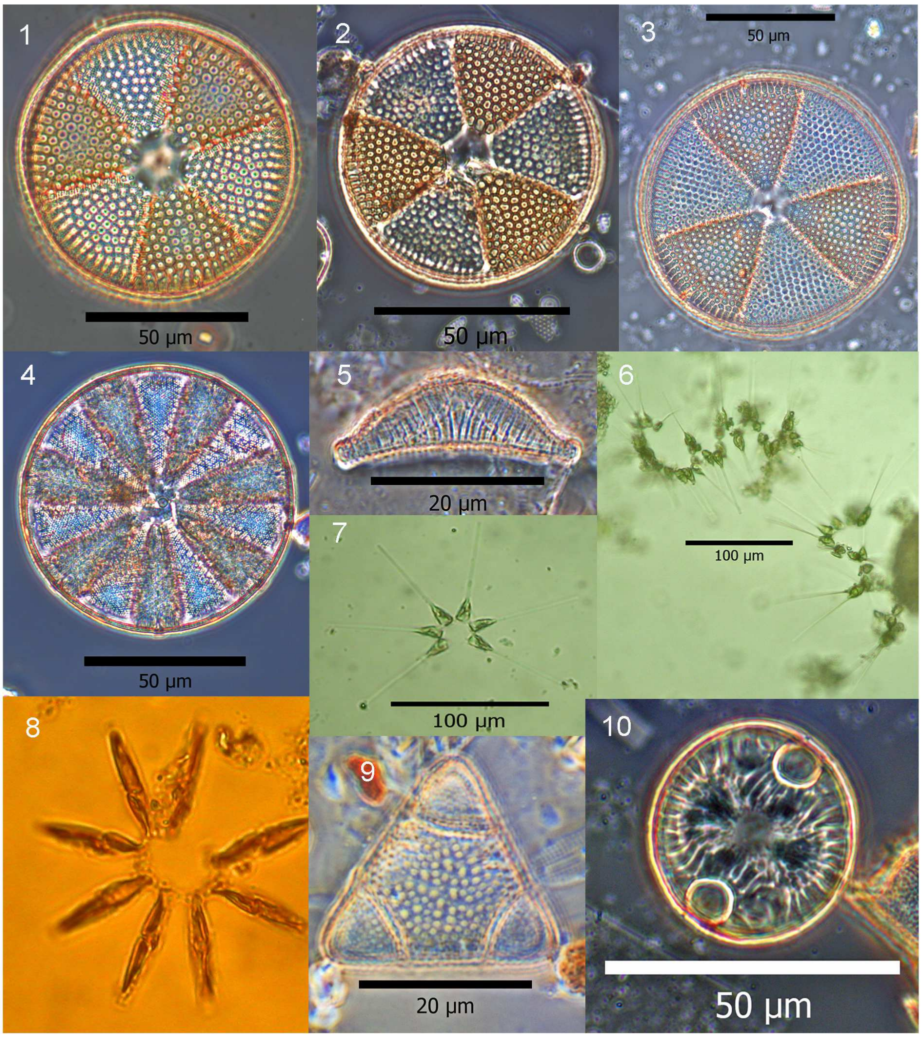

Table 1

1-3: *Actinoptychus senarius*
4: *Actinoptychus splendens*
5: *Achnanthes* sp.
6-7: *Asterionellopsis glacialis*
8: *Asteroplanus karianus*
9: *Biddulphia alternans*
10: *Auliscus sculptus*

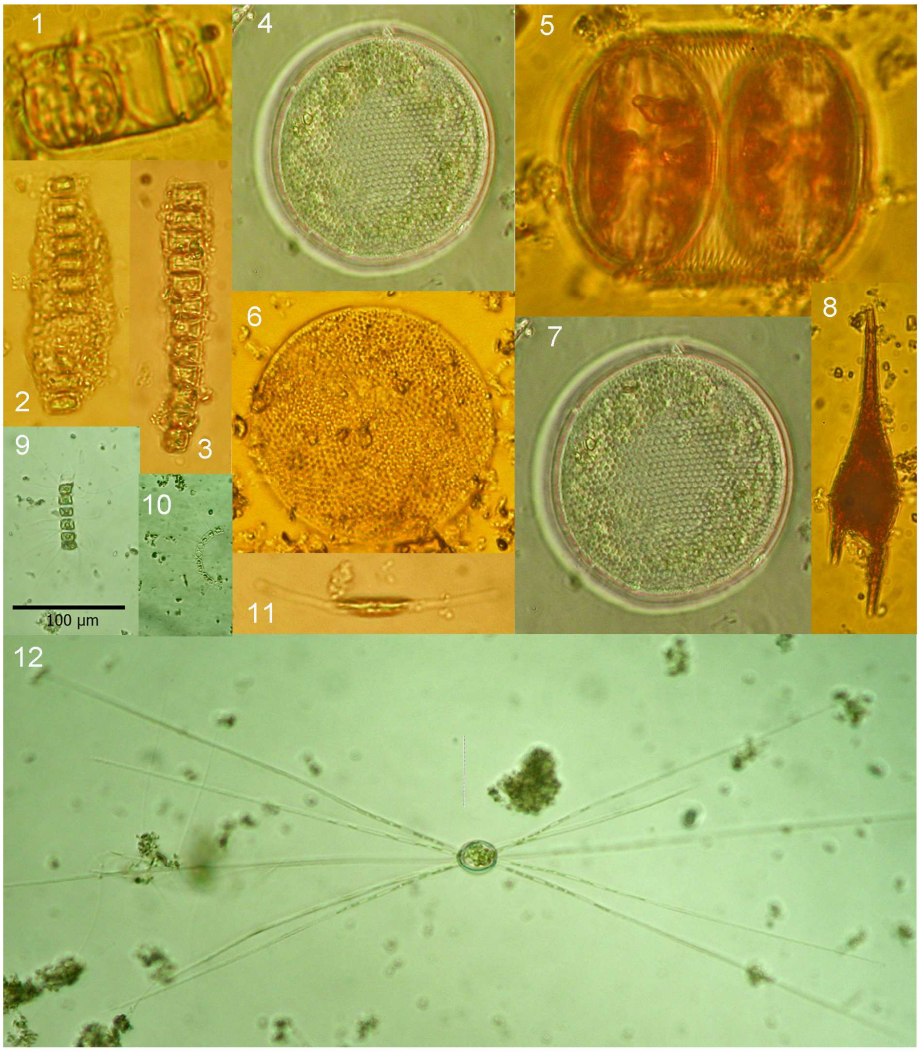

Table 2

1: *Biddulphia alternans*
2-3: *Brockmanniella brockmannii*
4-7: *Coscinodiscus* sp.
8: *Ceratium furca*
9: *Chaetoceros* sp.
10: *Chaetoceros debilis*
11: *Ceratoneis closterium*
12: *Chaetoceros densus*

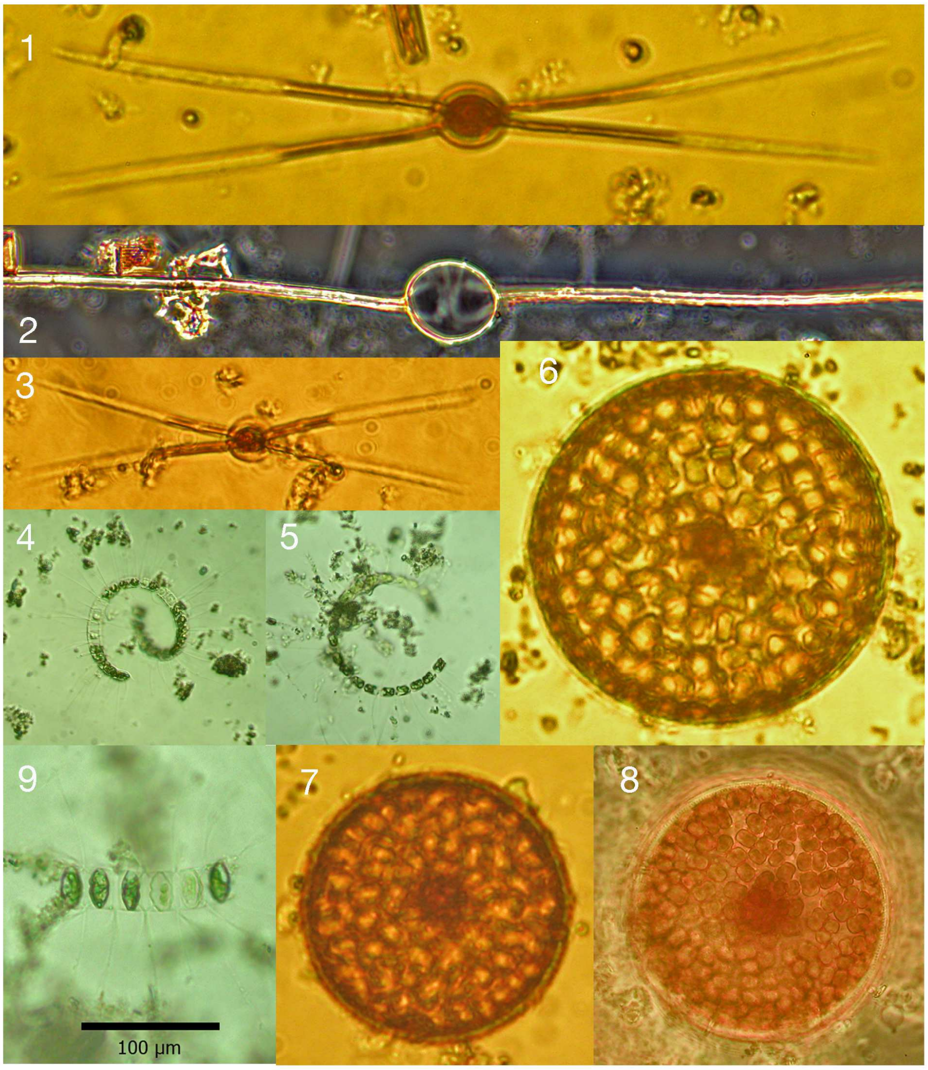

Table 3

1,3: *Chaetoceros danicus*
2: *Chaetoceros densus*
4-5: *Chaetoceros pseudocurvisetus*
6-8: *Thalassiosira* sp.
9: *Chaetoceros* sp.

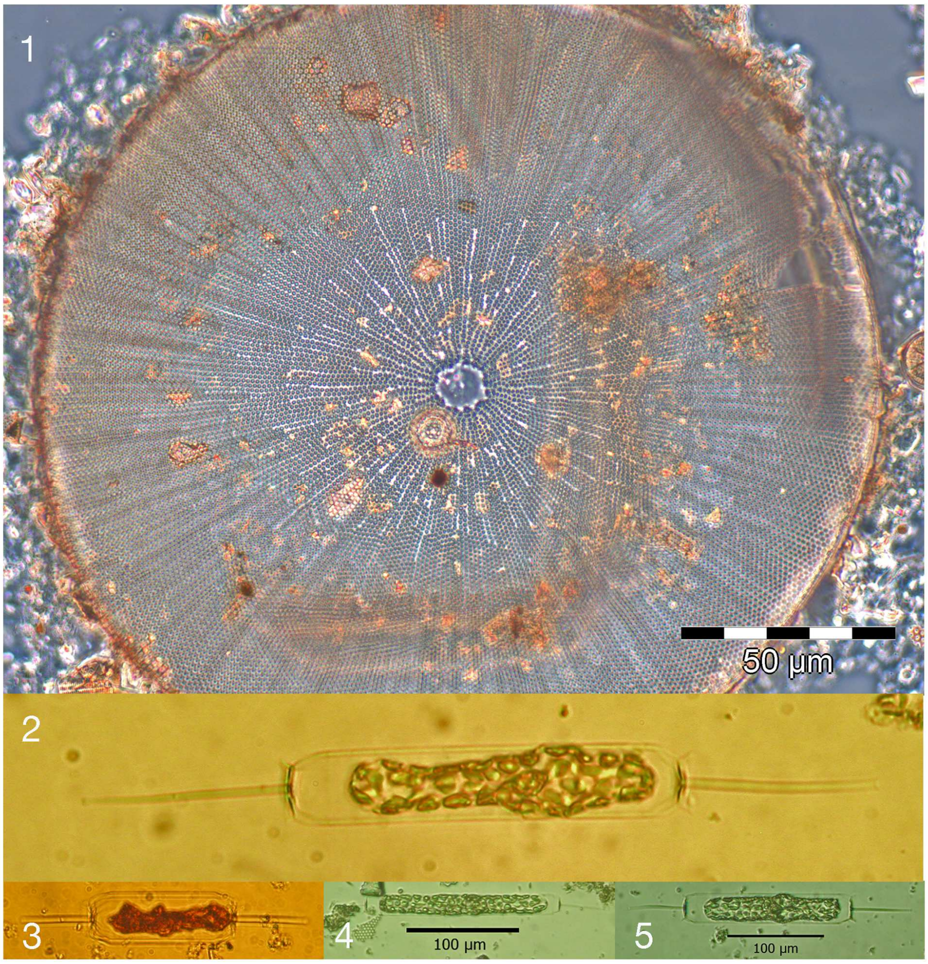

Table 4

1: *Coscinodiscus wailesii*
2-5: *Ditylum brightwellii*

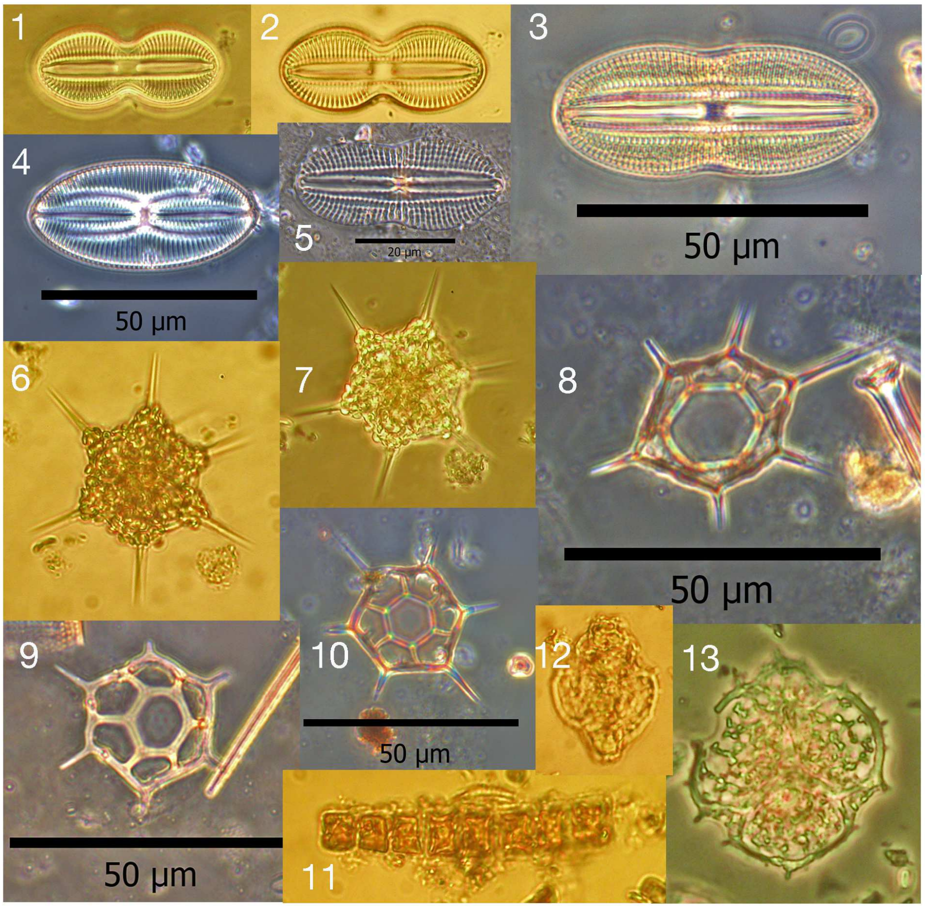

Table 5

1-4: *Diploneis bombus*
5: *Diploneis* sp.
6-10: *Distephanus speculum*
11: *Fragilaria* sp.
12-13: *Ebria tripartita*

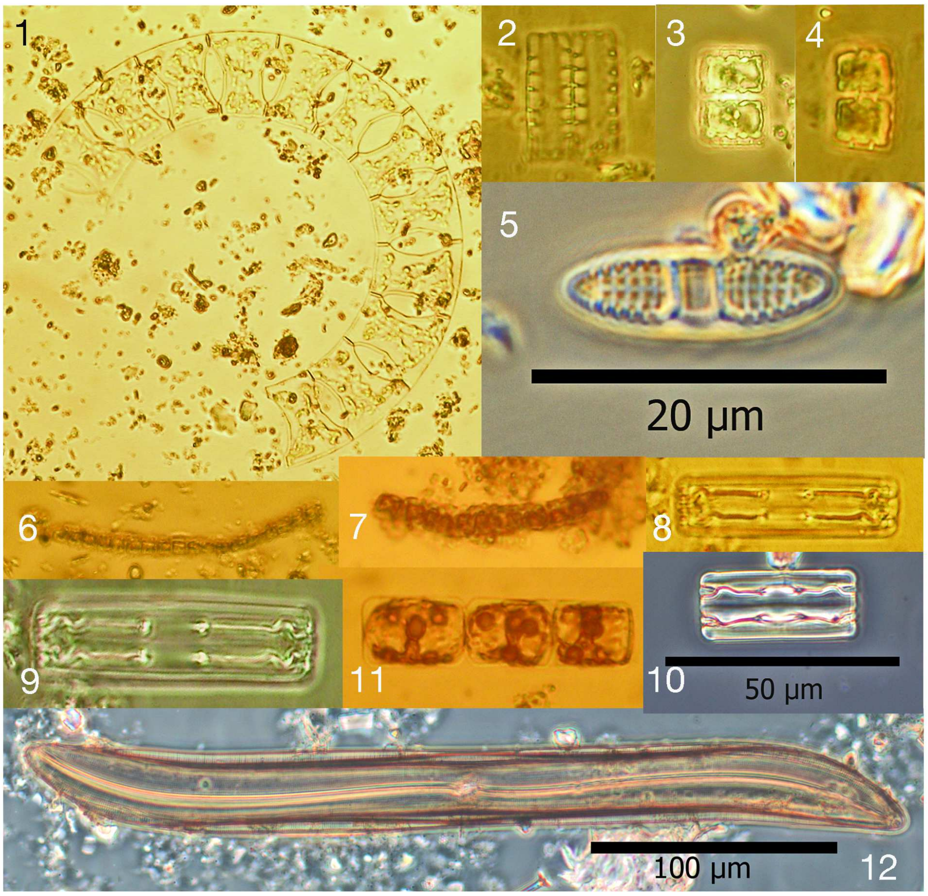

Table 6

1: *Eucampia zodiacus*
2-5: *Eunotogramma dubium*
6-7: *Fragilaria* sp.
8-10: *Grammatophora marina*
11: *Guinardia delicatula*
12: *Gyrosigma balticum*

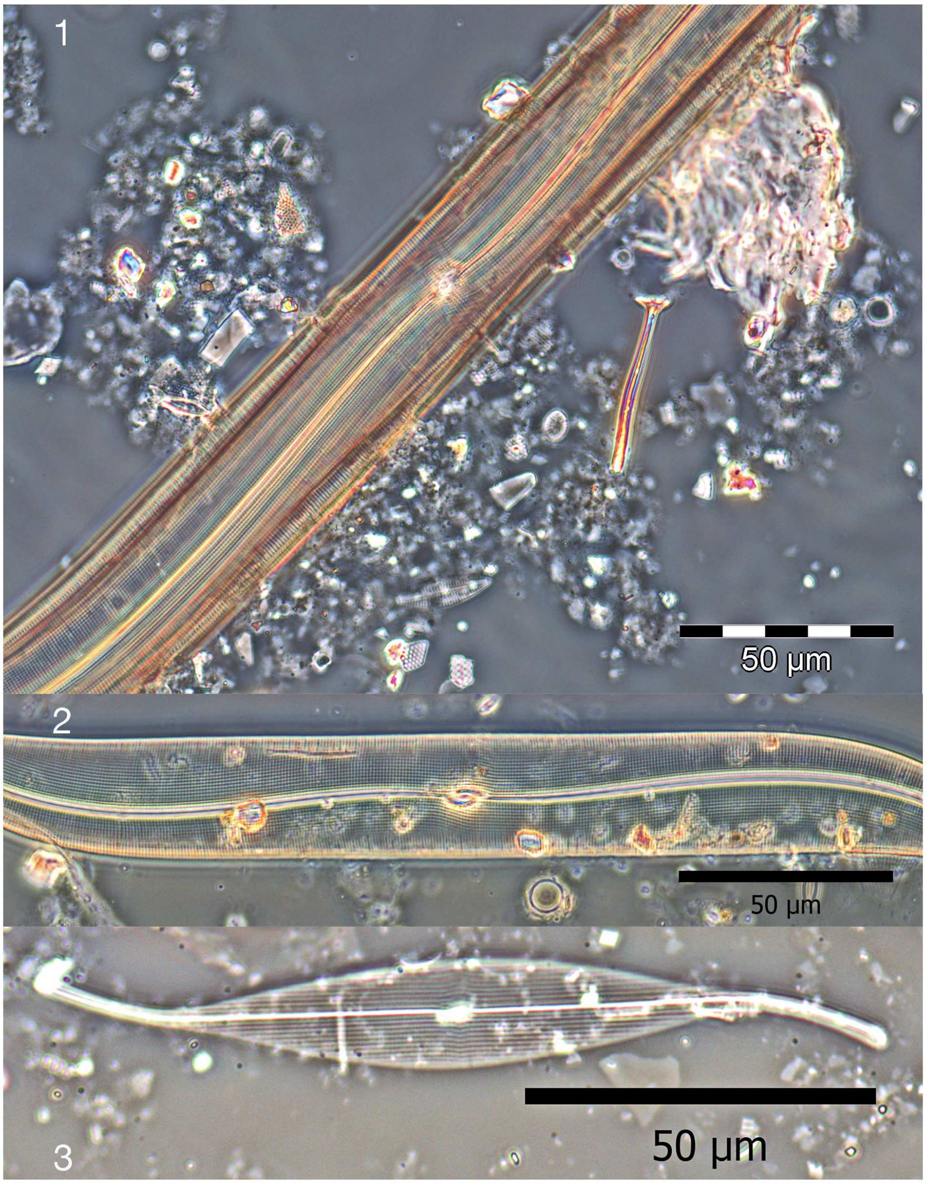

Table 7

1-2: *Gyrosigma balticum*
3: *Gyrosigma fasciola*

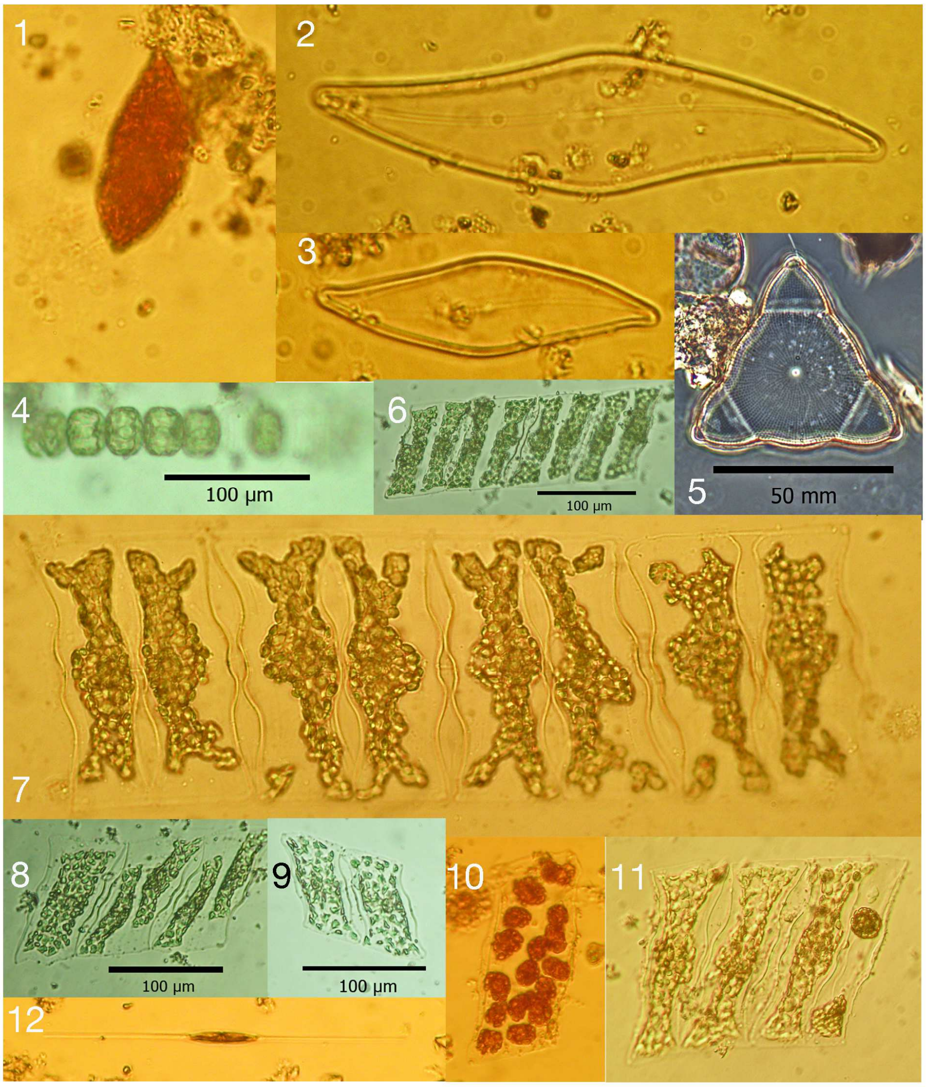

Table 8

1: *Gyrodinium* sp.
2-3: *Gyro- / Pleurosigma* sp.
4: *Lauderia borealis*
5: *Lithodesmium undulatum*
6-11: *Mediopyxis helysia*
9: *Paulsenella vonstoschii* cells in a *Mediopyxis helysia* cell
12: *Nitzschia longissima*

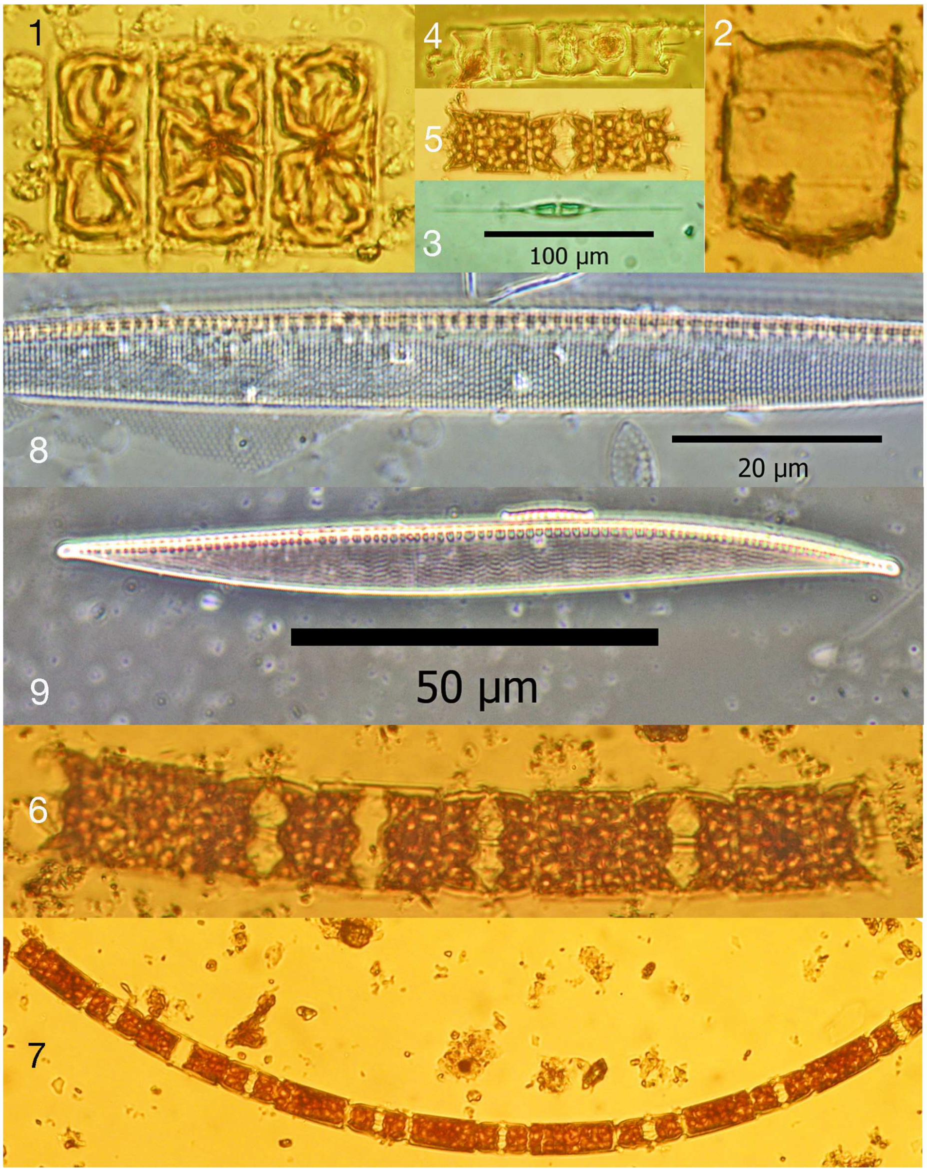

Table 9

1: *Meuneria membranacea*
2: *Odontella rhombus*
3: *Ceratoneis closterium*
4-7: *Odontella aurita*
8-9: *Nitzschia* sp.

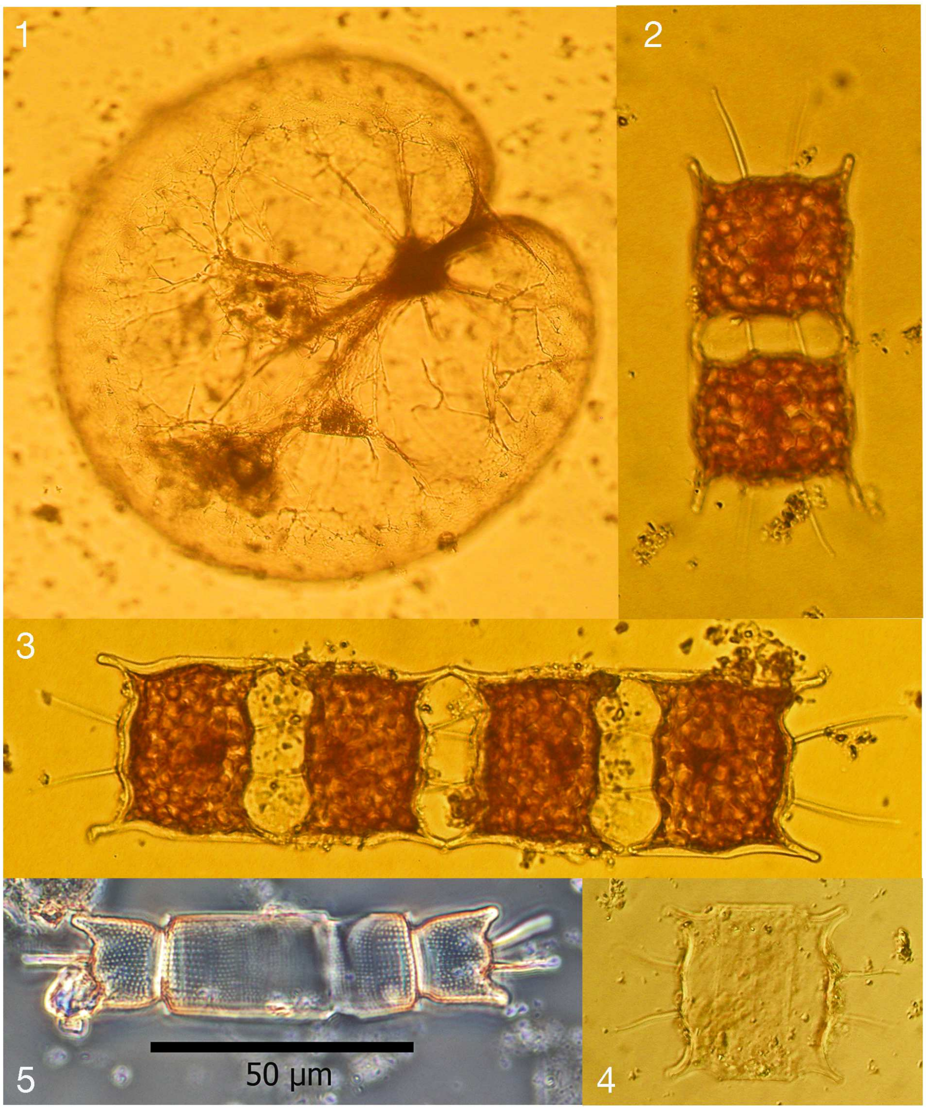

Table 10

1: *Noctiluca scintillans*
2-4: *Odontella mobiliensis / regia*
5: *Odontella aurita*

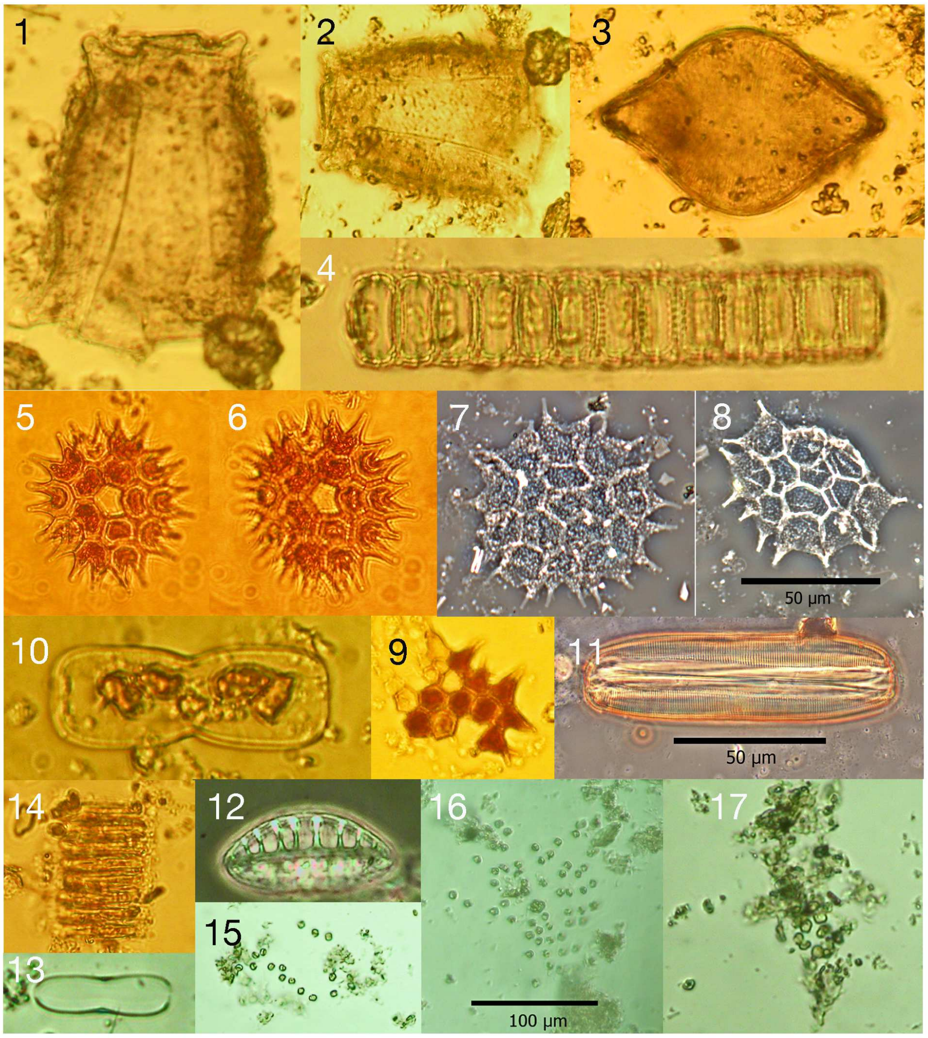

Table 11

1-3: *Odontella rhombus*
4: *Paralia sulcata*
5-8: *Pediastrum boryanum*
9: *Pediastrum kawraiskyi*
10-13: *Pennales* indet.
14: *Subsilicea fragilarioides*
15-17: *Phaeocystis* sp.

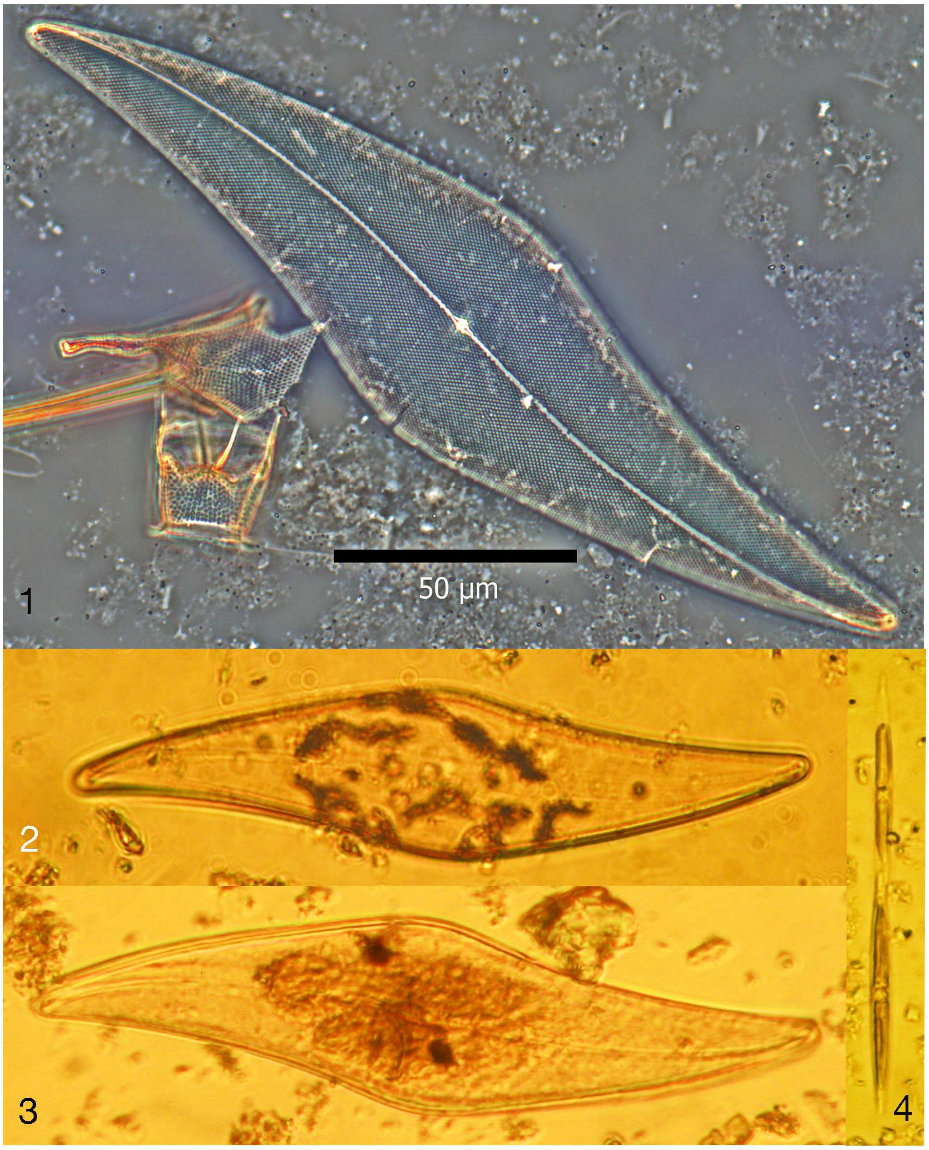

Table 12

1-3: *Pleurosigma* sp.
4: *Pseudonitzschia seriata*

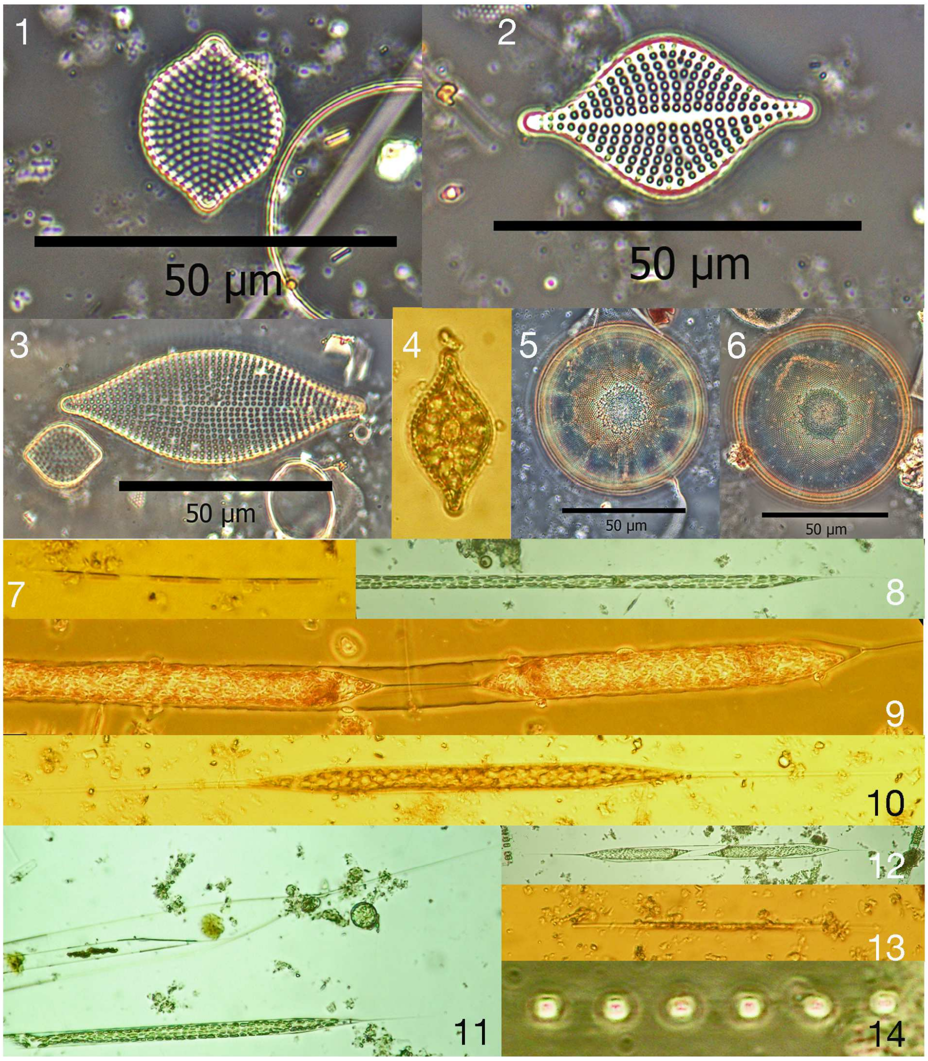

Table 13

1-4: *Rhaphoneis amphiceros*
5-6: *Podosira stelliger*
7: *Pseudonitzschia delicatissima*
8-12: *Rhizosolenia setigera*
13: *Rhizosolenia similis*
14: *Skeletonema costatum* complex

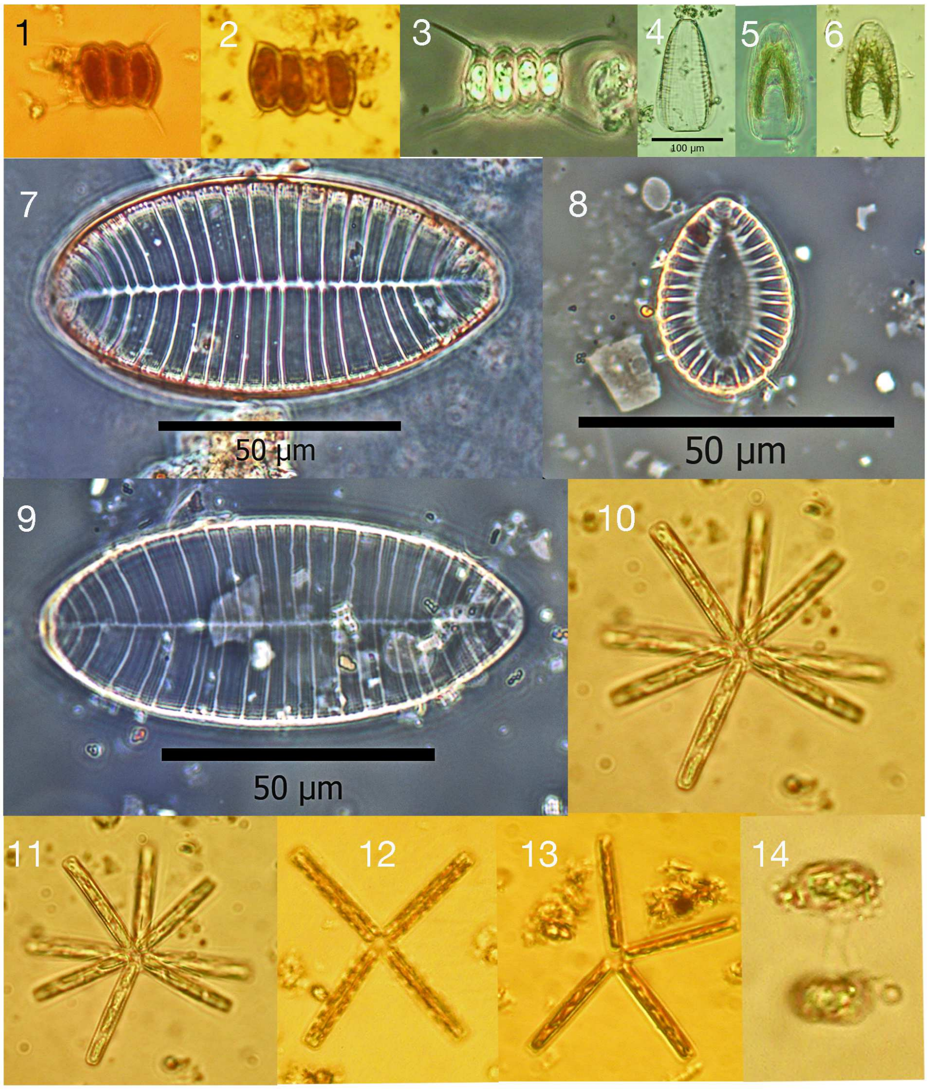

Table 14

1-3: *Scenedesmus* sp.
4-9: *Surirella* spp.
10-11: *Thalassionema frauenfeldii*
12-13: *Thalassionema nitzschioides*
14: *Thalassiosira curviseriata / tealata*

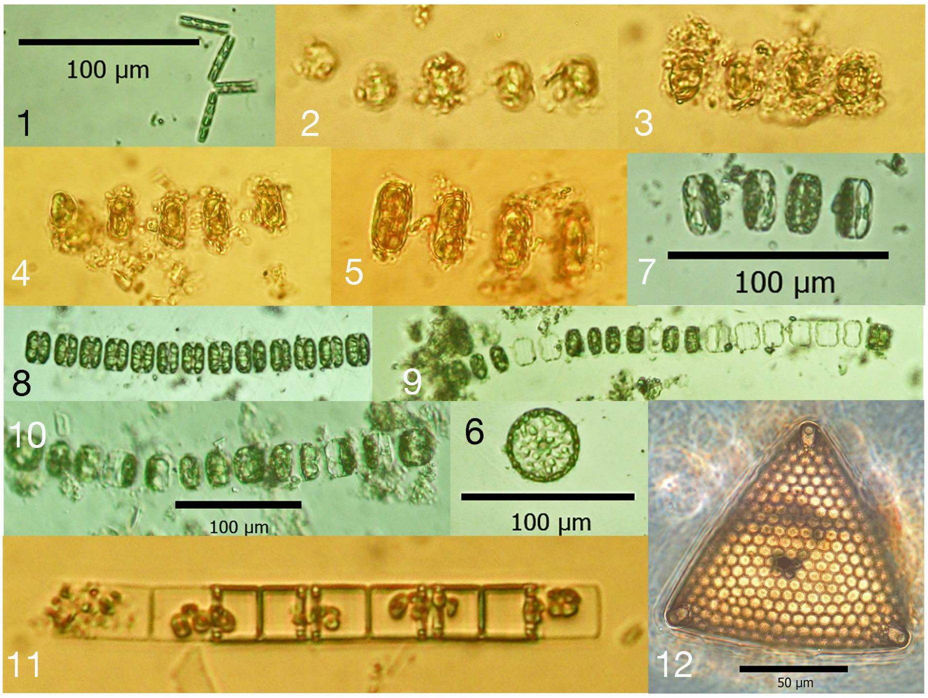

Table 15

1: *Thalassionema nitschioides*
2-4: *Thalassiosira curviseriata / tealata*
5-6: *Thalassiosira rotula*
7-10: *Thalassiosira nordenskioeldii*
11: indet. species
12: *Triceratium furca*

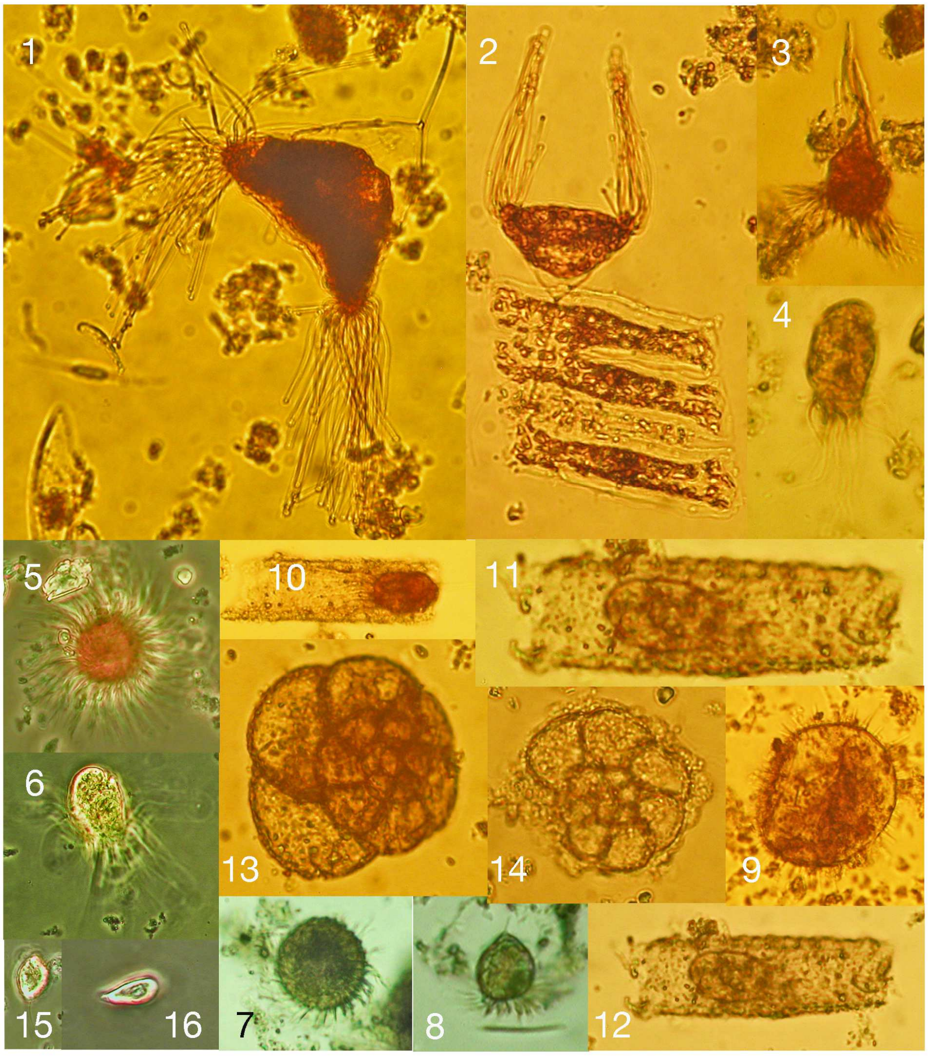

Table 16

1-2: *Acineta* sp. attached to a *Mediopyxis helysia* chain
3-9: different Ciliate species
10-12: Tintinnia indet.
13-14: Foraminifera indet.
15-16: Cryptophycea indet.

CURRICULUM
VITAE

10. CURRICULUM VITAE

Sandra Meier, born on 22 October 1980 in Krefeld

PROFESSIONAL EXPERIENCE

04|2013 – today	Researcher at the University of Helsinki, Finland, supervised by Dr. Janne Soininen
01|2009 – today	PhD student at the ICBM, University of Oldenburg. Doctoral thesis: 'Spatiotemporal dynamics of a phytoplankton metacommunity in a natural coastal system', supervised by Prof. Dr. Helmut Hillebrand

EDUCATION

07|2008	Diploma Diploma thesis: 'Phytoplankton dynamics in the tidal inlet Otzumer Balje', supervised by PD Dr. Holger Freund (ICBM, University of Oldenburg) and Prof. Dr. Richard Pott (University of Hanover)
04|2003 – 07|2008	Studies in biology at the Leibniz University of Hanover, Germany
10|2002 – 03|2003	Studies in biology at the Carl von Ossietzky University of Oldenburg, Germany
08|2000 – 06|2002	Apprenticeship as dairy industry lab assistant (Biolac GmbH, Harbarnsen, Germany)
06|2000	Abitur, Gymnasium Walsrode

DANKSAGUNG

11. *DANKSAGUNG*

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ERKLÄRUNG

ERKLÄRUNG

Hiermit bestätige ich, dass ich die von mir vorgelegte Dissertation selbstständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit − einschließlich Abbildungen und Tabellen − die ich anderen Werken im Wortlaut oder dem Sinn nach entnommen habe, in jedem Einzelfall kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorlag; dass sie − abgesehen von den im Folgenden angegebenen Teilpublikationen* − noch nicht veröffentlicht wurde sowie, dass ich solche Veröffentlichungen vor Abschluss des Promotionsverfahren nicht vornehmen werde. Die Leitlinien guter wissenschaftlicher Praxis an der Carl von Ossietzky Universität Oldenburg sind mir bekannt und wurden befolgt. Es wurden im Zusammenhang mit dem Promotionsverfahren keine kommerziellen Vermittlungs- und Beraterdienste (Promotionsberatung) in Anspruch genommen. Die Bestimmungen der Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation wurde von Prof. Dr. Helmut Hillebrand betreut.

KAPITEL 1

Dieses Kapitel beruht auf dem Manuskript: Meier S, Muijsers F, Badewien T, Beck M, Hillebrand H (2014) Temporal variability of the phytoplankton community in the backbarrier tidal flats of Spiekeroog. In prep., to be submitted. Friso Muijsers hat im Rahmen seiner Masterarbeit die Probenahme in 2010 durchgeführt und statistisch ausgewertet. Dr. Melanie Beck und Dr. Thomas Badewien stellten Daten des Messpfahls zur Verfügung.

KAPITEL 2*

Dieses Kapitel beruht auf dem Manuskript: Meier S, Muijsers F, Badewien T, Beck M, Hillebrand H (2014) Dominance of the non-indigenous diatom Mediopyxis helysia in Wadden Sea phytoplankton can be linked to broad tolerance to different Si and N supply. In review (Journal of Sea Research). Friso Muijsers hat im Rahmen seiner Masterarbeit die Probenahme in 2010 durchgeführt und ausgewertet. Dr. Melanie Beck und Dr. Thomas Badewien stellten Daten des Messpfahls zur Verfügung.

KAPITEL 3

Dieses Kapitel beruht auf dem Manuskript: Meier S, Muijsers F, Ptacnik R, Badewien T, Beck M, Hillebrand H (2014) Spatiotemporal turnover of a phytoplankton community in a natural coastal system. In prep., to be submitted. Friso Muijsers hat im Rahmen seiner Masterarbeit die Probenahme in 2010 durchgeführt, ausgewertet und war, wie auch Dr. Robert Ptacnik, bei der statistischen Analyse behilflich. Dr. Melanie Beck und Dr. Thomas Badewien stellten Daten des Messpfahls zur Verfügung.

KAPITEL 4

Dieses Kapitel beruht auf dem Manuskript: Meier, S., Sauer, S., Hillebrand, H. (2014)

Removing benthic diatoms from sediment particles for species composition analysis. In prep., to be submitted. Simon Sauer hat im Rahmen seiner Bachelorarbeit die Versuche durchgeführt und ausgewertet.

Ich führte die Probenahme in 2009 sowie das in Kapitel 2 beschriebene Laborexperiment durch und wertete diese aus. Zudem verfasste ich alle Manuskripte, deren Entstehungsprozess von Helmut Hillebrand begleitet wurde. Des Weiteren initiierte Helmut Hillebrand das Langzeitmonitoring und konzipierte den in Kapitel 2 beschriebenen Laborversuch.

Oldenburg, den 31.03.2014

(Sandra Meier)