Faculty of Environmental Sciences

Institute of Hydrobiology

Functional traits of phytoplankton communities, their dynamics and interactions with the abiotic environment – results from Rappbode Reservoir, Germany

DISSERTATION

in fulfillment of the requirements for the academic degree doctor rerum naturalium (Dr. rer. nat.)

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Date of oral defence: 22nd September 2020, Dresden

Declaration of conformity

I hereby confirm the accordance of this copy with the original dissertation on the topic: “Functional traits of phytoplankton communities, their dynamics and interactions with the abiotic environment – results from Rappbode Reservoir, Germany”

The thesis was submitted in November 2019 and defended on 22nd September 2020 in Dresden.

Valerie Wentzky

Flintbek, 11th December 2020

This dissertation is based on the following research articles:

A revised version has been published under the title: **Wentzky, V.C.**, Tittel, J., Jäger, C.G., Bruggeman, J., Rinke, K. (2020): Seasonal succession of functional traits in phytoplankton communities and their interaction with trophic state. *Journal of Ecology, 2020;00, 1–15*.

Wentzky, V.C., Tittel, J., Jäger, C.G., Rinke, K. (2018): Mechanisms preventing a decrease in phytoplankton biomass after phosphorus reductions in a German drinking water reservoir – results from more than 50 years of observation. *Freshwater Biology, 63(9), 1063-1076*.

Wentzky, V.C., Frassl, M.A., Rinke, K., Boehrer, B. (2019): Metalimnetic oxygen minimum and the presence of Planktothrix rubescens in a low-nutrient drinking water reservoir. *Water Research, 148, 208-218*.

Summary

Understanding and explaining spatio-temporal dynamics of ecological communities and their interaction with the environment is a central goal in ecology, but at the same time a very challenging task. Functional traits are a promising concept to achieve a better mechanistic understanding of variations in community structure. Traits are characteristics of organisms that directly or indirectly influence fitness and vary largely between different species. As traits functionally link communities with their bio-geochemical environment, they are well suited for explaining how community structure changes in response to changing environmental conditions, and how in turn communities shape their surrounding environment. Despite the potential of trait-based approaches to mechanistically explain the relationship between communities and their environment, they have rarely been applied in freshwater ecology. Therefore, this thesis explores spatio-temporal dynamics in functional traits of freshwater phytoplankton communities and their interaction with the environment, using the Rappbode Reservoir in Germany as a case study.

Phytoplankton community data from a seasonally resolved dataset with 50 years of observation from the Rappbode Reservoir were used to translate taxonomic composition into ecologically meaningful functional trait values. The studied functional traits showed consistent, reoccurring seasonal developments that clearly mirrored environmental pressures over the year. From late autumn to spring nutrients and turbulence were high. Hence small celled, fast growing species that are able to rapidly incorporate existing nutrients and tolerate poor light conditions dominated (dominant traits: maximum growth rate, light affinity). In contrast, when turbulence and nutrients were low in summer, large cell size and more complex mechanisms to efficiently exploit mineral nutrients or acquire previously unexploited nutrient pools were key ecological strategies (dominant traits: phosphate affinity, mixotrophy, motility, nitrogen fixation). The similarity in observed trait patterns over several years indicates that despite the diversity and complexity of phytoplankton species dynamics, the seasonal succession is a highly ordered, predictable process, driven by trade-offs between different ecological strategies.

During the 50 years of observation, nutrient concentrations in the Rappbode Reservoir decreased strongly (oligotrophication). A comparison between nutrient-rich and nutrient-deficient years revealed that the general intra-annual succession patterns of phytoplankton functional traits remained the same. However, the intra-annual succession patterns were more pronounced in nutrient-rich years. While functional community composition in summer changed little after oligotrophication, spring communities were largely affected by nutrient reductions and functional traits in spring became more similar to the ones in summer. Based on the quantitative analysis of functional traits, the thesis could generalize the existing patterns to provide a quantitative, functional template for seasonal succession patterns in lake ecosystems under nutrient-rich and nutrient-deficient conditions.

Over the entire observation period, phytoplankton biomass did not respond to reduced phosphorus concentrations. This unexpected resistance of algal biomass against reductions in the limiting resource phosphorus was caused by changes in internal processes and functional traits of the phytoplankton community, allowing them to adapt to lower nutrient levels without a loss in total biomass. The main casual mechanism for the decoupling of inorganic nutrients from phytoplankton biomasses was an increase in phagotrophic mixotrophs and their ability to make bacterial phosphorus available for the entire phytoplankton community. Further, in nutrient-deficient years biomass losses in spring were compensated by high biomasses in summer. Higher summer biomasses compared to spring biomasses in nutrient-deficient years were probably also linked to a decrease in diatoms in spring. Diatoms are characterized by high sinking velocities. This trait in combination with phosphorus uptake by diatom cells leads to high downward nutrient exports from the pelagic zone. The decrease of diatoms in nutrient-poor years was related to less phosphorus losses over the season, leaving more nutrients for summer phytoplankton. The effect of species with high sinking velocities on seasonal phosphorus processing illustrates the importance of functional trait composition on biogeochemical cycling.

A one-year monitoring campaign with a high temporal and depth resolution at Rappbode Reservoir allowed to investigate the impact of vertical phytoplankton trait distributions on the geochemical environment. During summer, a phytoplankton mass development occurred at the depth of the metalimnion, mainly represented by the cyanobacterium *Planktothrix rubescen*s. The positive net growth at larger depth of this metalimnetic species is enabled through the possession of specific traits, e.g. buoyancy regulation and the ability for efficient light harvesting. The data indicated that the occurrence of metalimnetic species was connected to the formation of a metalimnetic oxygen minimum. The oxygen minimum occurred after the metalimnetic algae peak disappeared from the thermocline. Metalimnetic phytoplankton induced oxygen depletion probably through one of the

following processes: Oxygen consumption by bacteria related to the degradation of dead organic material from metalimnetic algae or internal respiration of metalimnetic algae towards the end of the growing season leading to net oxygen losses due to the continuation of algal respiration. This constitutes a previously undiscovered mechanism for the development of metalimnetic oxygen minima.

In conclusion, the thesis illustrates how trait-based approaches enhance ecological understanding of phytoplankton community dynamics. The trait-based approaches shed light on how phytoplankton communities respond to environmental gradients and how communities can affect their geochemical environment. The thesis also shows that functional traits can be used to reduce the complexity of communities through converting species information into ecologically meaningful functions and that they allow to link changes in community composition to corresponding features in the biogeochemical environment. The quantitative, trait-based approaches used in this study therefore improve our mechanistic understanding of community dynamics and are a step forward to higher predictability and generality in limnology.

Contents

1 Scientific background

1.1 The importance of phytoplankton

Phytoplankton are unicellular, photoautotrophic organisms drifting with the currents in the water bodies of lakes, rivers and the ocean. Phytoplankters are too small to be seen individually with the unaided eye, but they are highly diverse, varying from photosynthesizing prokaryotic bacteria (cyanobacteria) to eukaryotic diatoms and green algae. Cyanobacteria were the first primary producers that evolved at least 2.7 billion years ago, when the atmosphere of the earth was still anoxic (Bekker *et al.*, 2004; Holland, 2006). It was their photosynthetic activity that converted carbon dioxide to oxygen and organic carbon. Oxygen was released into the atmosphere and thus provided the foundation for the evolution of aerobic, heterotrophic life. Later in time, other clades of phytoplankton developed through endosymbiosis, such as red and green algae, followed by diatoms and dinoflagellates (Falkowski *et al.*, 2004). They further contributed to oxygen release and when the first terrestrial plants evolved about 500 million years ago the concentration of oxygen in the atmosphere was already more than 50 percent of today’s level (Wellman, Osterloff & Mohiuddin, 2003; Holland, 2006).

Phytoplankton is a major primary producer on the global scale and is important to global-scale biogeochemical cycling of elements in freshwater and marine ecosystems (Falkowski, Barber & Smetacek, 1998; Field *et al.*, 1998). As phytoplankters provide the basal energy input to pelagic food chains, they represent a main component of aquatic ecosystems and their abundance and community structure largely affect higher trophic levels (Hallegraeff, 1993; Chassot *et al.*, 2010). Despite the local and global importance of phytoplankton, their excessive growth has severe negative consequences. Algal blooms can cause anoxia (Diaz & Rosenberg, 2008), fish kills (Shumway, 1990; Burkholder, Glasgow Jr & Hobbs, 1995) and problems for drinking water quality (Qin *et al.*, 2010). A good understanding of phytoplankton distribution and dynamics, their response to changes in the environment as well as their impact on ecosystem processes is therefore crucial to face today´s challenges for aquatic ecosystems, such as climate change, altered nutrient loads and the assurance of high drinking water quality (Paerl & Huisman, 2008).

1.2 Conceptual approaches for phytoplankton communities

1.2.1 Taxonomic approaches

The simplest and most commonly used way to approach phytoplankton communities is by aggregation to total biomass (or alternatively to total chlorophyll a), which provides satisfactory quantification for many purposes (Vollenweider, 1971; Scheffer *et al.*, 2003; Sommer *et al.*, 1986). Nonetheless, phytoplankton communities can be extremely diverse, often consisting of many different species with diverse ecological properties and functions. For instance, phytoplankter vary from small picocyanobacteria of less than 2 µm length (e.g. *Synenococcus*) to large dinoflagellates, such as *Ceratium hirundinella* with a size of up to 400 µm. Some species consist of single cells (e.g. *Cryptopmonas*), others of chains (e.g. *Planktothrix.*) or loose colonies (e.g. *Microcystis*), some are able to move through flagella (e.g. *Chlamydomonas*) or gas vacuoles (e.g. *Aphanizomenon*), and others are able to fix atmospheric nitrogen to overcome nitrogen limitation (cyanobacteria such as *Anabaena*). Species also differ in their physiological processes, e.g. in their growth rates (Banse, 1976) or light affinities (Schwaderer *et al.*, 2011). However, the analysis of phytoplankton communities at biomass level does not consider this diversity and gives no information about structural shifts in the plankton community, which is often necessary to understand the response of plankton to external factors (Schindler, 1990) and their impact on higher trophic levels and geochemical cycling. The species composition is also highly relevant for water management, human health and finally also has economical relevance, since some species produce toxins (e.g. cyanobacteria such as *Planktothrix*) or taste and odor (e.g. *Synura*), while others are rather harmless (Carmichael, 2001; Paterson *et al.*, 2004). Therefore it is sometimes inevitable to take a closer look at the phytoplankton community through the lens of a microscope.

Characterizing phytoplankton communities by their species composition (Tilman, Kilham & Kilham, 1982), gives the most complete information in terms of taxonomy, but a functional information can only be derived once the niche of a species is understood. However, since predictions at species level are difficult or maybe even impossible (Reynolds, 2000) and general principles about whole communities can rarely be derived (Simberloff, 2004), numerous attempts have been made to aggregate the high number of species into fewer ecologically meaningful groups. Traditionally, freshwater phytoplankton species have been grouped according to their broader taxonomic affiliation into diatoms, cyanobacteria, chlorophytes, cryptophytes, dinoflagellates, chrysophytes and desmids (Kalff, 2002). These coarse aggregations allowed for a few generalizations, e.g. the decrease in chrysophytes in nutrient rich lakes (Jeppesen *et al.*, 2005) or the increase in cyanobacteria with lake warming (Winder & Sommer, 2012) and eutrophication (Jeppesen *et al.*, 2005). However, taxonomic classifications have the drawback that the ecological functions of the species are heterogenous within these higher taxonomic units and hence often do not reflect their ecological niche. On the one hand a taxonomic group might include species with very different ecological adaptations, since even very closely related species can be functionally very different. For example, *Synura petersenii* releases ketones and aldehydes from their cells that cause a fish-like odor and taste, while there is no such evidence from other *Synura* species (Nicholls & Gerrath, 1985). Another example is the taxonomic group of diatoms, which contains species with small single cells, while others form large colonies or chains. On the other hand species from different taxonomic groups can share the same ecological strategy and hence functions can overlap between different groups (Salmaso, Naselli-Flores & Padisák, 2015). For instance, the mixotrophic strategy occurs in species from several taxonomic groups, including cryptophytes, chrysophytes and dinoflagellates, but not all species in these groups are mixotrophs.

1.2.2 Empirically derived functional groups

To overcome the limitations of taxonomic classifications, more recent approaches classify phytoplankton based on specific functional properties that species share. A classification according to function might be superior in describing the role of phytoplankton communities in an ecosystem and predicting their response to changing environments (Litchman & Klausmeier, 2008). One method of establishing functional groups is based on the assumption that species with similar functional properties often occur simultaneously and have similar environmental sensitivities and tolerances (Reynolds, 1980). When species increase or decrease in abundance at the same time or place, they may be grouped together, as they are assumed to respond similarly to changing environmental conditions. Hence, the occurrence of a certain functional group reflects the physical, chemical or biological conditions of the habitat and vice versa the habitat characteristics provide information about the occurrence of certain functional groups. The method of functional grouping was first applied in freshwater ecology by Reynolds (1980), who identified 14 functional groups of phytoplankton based on observations from field data. This system was later expanded

(Reynolds, 1984a) and now includes 40 associations described by Reynolds *et al.* (2002) and Padisák, Crossetti and Naselli-Flores (2008). Reynold´s approach of functional groups has been shown to give a good proxy for phytoplankton responses to environmental conditions (Kruk *et al.*, 2017; Kruk *et al.*, 2002) and has been used to describe seasonal developments (Rychtecký & Znachor, 2010). Since groups of species in Reynolds´s approach were identified based on similar dynamic behavior of species in the observed lakes, the classification is a posteriori and empirically derived.

1.2.3 Trait based approaches

Functional groups can also be derived a priori based on specific properties (e.g. morphology, behavior) or physiologic capabilities that species possess (Salmaso & Padisák, 2007; Litchman & Klausmeier, 2008). These morphological, behavioral and physiological characteristics of organisms can be called traits. Traits directly or indirectly influence fitness (growth, reproduction or survival) and largely differ between various algal species (Violle *et al.*, 2007). Traits evolved in response to abiotic conditions and biotic interactions and are therefore differently distributed across environmental gradients (Zakharova, Meyer & Seifan, 2019). Functional approaches that are based on the measurement of traits have been developed in terrestrial ecology (Grime, 1977; Weiher & Keddy, 1995; Díaz & Cabido, 1997; Chapin *et al.*, 1996; Weiher *et al.*, 1999; McGill *et al.*, 2006) and these concepts were later adopted to aquatic ecosystems (Weithoff, 2003; Litchman & Klausmeier, 2008). An early example for the classification based on functional properties of phytoplankton species is the C-R-S concept, where C represents the competitive, R the ruderal and S the stress tolerant strategy (Reynolds, 1988). This approach is trait-based since the functional groups were derived based on common features, e.g. functional growth and morphometric attributes. Another example is the work by Salmaso and Padisák (2007), who used morphological and functional characteristics (size, form, mobility, nutrient requirements, mixotrophy) to classify species into discrete functional groups with the use of multivariate methods.

Besides the usage of traits to derive functional groups, functional traits can be examined individually, e.g. in order to obtain averaged trait values for the whole community. While for discrete functional groups species are grouped because they use similar strategies, the use of continuous functional traits focuses on the similar characteristics underlying those strategies (Zakharova, Meyer & Seifan, 2019). Important functional traits which directly influence fitness of phytoplankton are maximum growth rate, nutrient affinity, light affinity and grazing resistance (Litchman & Klausmeier, 2008; Schwaderer *et al.*, 2011; Edwards *et al.*, 2012). Information about these traits have mainly been derived from physiological measurements on species grown in laboratory cultures. Obtaining these traits is very laborious and it is therefore notoriously difficult to get a complete list of trait values for all species (Edwards *et al.*, 2012). In order to bridge these gaps, models have been developed to estimate missing trait values (e.g. Bruggeman, Heringa & Brandt, 2009; Bruggeman, 2011; Edwards, 2016).

Most studies however choose functional traits that are easier to measure, e.g. morphological traits such as mobility, cell size or shape (e.g. Weithoff, 2003; Kruk *et al.*, 2011; Stanca, Cellamare & Basset, 2012; Acevedo-Trejos *et al.*, 2015). These morphological and behavioral traits indirectly influence fitness, since they are related to many physiological traits. For instance, cell size sets the physical and physiological limits to many processes within cells and can be used as some kind of master trait (Litchman *et al.*, 2010). Cell size is moreover positively correlated with grazing resistance (Thingstad *et al.*, 2005) and nutrient uptake affinity (Litchman, Edwards & Klausmeier, 2015), but scales negatively with maximum growth rate (Banse, 1976) and light affinity (Edwards *et al.*, 2015).

Though trait-based approaches are still in their early stages, characterizing communities by their trait distributions has been argued to give new insights into the temporal and spatial dynamics of plankton communities, reveal mechanisms that structure biological communities and facilitate scaling physiological processes to global scales (Litchman *et al.*, 2007; Litchman & Klausmeier, 2008; Zakharova, Meyer & Seifan, 2019). The use of functional traits has also been suggested to create a more quantitative and predictive community ecology that is capable of addressing issues of increasing relevance, such as the consequences of land-use and climate change for ecosystem processes (McGill *et al.*, 2006). Especially ecological modelling may benefit from trait based approaches, since mean trait values are less complex and difficult to predict than the dynamics of species (e.g. Wirtz & Eckhardt, 1996; Acevedo-Trejos *et al.*, 2015; Berge *et al.*, 2017). Trait-based libraries can also be used to identify relevant parameter values for phytoplankton community modules within lake models and by that enhance the transferability of such models. Hence rebuilding community ecology from functional traits may help to reduce the complex nature of communities while keeping important ecological information. This can help to find general rules, which is the central goal of science (McGill *et al.*, 2006).

1.3 Seasonal, vertical and inter-annual changes in phytoplankton communities

As species differ in their ecological preferences and functional traits, they respond differently to changing environmental conditions (Levasseur, 1984; Sterner, 1989). In freshwater ecosystems, major gradients in the abiotic environment exist along the seasonal and inter-annual time scale as well as along the vertical axis. The seasonal and vertical gradients are mainly caused by variations in nutrient and light availability as well as temperature.

Temperature is a main driver of vertical and seasonal heterogeneity. Towards summer, increasing surface water temperature leads to thermal stratification due to a change in water density with temperature. Seasonal thermal stratification is typical for lakes and reservoirs in temperate regions. It divides the water body into three layers: The warm top layer (epilimnion), the cold bottom layer (hypolimnion) and the layer in between (metalimnion or thermocline), which contains a steep temperature gradient (Boehrer & Schultze, 2008). The steep density gradient in a stratified lake implies that vertical exchange across gradients is limited and requires large mixing energy. As a consequence, vertical gradients of many environmental variables remain stable over the warm season and can only disappear by mixing when autumnal cooling has removed or diminished the temperature gradient.

Vertical fluxes of solutes, e.g. nutrients, are small during the stratified season. As nutrients are usually supplied from the sediment or deep unproductive layers, the water body is separated into the nutrient poor epilimnion and a nutrient richer hypolimnion. This changes in autumn when decreasing surface temperatures lead to an increasingly deeper recirculation in the lake through successive inclusion of deep water layers and their nutrients into the upper mixed water layers. Finally, nutrients become rather homogenously distributed over the water column once the recirculation has included the entire water body. These seasonal and vertical variations in environmental conditions shape seasonally re-occurring spatio-temporal dynamics in phytoplankton communities.

1.3.1 Seasonal developments

Understanding the distribution of species along the seasonal gradient has long been a major focus of freshwater biologists. The most popular and widely cited theoretical models about seasonal succession of phytoplankton species were developed by Margalef (1978) and by the plankton ecology group (PEG model; Sommer *et al.*, 1986). Margalef (1978) described seasonal succession by the occurrence of different life-forms, which are placed in a nutrient and turbulence space. Each group is associated with a different regime and ecological strategy (r- versus K-strategists).

The concept for the r and K selection strategies was first proposed in terrestrial ecology by Wilson and MacArthur (1967) and Margalef (1978) applied it to phytoplankton. Small sized phytoplankton species with high maximum growth rates, the ability to respond quickly to the availability of environmental resources are categorized as r-strategists. In contrast K-strategists are characterized by slower growth rates and higher nutrient affinities and thus higher tolerance against resource limitation. Margalef (1978) predicts that nutrient rich environments with high turbulence, as they exist during mixing in spring, select for r-strategists, while K-strategists are selected in summer under nutrient-poor, stratified conditions. The r-K selection strategy was applied to explain phytoplankton distribution patterns (e.g. Kilham & Hecky, 1988; Sommer, 1981) and was later extended by Reynolds (1988) to the C-R-S model.

In 1986, the plankton ecology group (PEG) model was developed (Sommer *et al.*, 1986). It is a conceptual model describing the factors driving seasonal changes and providing a verbal template for the intra-annual development of phyto- and zooplankton biomass and community composition in eutrophic and oligotrophic (deep) lakes of the temperate zone. According to the PEG model, in winter and early spring phytoplankton growth is limited by light availability, since turbulence is excessive and algal cells get dispersed out of the photic zone. Due to the deep mixing, cells spend most of the time in the dark and cannot absorb enough light for growth, even though ample nutrients are available (Sverdrup, 1953). As soon as temperature increases and stratification sets on, phytoplankton spends a greater proportion of time closer to the surface in better light conditions and phytoplankton blooms can develop (Sverdrup, 1953). The spring bloom mainly consists of fast-growing, small, edible species with high tolerances towards low light (Sommer *et al.*, 1986; Reynolds *et al.*, 2002). Due to the intensive algal growth, biomass is produced which zooplankton can feed on and zooplankton biomass can increase. Nutrient depletion and high grazing pressure result in a breakdown of the spring bloom and phytoplankton biomass drops to very low levels for an extended period, called the clearwater phase (Lampert *et al.*, 1986). After this clearwater phase, algae develop that can cope with the increased nutrient limitation and grazing pressure: The community composition then shifts towards larger, inedible cells with higher grazing resistance and higher nutrient competitive ability (Sommer *et al.*, 1986). With decreasing temperatures in autumn, complete mixing of the water column recharges the surface layers with nutrients, which may induce another small growth phase of fast growing algae. But ultimately low light conditions during the mixing phase hamper phytoplankton growth. The description of drivers of plankton seasonal succession in the PEG model was later extended by other factors, such as food quality limitation and the role of parasites (Sommer *et al.*, 2012).

1.3.2 Vertical distribution and deep chlorophyll maxima

Along the vertical axis, the distribution of phytoplankton species is strongly shaped by a trade-off between light and nutrient availability (Klausmeier & Litchman, 2001; Jäger, Diehl & Emans, 2010; Mellard *et al.*, 2011). Theoretical studies predict that different species with different competitive abilities can coexist along the vertical gradient (Huisman & Weissing, 1995; Yoshiyama *et al.*, 2009; Ryabov & Blasius, 2011; Kerimoglu, Straile & Peeters, 2012). These predictions are supported by field enclosure experiments (Jäger, Diehl & Schmidt, 2008) and a survey of lakes in southern Québec (Beisner & Longhi, 2013), indicating vertical niche partitioning of algal species along opposing gradients of light and nutrients in the water column. The proportion of algae with high maximum growth rates, high metabolic respiration, better nutrient utilization, but lower nutrient storage capacity and lower sinking velocity decreases along the vertical gradient from the surface to the bottom layer (Jäger, Diehl & Emans, 2010).

A special feature of vertical phytoplankton distributions are deep chlorophyll maxima (DCM). A DCM is an absolute maximum of phytoplankton biomass along the vertical profile that can be found in deep layers of stratified lakes. DCMs usually form in the metalimnion or in the upper hypolimnion during the stratification period in summer. The formation of these maxima would not be possible during the mixing period, since turbulence prevents the accumulation of phytoplankton at a certain depth (Camacho, 2006; Leach *et al.*, 2017). DCMs are usually found in meso- and oligotrophic lakes, since in these lakes nutrients get depleted in the epilimnion during the stratified period, allowing light to penetrate deeper into the meta- and upper hypolimnion (Reynolds, 1992; Moll, Brache & Peterson, 1984). There the simultaneous availability of inorganic nutrients (from the sediments or from recycling of organic material that settled down from upper layers) and sufficient light from above favors the growth of certain phytoplankton species that are adapted to the low light conditions in this habitat (Gervais, 1997; Gong *et al.*, 2015). Populations of these species can accumulate in high densities in the metalimnion, while the growth of possible competitors is inhibited by low light availability. In addition to the vertical distribution of resources, different grazing pressure in the different lake strata have been proposed as a mechanism forming DCMs (e.g. Tittel *et al.*, 2003). Hartwich, Wacker and Weithoff (2010) suggested that a lower food quality of algae in deeper layers prevents zooplankton from exploiting this quantitatively rich food resource, contributing to the formation of the DCM. However, modelling studies claim that zooplankton grazing determines the magnitude of the DCM, but do not initiate its formation (White & Matsumoto, 2012; Pannard, Planas & Beisner, 2015).

The DCM is normally formed by one or very few species (Selmeczy *et al.*, 2015). These species possess special traits, allowing them to develop population maxima in the metalimnion of stratified lakes. One important feature is the ability to harvest enough light for photosynthesis under the low-light conditions existing in such deeper layers (Vila & Abella, 2001). The light arriving in deeper layers of the water column mainly consists of the central part of the PAR spectrum, since light selectively gets extinct in upper layers (Camacho, 2006). Wavelength of the central PAR spectrum can be harvested by the accessory pigments phycobilins, such as phycoerythrin and phycocyanin (Callieri *et al.*, 1996). Hence, the possession of these phycobilins gives a competitive advantage under low light availability in deeper layers (Gervais, 1997; Camacho, Vicente & Miracle, 2000; Camacho, Vicente & Miracle, 2001). Another important trait for the formation of DCMs in the metalimnion is the capacity for vertical movement. Mobility is either obtained by flagella or by buoyancy mechanisms and enables algae to migrate across the strong chemical and physical gradients in the metalimnion and to position themselves in the depth with optimal growth conditions. The mechanism of vertical movement allows algae to get nutrients from deeper water layers and then migrate to upper layers with higher light availability (Salonen, Jones & Arvola, 1984; Camacho, Vicente & Miracle, 2001). Since both traits, the ability to regulate their vertical position in the water column and the capacity to synthesize phycobilins for light harvesting, simultaneously occur in some cyanobacteria and cryptophytes, they are the prominent algae groups forming DCMs.

1.3.3 Inter-annual trajectories

In addition to variations in distribution patterns along the seasonal and vertical axis, phytoplankton community composition can also fluctuate from year to year. Inter-annual variations can have several causes, including changes in nutrient inputs (Jeppesen *et al.*, 2005), land use (Katsiapi *et al.*, 2012; Friese *et al.*, 2014), acidification (Findlay & Kasian, 1986; Findlay, 2003), water level fluctuations (Nõges, Nõges & Laugaste, 2003) or changing climate (Winder & Sommer, 2012). The effects of climate change are in the current focus of environmental science. Changing climatic conditions can impact phytoplankton community composition in many different ways (Adrian *et al.*, 2009), e.g. via warmer water temperatures (Paerl & Huisman, 2008), changes in stratification patterns (Yankova *et al.*, 2017), changes in rainfall and drought (Harris & Baxter, 1996; Findlay *et al.*, 2001), changes in wind (Mi *et al.*, 2018) or changes in runoff and therefore nutrient loading (Jeppesen *et al.*, 2009). Most studies predict that climate change favors cyanobacteria (Mooij *et al.*, 2005; Carey *et al.*, 2012; Paerl & Huisman, 2008; Elliott, Jones & Thackeray, 2006), firstly because they grow better at higher temperatures (Jöhnk *et al.*, 2008; Reynolds, 2006), secondly because increased nutrient discharge may favor their growth (Paerl & Huisman, 2008) and thirdly because reduced vertical mixing allows the buoyant cells of cyanobacteria to float upward and accumulate in dense surface blooms, suppressing the underlying non-buoyant species through light reduction (Huisman & Hulot, 2005).

In addition, oligotrophication is another important driver of inter-annual shifts in plankton composition, since nutrient inputs to freshwaters were reduced in many developed countries over the last decades as a result of combating external nutrient loading (Sas, 1990), sometimes combined with additional in-lake restoration measures such as chemical-physical methods (Cooke *et al.*, 2016) or biomanipulation (Mehner *et al.*, 2002). Several studies show inter-annual changes in phytoplankton community composition in response to nutrient reductions, sometimes even if no changes in total biomass were detected (Dokulil & Teubner, 2005; Ruggiu *et al.*, 1998; Anneville *et al.*, 2010; Gaedke & Schweizer, 1993; Sommer, Gaedke & Schweizer, 1993). The oligotrophication process usually favored dinophytes, cryptophytes and chrysophytes (Sommer, Gaedke & Schweizer, 1993; Jeppesen *et al.*, 2005; Kamjunke, Straile & Gaedke, 2009; Anneville *et al.*, 2010; Anneville, Gammeter & Straile, 2005), algal groups that are mobile, mixotrophic or adapted to low nutrient concentrations. A review of 35 re-oligotrophication studies found that cyanobacteria declined in deep lakes, but showed no response in shallow lakes in response to reduced nutrient concentrations (Jeppesen *et al.*, 2005). In shallow lakes there was a tendency towards an increased share of diatoms during oligotrophication, probably due to a relaxation of silica limitation (Jeppesen *et al.*, 2005). However, different pressures often interactively influence the inter-annual development of phytoplankton communities and combined effects are possible or even likely. For example, empirical data (Horn, 2003; Pomati *et al.*, 2015; Horn *et al.*, 2015) and modelling studies (Huber, Adrian & Gerten, 2008; Elliott, Jones & Thackeray, 2006) showed that effects of oligotrophication on plankton communities are modified by climate change and disentangling both effects is often a challenging task.

1.4 Impact of phytoplankton communities and their traits on the geochemical environment

Plankton is influenced by its abiotic environment, but it also shapes its environment. As major aquatic primary producers phytoplankton significantly impact oxygen concentrations in the atmosphere (Field *et al.*, 1998) as well as in the water body (Stefan *et al.*, 1995) by producing oxygen and organic carbon from water and carbon dioxide during photosynthesis. Also the underwater light climate strongly depends on phytoplankton biomass, since phytoplankton absorbs light and thus changes light quantity and quality in the water layers below (Kalff, 2002). Moreover, phytoplankton affects nutrient concentrations in the water body by using nutrients for their own metabolism. For example, the commonly observed nutrient depletion in summer in the epilimnion is caused by nutrient uptake by phytoplankton. For phosphorus, it is even often the case that phytoplankters take up more phosphorus than they need (luxury uptake) in order to store it for later times. This overproportional P-uptake can be an important component shaping vertical profiles of phosphorus concentrations (Frassl, Rothhaupt & Rinke, 2014).

Sometimes the effect on the environment not only depends on the concentration of phytoplankton in the water body, but also on the functional traits that the species in the community possess. As different functional groups differ in their nutrient requirements and stoichiometry, they also shape the chemical composition of their environment differently (Finkel *et al.*, 2006; Boyd, 2013; Litchman *et al.*, 2015). For example, fast growing species invest more resources into ribosomes and since ribosomes are relatively rich in phosphorus, they acquire more phosphorus from the environment than slowly growing species (Elser *et al.*, 1996; Klausmeier *et al.*, 2004; Litchman, Edwards & Klausmeier, 2015). As another example, the unique metabolic requirements of diatoms for silica result in reduced silica concentrations in the water body when diatoms are abundant (Schelske & Stoermer, 1971). The high sinking rates of diatoms (Sommer, 1984) contribute to the removal of nutrients from the epilimnion and their burial in the sediments, as shown for e.g. carbon, silica and phosphorus (Benndorf, 1968; Nelson *et al.*, 1995; Tréguer & De La Rocha, 2013; Frassl, Rothhaupt & Rinke, 2014; Horn *et al.*, 2015). Thus diatoms with their high sinking velocity significantly affect biogeochemical cycles. Moreover, the nutrient concentrations in the water are affected by differences in nutrient uptake kinetics, such as higher or lower nutrient uptake rates and affinities (Litchman *et al.*, 2007), or the ability to acquire nutrients from substrates that are not accessible for the whole community, such as the uptake of bacteria by phagotrophic mixotrophs (Mitra *et al.*, 2014) or the capacity to fix atmospheric nitrogen (Mahaffey, Michaels & Capone, 2005). Also the mobility of organisms affects nutrient distributions, especially along vertical gradients. For example, it has been shown that motile species impact the phosphorus distribution in lakes by transporting nutrients from the hypolimnion upwards into the epilimnion during regular vertical migration (Salonen, Jones & Arvola, 1984). All these examples lead to the conclusion that the geochemical environment of aquatic ecosystems is largely influenced by the dynamics of phytoplankton communities and their functional composition.

2 Motivation and objectives: How to exploit the trait based approach to gain a better understanding of plankton dynamics

A good mechanistic understanding is required to better grasp the complexity of ecosystems and their spatio-temporal dynamics. This can be achieved by functional traits, since they reduce the complexity and directly link changes of communities with changes in their environment via functions. However, trait based approaches have rarely been applied to quantify seasonal, vertical and long-term dynamics of freshwater ecosystems. Therefore this thesis focuses on spatio-temporal trait dynamics of phytoplankton communities and their interaction with the environment. Chapter 3 and 4 focus on the importance of the biotic and abiotic environment for phytoplankton trait dynamics, while chapter 5 addresses the impact of species with specific functional characteristics on the geochemical environment. The thesis covers different temporal and spatial gradients (seasonal: chapter 3 and 5; inter-annual: chapter 3 and 4; vertical: chapter 5) and several functional traits (see table 2.1 for an overview). As a study site, Germany´s largest drinking water reservoir, the Rappbode Reservoir, was chosen, since I could make use of a seasonally and vertically resolved dataset from the local water-company ranging back to 1961, which has never been subject to scientific studies before. Moreover, the Rappbode Reservoir was suitably located for additional field work. Chapter 3 and 4 are based on the long-term dataset from the local-water company. These data were digitalized and brought into a useful format by myself. Most data for chapter 5 were collected on our own monitoring program during a one-year field campaign. The overall goals of the thesis are:

- Establish a methodology for translating taxonomic information into functional trait information.
- Use traits to gain a better mechanistic understanding of phytoplankton dynamics and aquatic ecosystems in general.
- Quantitatively investigate the seasonal succession of functional traits.
- Examine the long-term response of phytoplankton functional traits to nutrient reductions.
- Identify mechanisms for the decoupling of phytoplankton biomass from nutrient concentrations with the help of functional traits.

- Investigate the impact of functional traits on the geochemical environment, especially on oxygen dynamics.

The following sections describe the motivation, research gaps and objectives of the three main chapters in more detail.

Table 2.1: Overview about phytoplankton functional traits used in this thesis, including their trait type, definition and ecological function. Trait type and ecological function are assigned according to Litchman and Klausmeier (2008). The last column states in which chapter of the thesis the traits are addressed.

Trait	Trait type	Definition	Ecological function	Addressed in chapter
Cell size	Morphological	Volume of a single cell	Reproduction, resource acquisition and predator avoidance	3
Edibility for *Daphnia*	Behavioral and morphological	Susceptibility against predation by *Daphnia*. The rate of prey consumption relative to the rate at which the favorite prey is consumed.	Predator avoidance	3
Maximum growth rate	Physiological	Ability for fast uptake of nutrients and fast growth. Competitive ability under high nutrient concentrations.	Resource acquisition	3
Phosphate affinity	Physiological	Ratio of maximum growth rate to half-saturation coefficient. Phosphorus uptake ability. Competitive ability under phosphate limitation.	Resource acquisition	3
Light affinity	Physiological	Initial slope of the growth-irradiance curve. Growth ability under light limitation. Ability to tolerate low irradiances.	Resource acquisition	3, 5

Motility	Behavioral	The possession of flagella. Ability to actively move in the water column to a position with optimal conditions.	Resource acquisition and predator avoidance	3, 4
Buoyancy	Behavioral	Possession of gas vacuoles. Ability to adjust position in the water column to a depth with optimal conditions.	Resource acquisition and predator avoidance	3, 4, 5
Mixotrophy	Physiological and behavioral	Potential to ingest bacteria (phagotrophy)	Resource acquisition	3, 4
Silica use	Physiological	Need to use silica as cell wall material	Resource acquisition	3, 4
Nitrogen fixation	Physiological	Potential to fix atmospheric nitrogen. Competitive advantage under nitrogen limitation.	Resource acquisition	3
Chain and colony formation	Morphological	The potential to form colonies or chains.	Resource acquisition and predator avoidance	3
Possession of phycobilins	Physiological	The possession of phycobilins (the accessory pigments phycocyanin or phycoerythrin) increases the range of the usable light spectrum.	Resource acquisition	5

2.1 Using trait based approaches to understand temporal dynamics of plankton communities

Identifying generalized rules for spatio-temporal dynamics in phytoplankton communities by using functional traits is a logical step towards a more quantitative, predictive aquatic ecology. In terrestrial ecology, a growing body of work applies trait based approaches to understand changes in natural communities across environmental gradients. For instance, Cornwell and Ackerly (2009) showed that plant traits shifted in regular ways across gradients in soil water content in coastal California, while Swenson and Weiser (2010) found a correlation of mean trait values of forest communities with climate variables in North America. However, in aquatic ecosystems trait based-approaches have rarely been applied to study seasonal, vertical or inter-annual dynamics in phytoplankton communities, despite increasing knowledge about relevant ecological traits directly or indirectly affecting the fitness (Litchman & Klausmeier, 2008). Some studies focused on the temporal dynamics of easily identifiable traits indirectly influencing fitness, such as morphological or behavioral traits. Weithoff and Gaedke (2016) studied the seasonal dynamics of the phytoplankton community in Lake Constance and found a distinct recurrent annual pattern within a multi-dimensional trait space. In their study, they aggregated the traits cell size, longest linear dimension, motility, mixotrophy and silica demand to one community trait mean, but did not look at dynamics of individual traits. Klais *et al.* (2017) studied the seasonal dynamics of individual traits (cell size, silica demand, formation of chains or colonies, motility, accessory pigment composition, ability to fix atmospheric nitrogen, mixotrophy) along a gradient from marine to brackish waters in the Baltic Sea. The traits used in Klais *et al.* (2017) are morphological and behavioral. I am aware of only two articles studying the spatio-temporal dynamics of physiological traits in aquatic ecosystems. Edwards, Litchman and Klausmeier (2013a) tested whether physiological traits could explain seasonal dynamics in phytoplankton communities in a marine ecosystem. They showed that responses in the lab-measured traits light utilization, nitrate utilization and maximum growth rate could be related to variations in the limiting resources light and nitrate. In a second study, Edwards, Litchman and Klausmeier (2013b) studied the response of phytoplankton functional traits to gradients of light and phosphorus across lakes in the United States. Again, they could predict the response of the physiological traits light utilization and maximum growth rate to the availability of resources. Both studies support the idea that functional traits, including traits measured on laboratory cultures, can result in a better understanding of the structure and dynamics of phytoplankton communities in natural environments.

In lakes and reservoirs, the seasonal succession has traditionally been analyzed from a taxonomic point of view (e.g. Padisak, 1992; Tallberg *et al.*, 1999; Kalff, 2002; Rychtecký & Znachor, 2010). When traits were considered in the description of seasonal succession, this was done in a descriptive, qualitative way (Sommer, 1985; Sommer *et al.*, 1986), but the seasonal development of traits has never been quantitatively analyzed. Hence the objective of chapter 3 is the application of trait-based approaches to the seasonal succession of phytoplankton. The goal is to provide a quantitative trait-based template for seasonal succession patterns under eutrophic and oligotrophic conditions. I use a more than 50-year long seasonally resolved dataset from the Rappbode

Reservoir, which experienced an abrupt shift in nutrient conditions, to address the following questions:

- Are there reoccurring seasonal patterns in phytoplankton trait dynamics? How does the occurrence and dominance of specific trait combinations develop along the season? (Q1.1)
- Does the trait composition mirror biotic and abiotic environmental pressures along the season? (Q1.2)
- Are there differences in the seasonal succession of traits between nutrient rich and nutrient deficient years? (Q1.3)
- Is the functional trait composition of phytoplankton communities influenced by nutrient reductions? (Q1.4)

2.2 Using trait based approaches to identify causal relationships for the decoupling of nutrients from its ecological response

While chapter 3 focused on seasonal dynamics of functional traits, chapter 4 concentrates on long-term trends. Phosphorous is the main limiting element for phytoplankton growth in the Rappbode Reservoir (other nutrients such as nitrogen can be excluded), like in most other freshwater systems (Hecky & Kilham, 1988). After phosphorus concentrations have been reduced around 1990 in the Rappbode Reservoir, phytoplankton biomass did not decrease in the long run. This decoupling of nutrients from its biological response is unexpected given Vollenweider´s (1971) assumption of a linear response of phytoplankton biomass to phosphorus concentrations. However, just like the Rappbode Reservoir, a considerable number of other lakes and reservoirs deviate from Vollenweider´s rule, showing no response of phytoplankton biomass to decreasing nutrient concentrations. The reasons for these nonlinearities are largely unresolved (Jeppesen *et al.*, 2005; Tadonleke *et al.*, 2009; Weyhenmeyer & Broberg, 2014; Horn *et al.*, 2015), though highly relevant, since regulatory frameworks are based upon the assumption that ecosystem processes are reversible and hence reductions in nutrients lead to ecological improvements. Therefore, by using a trait-based approach, chapter 4 aims to shed light on the processes and mechanisms leading to a decoupling of nutrient availability (total phosphorus) from phytoplankton biomass. The following questions will be addressed:

- In which way did the phytoplankton community quantitatively react to strong nutrient reductions? (Q2.1)
- What do the results from Rappbode Reservoir imply for the reversibility of ecosystems? (Q2.2)
- What causes the decoupling of phytoplankton biomass from inorganic nutrients? Can a functional approach help to shed light on the mechanisms preventing a decrease in biomass after phosphorus decreased? (Q2.3)

2.3 Using trait-based approaches to understand the impact of phytoplankton composition on the geochemical environment

While chapters 3 and 4 focus on the impact of the abiotic environment on phytoplankton functional traits and their dynamics, chapter 5 studies how phytoplankton shapes its geochemical environment, especially the distribution of oxygen. As outlined before in section 1.4, the dynamics of phytoplankton communities and their functional traits also influence the abiotic environment, e.g. the distribution of nutrients in the water column and their burial in the sediments. Also the concentrations and distribution of oxygen in the water body are largely influenced by phytoplankton. It is well studied, that microbial degradation of dead phytoplankton that has settled to the bottom sediments can cause oxygen depletion in the lower hypolimnion (Kalff, 2002). It is also well known, that autotrophic organisms can cause supersaturation with oxygen in the epilimnion when mixing is absent and photosynthetic activity is high (Kalff, 2002). Oxygen maxima also commonly occur in the metalimnion of lakes (Leach *et al.*, 2017) and have been attributed to oxygen production by photosynthesizing phytoplankton, which form a deep chlorophyll maxima in the metalimnion (Parker, Wenkert & Parson, 1991; Stefan *et al.*, 1995; Wilkinson *et al.*, 2015). The phytoplankton species, which are responsible for metalimnetic oxygen maxima, possess specific functional traits to be able to grow in the metalimnion of lakes. Already Reynolds *et al.* (2002) grouped a couple of species to form a functional group that is typical for metalimnia. As mentioned in section 1.3.2, these metalimnetic species are characterized by low light requirements and the possession of phycobilin pigments, which facilitates the use of a wider range of the light spectrum (Vila & Abella, 2001; Camacho, 2006). The ability for vertical movement through flagella or buoyancy is another important trait for metalimnetic species in order to migrate between nutrient-rich deeper layers and upper layers with higher light availability

(Salonen, Jones & Arvola, 1984; Camacho, Vicente & Miracle, 2001). While the effect of metalimnetic species on the occurrence of oxygen maxima in the metalimnion is rather well studied, it is unknown if metalimnetic species could also be indirectly responsible for metalimnetic oxygen minima by loading the metalimnion with organic biomass that can sustain high oxygen consumption rates. Metalimnetic oxygen minima are a phenomenon commonly observed in stratifying lakes during summer, but the reasons for their formation are to my surprise only rarely studied (Wetzel, 2001; Kreling *et al.*, 2017). Hence, in chapter 5 of this thesis I want to close this knowledge gap and investigate if metalimnetic oxygen minima can be caused by metalimnetic algae and their functional traits. The following questions will be addressed:

- Is there evidence from the Rappbode Reservoir that phytoplankton traits shape their abiotic environment? (Q3.1)
- What implication has the vertical distribution of phytoplankton and their functional traits for oxygen depletion? Does the functional group of metalimnetic species contribute to the formation of metalimnetic oxygen minima? (Q3.2)

3 Seasonal succession of functional traits in phytoplankton communities and their interaction with trophic state

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Under review in Journal of Ecology

3.1 Abstract

1. Understanding and explaining the structure of communities in response to environmental gradients is a central goal in ecology. Trait-based approaches are promising but yet rarely applied to understand community dynamics in response to changing environmental conditions.
2. Here we investigate seasonal succession patterns of functional traits in phytoplankton communities and how nutrient reductions (oligotrophication) alter these patterns. We used phytoplankton data from 40 years of observation from the Rappbode Reservoir (Germany), which underwent a strong shift in trophic conditions, and translated taxonomic composition into functional traits by assigning trait values compiled from the literature.
3. All studied traits (morphological, behavioral and physiological traits) responded to changing environmental conditions and showed consistent, reoccurring seasonal developments. The seasonal succession of phytoplankton communities was shaped by a trade-off between small celled, fast growing species that are able to rapidly incorporate existing resources (*r*-strategists) and large celled species with more complex and efficient mechanisms to exploit scarce mineral nutrients or acquire previously unexploited nutrient pools (*k*-strategists). In summer, when nutrients were scarce, the *k*-strategy was prevailing (important traits: phosphate affinity, nitrogen fixation, motility and mixotrophy). During the rest of the year, nutrients and turbulence were high and *r*-strategists dominated (important traits: maximum growth rate, light affinity).

4. A comparison between eutrophic and oligotrophic years revealed that the main features of functional trait succession were largely preserved, but intra-annual fluctuations from spring to summer were stronger during eutrophic years. Nutrient reductions mainly affected functional traits and biomass in spring, while in summer the functional community composition changed little.
5. Synthesis. This study provides for the first time a quantitatively supported functional template for trait-based succession patterns in lakes under different nutrient conditions. By translating taxonomic composition into trait information, we demonstrate that the quantification of functional characteristics enables ecological interpretation of observed community dynamics and provides not only a testable template but also a powerful tool towards a more mechanistic understanding. The quantification of functional traits further improves the predictability of community shifts in response to changing environmental conditions and thus opens new perspectives for predictive limnology using lake-ecosystem models.

3.2 Introduction

Understanding and explaining the structure and dynamics of biotic communities in response to environmental gradients is a central goal in ecology. As planktonic organisms in aquatic systems have short generation times (Collins, Rost & Rynearson, 2014), are very dynamic and are highly influenced by abiotic factors as well as biotic interactions, they are well-suited to study the reaction of communities to environmental changes. In temperate lake ecosystems, seasonal changes in environmental factors such as temperature, light intensity, nutrient concentration or grazers induce shifts in phytoplankton abundance and species composition (Tilman, Kilham & Kilham, 1982; Bergquist, Carpenter & Latino, 1985; Vrede *et al.*, 1999; Stomp *et al.*, 2007), referred to as seasonal succession. Explaining and predicting these distinct, reoccurring seasonal patterns has long been in the focus of freshwater ecologists (Sommer *et al.*, 1986; Margalef, 1978; Reynolds, 1984a). Early theoretical models describe phytoplankton succession mainly as a consequence of turbulence and nutrient availability (Margalef, 1978; Reynolds, 1988). They predict the occurrence of *r*-strategists, which are characterized by small cell sizes and high maximum growth rates, under high nutrient and high turbulence conditions, as they prevail during spring. In summer, when nutrient availability and turbulence are low, *k*-strategists with larger cells, slow growth, but high nutrient affinities and diverse strategies for nutrient acquisition (e.g. mixotrophy, nitrogen fixation) are expected to dominate (Margalef, 1978; Reynolds, 1988). The most popular and widely cited conceptual model about plankton succession is the verbally formulated plankton ecology group (PEG) model, which provides a standard template to describe dynamics of total biomass and composition of plankton communities in response to specific driving environmental factors in the temperate zone (Sommer *et al.*, 1986; Sommer *et al.*, 2012). For example, the PEG model predicts a shift from small, edible algae in spring towards larger, inedible algae in summer as a response to increased grazing pressure from zooplankton. Besides changes along the seasonal development, the species composition of phytoplankton communities has also been shown to vary along nutrient gradients, e.g. during oligotrophication (Gaedke, 1998; Anneville *et al.*, 2002b; Jeppesen *et al.*, 2005). Interestingly, studies about oligotrophication focus mostly on inter-annual changes, while intra-annual changes in succession patterns with trophic status have rarely been addressed.

Community dynamics of phytoplankton along seasonal or along nutrient gradients are traditionally described taxonomically. As the basal level in taxonomy, species can be conceptualized by a characteristic information about morphological and physiological features, however predictions at species level are notoriously difficult or maybe even impossible to make (Reynolds, 2000). Therefore, higher taxonomic units (e.g. diatoms, cyanobacteria) are widely used to evaluate phytoplankton distributions (Wetzel, 2001). However, phylogenetic classifications of organisms have the disadvantage that their ecological functions are heterogeneous within these higher taxonomic units and hence often do not reflect their ecological niche. For instance species from the same taxonomic group might show very different ecological adaptations, while species from different taxonomic groups can share similar ecological strategies (e.g. mixotrophy or the ability to form colonies; Salmaso, Naselli-Flores & Padisák, 2015).

Trait-based approaches are a promising tool to overcome these drawbacks and to better reflect the ecological properties of (and diversity within) a community. While much work has been done on classifying species into functional groups (e.g. Reynolds, 1984a; Reynolds *et al.*, 2002; Reynolds, 1980; Padisák, Crossetti & Naselli-Flores, 2008; Kruk *et al.*, 2002; Kruk *et al.*, 2017; Salmaso, Naselli-Flores & Padisák, 2015), the study of individual functional trait dynamics in natural communities and their links to abiotic drivers as well as to fitness and survival (e.g. maximum growth rate or phosphate affinity) is still in its early stages in aquatic ecology (Litchman *et al.*, 2007; Litchman & Klausmeier, 2008; Litchman *et al.*, 2012; Weithoff, 2003). Functional traits can provide a mechanistic foundation for understanding and predicting community structure and dynamics across environmental gradients (Edwards, Litchman & Klausmeier, 2013b; Thomas *et al.*, 2012) and bridge from the level of organisms to that of ecosystems (Falkowski, Barber & Smetacek, 1998; Litchman *et al.*, 2015). However, studies about the seasonal dynamics of phytoplankton traits are rare, especially for physiological traits requiring detailed lab-measurements. We are only aware of Edwards, Litchman and Klausmeier (2013a) and Edwards (2016), who studied the seasonality of maximum growth rate, light and nutrient utilization traits in a marine ecosystem.

To the best of our knowledge there are no studies investigating the seasonal dynamics of eco-physiological traits (i.e. derived from quantitative lab-measurements, for simple binary traits refer to Weithoff, Rocha & Gaedke, 2015) in a freshwater habitat. Our study aims at closing this knowledge gap and investigates to which extent eco-physiological traits conceptualize functional changes in phytoplankton communities along inter- and intra-annual environmental gradients in lakes. Additionally, we analyze how the impact of nutrient reductions alters the seasonal patterns of these functional traits. We take advantage of a 50-year long, seasonally resolved data set from the German Rappbode Reservoir, which underwent a strong and abrupt shift in trophic conditions in the nineties (Wentzky *et al.*, 2018). This allows us to analyze functional trait succession under nutrient deficient and under enriched conditions (average TP concentrations: 0.13 mg/L and 0.02 mg/L respectively), without the confounding effects of geographical location and lake morphometry that are problematic when making cross-system comparisons (e.g. Edwards, Litchman & Klausmeier, 2013b). In contrast to previous studies (Edwards, Litchman & Klausmeier, 2013b; Edwards, Litchman & Klausmeier, 2013a; Klais *et al.*, 2017; Kruk *et al.*, 2015; Weithoff & Gaedke, 2016), we describe phytoplankton communities by a variety of relevant traits from independent categories, including morphological, behavioral and physiological traits (cell size, silica use, mixotrophy, motility, nitrogen fixation, buoyancy, ability to form chains and colonies, edibility for *Daphnia*, maximum growth rate, phosphate affinity and light affinity). With our trait-based approach we intend to achieve an understanding of the composition and dynamics of freshwater phytoplankton communities in response to seasonal and long-term environmental changes. Moreover, our goal is to generalize the existing patterns in order to provide a functional template for trait-based succession patterns in temperate lake ecosystems, which is quantitative and therefore largely extends the verbally formulated PEG model. Such a trait-based, quantitative approach will push forward research about seasonal phytoplankton developments, since it allows for a predictive community ecology that can be statistically tested and is capable of making comparisons across different environments.

3.3 Methods

3.3.1 Study site and sampling

The Rappbode Reservoir is Germany´s largest drinking water reservoir and is located in Harz Mountains, a mid-mountain reach in central northern Germany. The Rappbode Reservoir is a mono- to dimictic water body, which underwent a re-oligotrophication process around 1990. Within a very short time period of 2-3 years total phosphorus concentrations in the epilimnion declined from approximately 0.12 to 0.02 mg/L (Wentzky *et al.*, 2018). For more details about the Rappbode system we refer to Rinke *et al.* (2013), Friese *et al.* (2014) and Wentzky *et al.* (2019). For this study, we used water samples collected at 0, 5 and 10 m depth between 1970 and 2016 approximately six times a year in monthly intervals during the growing season (March until October). Additionally, mixed samples were taken in the water layer from 0 to 10 m depth every week between 1980 and 2016 for environmental abiotic parameters and between 1980 and 2008 for phytoplankton. More details about sampling methods and sample analysis are given in Wentzky *et al.* (2018). For further analysis, we calculated depth-weighted average values from the data collected at 0, 5 and 10 m depth in order to make them comparable with the mixed water samples collected at 0 to 10 m depth and both data sets were merged. These measurements cover most of the epilimnetic layer. In this study, we used data for phytoplankton community composition, soluble reactive phosphorus (SRP), water temperature, nitrate (NO_3), silica (Si), oxygen, pH and secchi depth (for details on measurement methods see Wentzky *et al.*, 2018).

3.3.2 Trait selection

For the present study we selected functional traits that are considered crucial for survival, growth or reproduction of phytoplankton (see Table 3.1): Size of individual cells, phosphate affinity, light affinity, maximum growth rate, silica use, motility, buoyancy, mixotrophy, nitrogen fixation, ability to form chains/colonies and edibility for *Daphnia*. Detailed descriptions of the selected phytoplankton traits and their importance for fitness are given in Table 3.1 and in Weithoff (2003), Litchman and Klausmeier (2008), Litchman *et al.* (2010) and Klais *et al.* (2017). For each species in the dataset the mentioned functional trait values were assigned. Cell sizes were taken from local measurements conducted on the organisms present in the Rappbode Reservoir. The cell size always refers to the volume of one single cell, even when organisms form chains or colonies, and hence does not exactly represent grazing resistance of algae. Trait values for morphological and behavioral traits (motility, mixotrophy, buoyancy, nitrogen fixation, silica use and chain and colony formation) were assigned based on available trait compilations (e.g. https://www.riinaklais.com/phytotraits, Weithoff (2003)) and additional literature review and web search. These latter traits are binary, where a value of 1 means possession of this trait and 0 means absence. While information about morphological and behavioral traits were relatively easy to compile, physiological trait values only exist for a subset of species, since they are measured on cultures in the laboratory. Hence, to be able to assign trait values to every member of the community we took advantage of a method developed by Bruggeman, Heringa and Brandt (2009) and Bruggeman (2011) allowing to estimate the missing values for the traits maximum growth rate, phosphate affinity, light affinity and edibility for *Daphnia*. Missing trait values were inferred from available laboratory measurements on related species with the help of phylogenetic relationships and morphology-based power-law relationships. For this study, Bruggeman´s model was extended to estimate the light affinity trait, since it was originally not included. For more details on the model see supporting information S9.1.

Table 3.1: Overview about phytoplankton functional traits used in this study, including their trait type, range and categories, definition and ecological function. Trait type and ecological function are assigned according to (Litchman & Klausmeier, 2008).

Trait	**Trait type**	**Range and categories**	**Definition**	**Ecological function**
Cell size	Morphological	8 - 200000 µm^3	Volume of a single cell	Reproduction, resource

				acquisition and predator avoidance
Phosphate affinity	Physiological	1.52 – 1504.98 L µmol^{-1} d^{-1}	Ratio of maximum growth rate to half-saturation coefficient. Phosphorous uptake ability. Competitive ability under phosphate limitation.	Resource acquisition
Light affinity	Physiological	0.004-0.07 µmol quanta^{-1} m^{2} s day^{-1}	Initial slope of the growth-irradiance curve. Growth ability under light limitation. Ability to tolerate low irradiances.	Resource acquisition
Maximum growth rate	Physiological	0.20 - 1 .18 d^{-1}	Ability for fast uptake of nutrients and fast growth. Competitive ability under high nutrient concentrations.	Resource acquisition
Silica use	Physiological	Presence or absence	Need to use silica as cell wall material	Resource acquisition
Motility	Behavioral	Presence or absence	The possession of flagella. Ability to actively move in the water column to position with optimal conditions.	Resource acquisition and predator avoidance

Mixotrophy	Physiological and behavioral	Presence or absence	Potential to ingest bacteria (phagotrophy)	Resource acquisition
Buoyancy	Behavioral	Presence or absence	Possession of gas vacuoles. Ability to adjust position in the water column to depth with optimal conditions.	Resource acquisition and predator avoidance
Nitrogen fixation	Physiological	Presence or absence	Potential to fix atmospheric nitrogen. Competitive advantage under nitrogen limitation.	Resource acquisition
Chain and colony	Morphological	Presence or absence	The potential to form colonies or chains	Resource acquisition and predator avoidance
Edibility for *Daphnia*	Behavioral	0.07 – 2.5	Susceptibility against predation by daphnia. The rate of prey consumption relative to the rate at which the favorite prey is consumed.	Predator avoidance

3.3.3 Phytoplankton community data

To compare the seasonal development between nutrient rich and nutrient poor years, the dataset was split into two periods of equal length, based on TP concentrations: The eutrophic period covered the nutrient rich years between 1970 and 1990. During the eutrophic period the annual mean TP concentration was on average 0.13 mg/L and ranged from 0.11 to 0.20 mg/L. The oligotrophic period covered the nutrient poor years from 1996 till 2016, with an average annual mean TP concentration of 0.022 mg/L, a minimum of 0.006 mg/L and a maximum of 0.048 mg/L. The number of phytoplankton samples (after aggregation of vertically resolved samples into vertically averaged values between 0-10 m) was 555 for the eutrophic period and 596 for the oligotrophic period. For environmental parameters, 557 samples were available for the eutrophic period and 1050 samples for the oligotrophic period.

To compare different seasons (see section: Synthesis of seasonal differences in trait composition), the dataset was further divided into spring, clearwater, summer and winter phase. The spring phase covered the months March, April and May (DOY 60-151); summer was defined as the period from July until October (DOY 182-304), winter from December until February (DOY 305-59); in June usually the clearwater phase appeared (DOY 152-181). The number of available samples during the eutrophic period was 158 for spring, 67 for clearwater phase, 215 for summer and 115 for winter season. For the oligotrophic period 161 samples were available for spring, 63 for clearwater phase, 238 for summer and 134 for winter months. The most abundant species during the different seasons in the Rappbode Reservoir are presented in Table S.9.2.1 in the supporting information.

3.3.3.1 Ecological trait space of the phytoplankton community

After assigning trait values to each species, we transformed this trait matrix of species into a distance matrix using principal components analysis (PCA) based on Euclidean distances, which is an ordination technique used for visualization of multivariate data. The PCA result can be interpreted as a functional trait space, where the species are separated according to their ecological traits. This trait space gives information about the location of species in relation to their traits and shows how close different traits are related. There were 87 species with unique trait combinations present in the dataset. Species in the PCA plot were phylogenetically aggregated into one of the following groups: diatoms, cyanobacteria, chlorophytes, dinoflagellates, cryptophytes, chrysophytes, euglenophytes and desmids.

3.3.3.2 Seasonal development of environmental parameters, phytoplankton biomass and traits

The taxonomic composition of each sample in our data set was translated into a matrix of trait values by adding the characteristic combination of traits to each species. This converted the list of species and their corresponding biovolumes into a matrix of biovolumes and trait values. Subsequently, community-weighted mean (CWM) values were calculated for each sample and each functional trait, in order to describe temporal variability of the individual traits. For quantitative traits and cell size the CWM is the biomass-weighted mean trait value (or mean cell size, respectively) of organisms in the sample. For the qualitative traits the CWM represents the biomass proportion of species possessing the trait value 1, hence the CWM will have a value between 0 and 1.

For comparison of the seasonal development of environmental parameters and individual trait values between the eutrophic and oligotrophic period, generalized additive models (GAM) were fitted to the intra-annual development of environmental variables and community-weighted mean trait values, using the method gam() from the R-package mgcv (Wood, 2017). In a GAM, relationships between predictors and dependent variables follow smooth patterns and can be nonlinear. Due to their flexible predictor functions and their easy interpretation, GAMs can uncover hidden patterns in the data, particularly in case of non-linearities and abrupt changes, and are hence an attractive tool for analyzing environmental time series. Since the dependent variable was specified by a Gaussian normal distribution (default setting for family chosen in the mgcv package), the GAM fit can have negative values.

3.3.3.3 Synthesis of seasonal differences in trait composition

As a graphical method to synthesize the information obtained from the individual traits and to evaluate the importance of selected traits for the eutrophic and oligotrophic period, radar charts were created for each season, using the “radarchart” function from the R-package fmsb (Nakazawa & Nakazawa, 2018). In radar charts, multiple variables, here traits, can be represented on axes starting from the center. The axes have equal distances between each other and are arranged radially around the center. For the charts, the average of the community-weighted mean trait values was calculated for each period (eutroph vs. oligotroph) and each phase (spring, clearwater, summer, winter). These trait mean values were drawn into the radar chart, where the data length of a spoke is proportional to the magnitude of the trait value relative to the maximum magnitude across all sampling points. Minima and maxima of the axes are the same for all plots.

3.4 Results

3.4.1 Ecological trait space spanned by the species

Separating the phytoplankton species according to their functional traits in a PCA (Fig. 3.1) yielded 27 % and 20 % of explained variation in the first two principal components. The traits buoyancy and nitrogen fixation were closely related because both only occurred in cyanobacteria. The silica use trait was located opposite of the traits nitrogen fixation and buoyancy, indicating a good separation between diatoms (mostly in the upper half of Fig. 3.1) and cyanobacteria (lower half of Fig. 3.1). Larger cell size was associated with motile and mixotrophic species. In contrast, species with smaller cell size occurred together with higher maximum growth rate, edibility for *Daphnia*, light affinity and chain and colony forming ability. The traits mixotrophy and motility were ordinated in far distance to high maximum growth rate indicating a trade-off between mixotrophy and fast growth, or in other words, characterize mixotrophs as *K*-strategists. Species from the taxonomic groups diatoms and chlorophytes, dinoflagellates, desmids, chrysophytes and cryptophytes showed different degrees of overlap in trait-space. While most of the groups were well separated and obviously occupy specialized areas in the trait space, e.g. diatoms, dinoflagellates and cyanobacteria, the chlorophytes apparently cover a wider trait space and constitute the most trait-diverse phylogenetic group in our analysis. This implies that species from different phylogenetic groups can share similar functional traits. Among the well separated groups, diatoms and cyanobacteria stand out in terms of the large area they occupied in the trait space while dinoflagellates, chrysophytes and euglenophytes remain relatively constrained to a narrower trait space. This observation has a sampling bias because far more diatom and cyanobacterial species are in the data set than species from the other groups; but nevertheless it is worth mentioning that trait diversity in our 46 years long record of phytoplankton communities are higher for diatoms and cyanobacteria compared to the other groups. Phylogenetic relatedness was therefore a poor predictor for functional characterization, particularly for chlorophyte and diatom species.

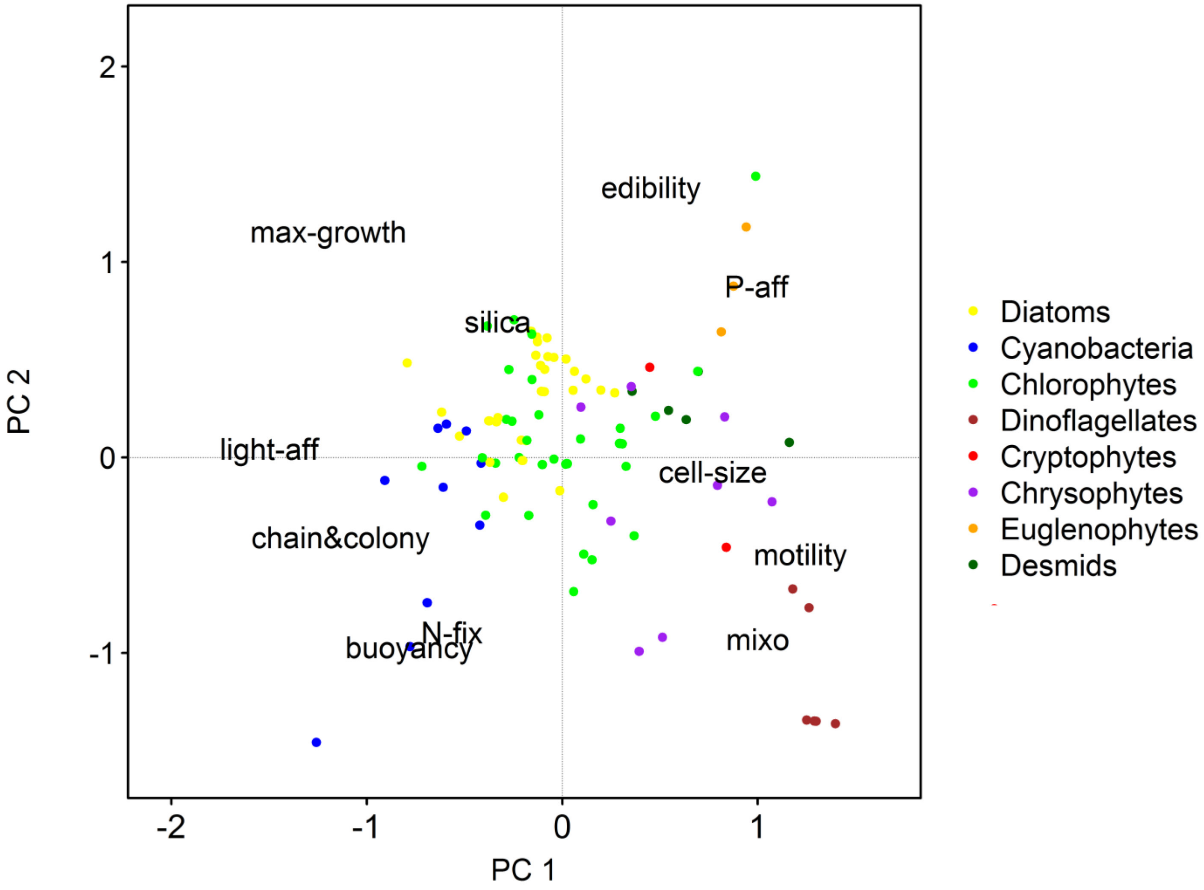


Fig. 3.1: Trait based ordination (PCA) of phytoplankton species along the two main axes, representing the 2-dimensional trait space. The different colors represent the algal group, where the species belongs to.

3.4.2 Seasonal development of environmental variables and phytoplankton biomass

The phytoplankton biomass and environmental parameters, including water temperature, soluble reactive phosphorus, nitrate, silica, oxygen, pH and secchi depth showed clear seasonal patterns (Fig. 3.2) and seasonality explained between 3.6 % (Secchi depth) and 94.6 % (water temperature) of variability in the data (Table S.9.2.2 supporting information). As indicated by the GAMs, the annual time series of biomass during the eutrophic period differed substantially from that of the oligotrophic period (Fig. 3.2a). While eutrophic years showed a clear biomass maximum during spring between day 100 and 150, followed by a biomass minimum, representing the clearwater phase, seasonal fluctuations were less pronounced during oligotrophic years and biomass was more equally distributed over the season. Water temperature was very well explained by seasonality (>90% explained deviance, Table S.9.2.2 supporting information). Temperatures were higher during the oligotrophic period, especially in summer (Fig. 3.2b). This points towards increased summer stratification due to climate warming during recent years.

SRP concentrations in the epilimnion were high during winter and early spring, decreased after the spring bloom from day 150 onwards, and then increased again in autumn after the offset of stratification when nutrients got re-mixed into upper water layers (Fig. 3.2c). Besides higher SRP concentrations during the whole year in eutrophic years, the seasonal differences in SRP concentrations were also far more pronounced than during oligotrophic years. Nitrate concentrations peaked around day 100 and decreased from then on until late summer (Fig. 3.2d) but never reached limiting concentrations for algae. The seasonality for both periods showed synchronous dynamics, with higher NO_3 concentrations during eutrophic years. Silica concentrations during the eutrophic period were higher in spring and lower in summer compared to the oligotrophic period (Fig. 3.2e). The lower Si concentrations in eutrophic summers were associated with higher shares of silica using phytoplankton in spring, which removed Si from the epilimnion due to sedimentation.

Oxygen concentrations in the epilimnion were higher during eutrophic years (Fig. 3.2f). Also a more pronounced oxygen peak was visible in spring from day 100 to 150, which indicated higher photosynthetic activity during eutrophic years. This corresponds to a stronger seasonality of pH during high nutrient years, the highest pH values were found in eutrophic summers (Fig. 3.2g). The light conditions were poorest at the time of the spring bloom around day 140 for both periods, as indicated by low secchi depths (Fig. 3.2h). Secchi depth increased afterwards and peaked in late summer during the time of maximum stratification. The seasonality in secchi depths for the eutrophic period was not as clear as for other environmental parameters, as displayed by wide confidence intervals and low explanatory power of the annual GAM. In summary, phytoplankton biovolume and most of the abiotic environmental variables showed a stronger seasonal development in eutrophic years while dynamics during the oligotrophic years remained lower, and in some variables no clear seasonal patterns could be identified under nutrient-poor conditions.

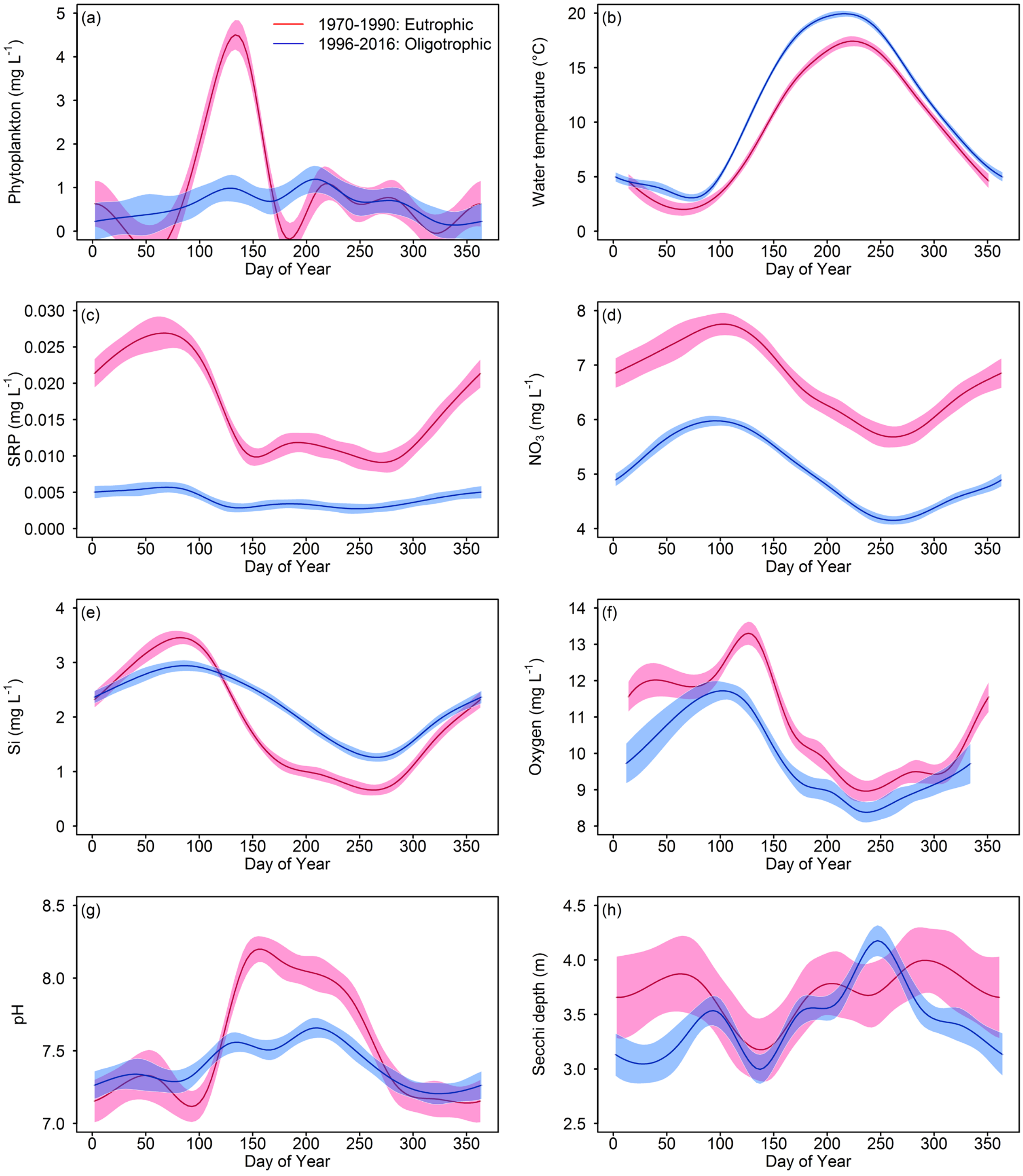
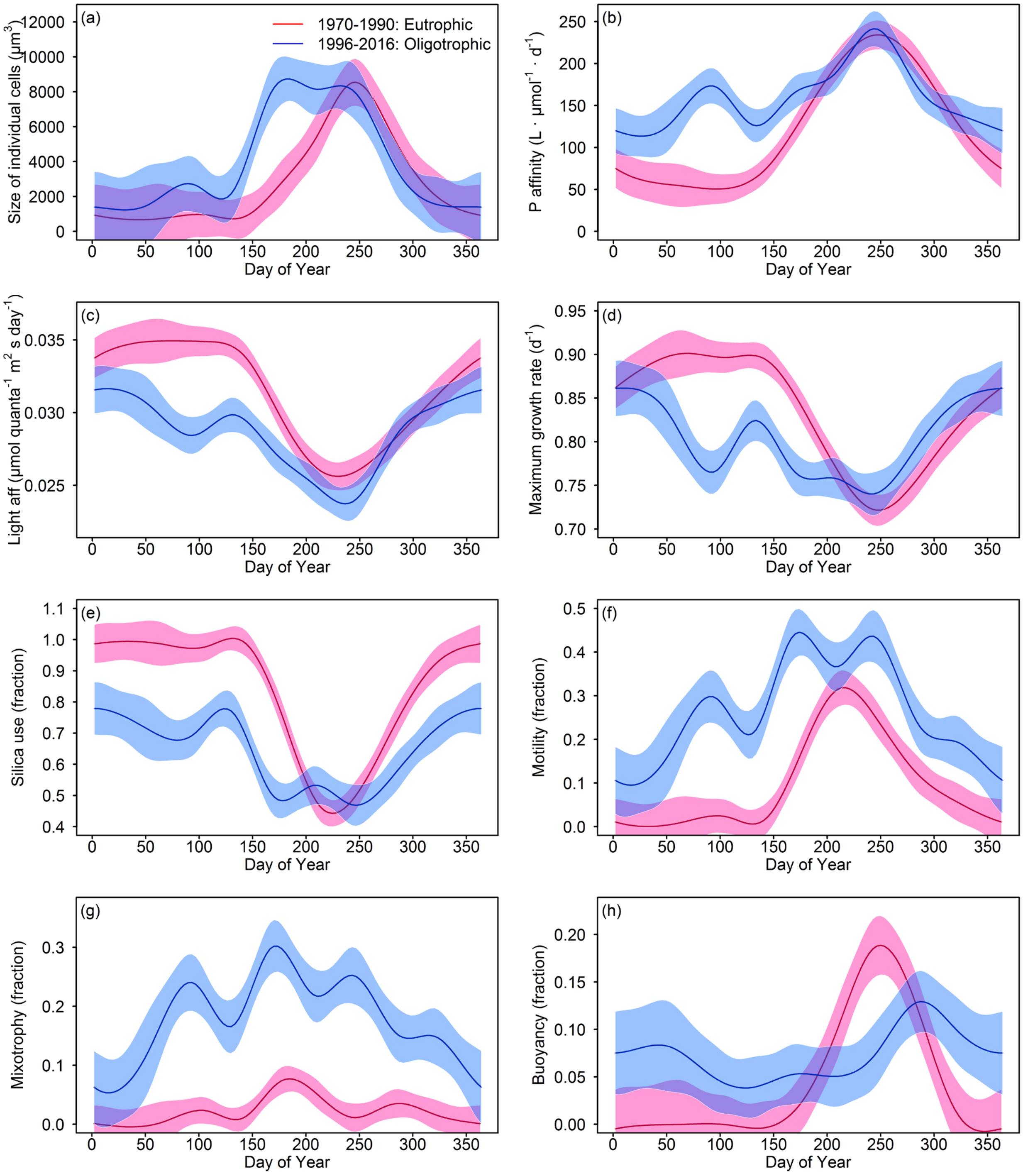


Fig. 3.2: Seasonal development of (a) phytoplankton biomass and environmental parameters, including (b) water temperature, (c) soluble reactive phosphorus (SRP), (d) nitrate (NO_3), (e) silica (Si), (f) oxygen, (g) pH and (h) light conditions (secchi depth),

during the eutrophic (red) and oligotrophic (blue) period in the Rappbode Reservoir. The solid lines are the smooth terms from the generalized additive models fitted to the data; the shades indicate the confidence intervals of these fits.

3.4.3 Seasonal development of phytoplankton functional traits

Many individual functional traits exhibited a recurrent seasonal pattern during the eutrophic as well as during the oligotrophic period, depicted by the GAMs of the annual time series (Fig. 3.3). The variation in trait data explained by seasonality, varied between 5 % and 48.9 % (Table S.9.2.2 supporting information). Similar to the seasonal variations in abiotic variables, for most traits a more pronounced seasonality was found during eutrophic years. This was indicated by the larger differences in trait composition between spring and summer, shown by the radar plots (Fig. 3.4), as well as by the higher explanatory power of the GAMs during nutrient-rich compared to nutrient-poor years (Table S.9.2.2 supporting information). For many traits, the values for the eutrophic and oligotrophic time series were very different during spring, while they became more similar in summer. This higher overlap in summer was also visible in the GAMs (Fig. 3.3, e.g. for the traits maximum growth rate, P-affinity, light affinity, motility, silica use) and in the radar plots (Fig. 3.4). This converging trait composition towards summer indicates that nutrient limitation is a dominant driver of phytoplankton community composition irrespective of the trophic state. In contrast to this, the diverging trait composition between oligotrophic and eutrophic states during spring clearly reflects the difference in nutrient availability. While oligotrophic spring communities were already under the influence of nutrient limitation, eutrophic spring communities showed no sign of nutrient limitation and were selected for *r*-strategists having high maximum growth rates and high light utilization.


(a)
1970-1990: Eutrophic
1996-2016: Oligotrophic
Size of individual cells (μm^3)
0
2000
4000
6000
8000
10000
12000
Day of Year
0
50
100
150
200
250
300
350
(b)
P affinity ($L \cdot \mu mol^{-1} \cdot d^{-1}$)
0
50
100
150
200
250
(c)
Light aff ($\mu mol\ quanta^{-1}\ m^2\ s\ day^{-1}$)
0.025
0.030
0.035
(d)
Maximum growth rate (d^{-1})
0.70
0.75
0.80
0.85
0.90
0.95
(e)
Silica use (fraction)
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
(f)
Motility (fraction)
0.0
0.1
0.2
0.3
0.4
0.5
(g)
Mixotrophy (fraction)
0.0
0.1
0.2
0.3
(h)
Buoyancy (fraction)
0.00
0.05
0.10
0.15
0.20

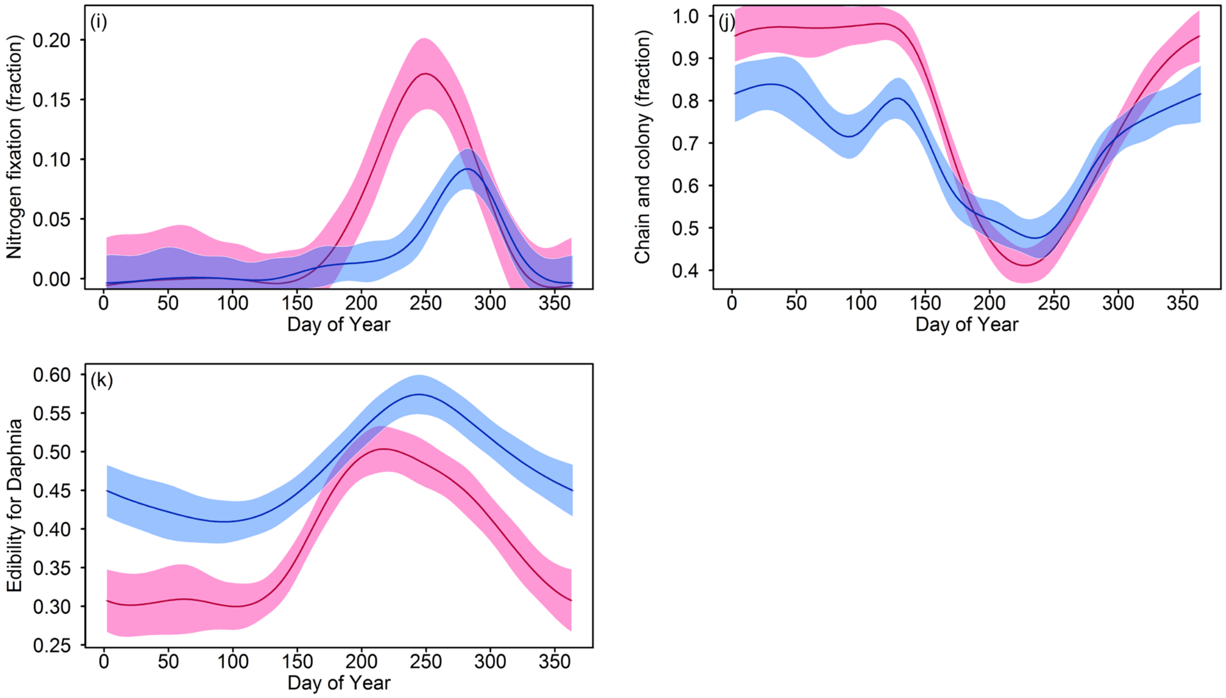


Fig. 3.3: Seasonal development of different phytoplankton traits, including (a) size of individual cells, (b) phosphate affinity, (c) light affinity, (d) maximum growth rate, (e) need to use silica for cell walls, (f) motility, (g) mixotrophy, (h) buoyancy, (i) ability to fix nitrogen, (j) ability to form chains or colonies, (k) edibility for Daphnia, during the eutrophic (red) and oligotrophic (blue) period in the Rappbode Reservoir. The solid lines are the smooth terms from the generalized additive models fitted to the data; the shades indicate the confidence intervals of these fits.

At the same time, the general succession patterns of plankton traits along the season also displayed some similarities between nutrient-rich and nutrient-poor years (Fig. 3.3 and 3.4). Independent of nutrient status, the spring community was characterized by algae with small size of individual cells, higher maximum growth rates, higher light affinities, the need to use silica and the ability to form chains and colonies. Towards summer these traits became less important and the abundance in large, phosphate affine, motile, mixotrophic, nitrogen fixing and buoyant species increased. For example, from spring to summer the average size of individual cells increased from around 1500 to 8000 μm^3 and phosphate affinity from around 100 to 240 L μmol^{-1} d^{-1}, while maximum growth rate decreased from around 0.86 to 0.73 d^{-1} and the share of silica users from almost 100 to 45 %. In winter, the community developed back towards higher maximum growth rate and light affinity and a higher share of silica users and colonial algae - a community composition similar to spring. Noteworthy, the seasonal trends in coloniality were mainly shaped by diatoms, as the community changed from a dominance of chain- forming diatoms in spring (e.g. *Asterionella formosa* and *Tabellaria fenestrata*) towards a more diverse community in summer with lower shares of colonial diatoms, but higher shares of single celled organisms such as *Cryptomonas*, *Ceratium hirundinella* or *Peridinium*. Throughout the year, mobile and mixotrophic species as well as algae edible for *Daphnia* were more abundant during the oligotrophic period (Fig. 3.3f, 3.3g and 3.3k). Especially the increase in mixotrophy with oligotrophication was very prominent, which have gone up from less than 10 % throughout the year in eutrophic years to almost 30 % in nutrient-poor summers. In summary, the calculation of community-averaged traits (Fig. 3.3 and 3.4) allowed for a quantitative assessment of changes in functional characteristics of the plankton community over seasonal and nutrient gradients.

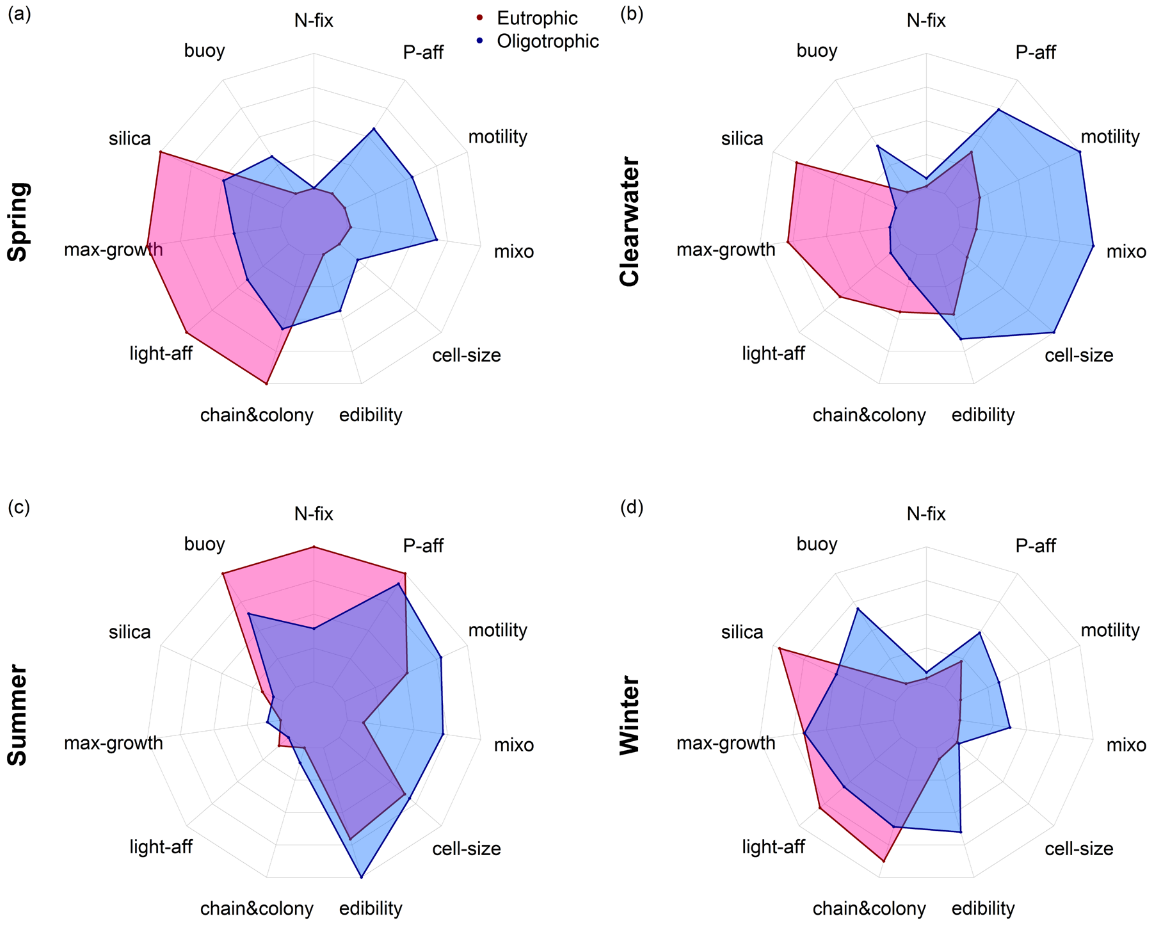


Fig. 3.4: The radar charts represent the importance of selected phytoplankton traits (each spoke represents one trait) for the eutrophic (red) versus the oligotrophic period (blue), during (a) spring (March, April, May), (b) clearwater phase (June), (c) summer (July, August, September, October) and (d) winter (November, December, January, February). The data length of a spoke is proportional to the magnitude of the trait value relative to the maximum magnitude across all data points. The axis minima and maxima are the same for all plots.

3.5 Discussion

The trait space spanned by the phytoplankton species (Fig. 3.1) showed that phosphate affinity, mixotrophy and motility increased with increasing cell size, while maximum growth rate and light affinity decreased (Banse, 1976; Finkel, 2001; Tang, 1995; Edwards *et al.*, 2015). This basically indicates a trade-off between *r* strategists (small cell size, high maximum growth rate and light affinity, low efficiency of resource use) and larger celled *k* strategists with slower growth rates, but more complex mechanisms for survival (high mixotrophy, motility, N-fixation) and high efficiency to use mineral nutrients (high P-affinity) (Leibold, 1997; Huisman & Weissing, 1995; Grover, 1991; Litchman & Klausmeier, 2001; Sommer, 1986b). These trade-offs among functional traits drive species replacements along environmental gradients and are therefore the basis for the seasonal succession patterns observed in Rappbode Reservoir.

3.5.1 Functional traits quantitatively show a change from *r* to *k* strategists from spring to summer

The development of phytoplankton traits showed distinct reoccurring patterns over the season, which are conceptualized in Fig. 3.5. These successional trait patterns were largely retained with trophic status, which is considerable given the large differences in nutrient concentrations between the two trophic periods (average TP concentrations: 0.13 mg/L for eutrophic and 0.02 mg/L for oligotrophic years). All traits, except the edibility for *Daphnia* trait (which is discussed separately below), clearly mirrored the environmental pressures over the year, e.g. high P-affinity during P limitation in summer and high light affinity during light limitation in spring. Major differences in functional trait composition exist between the summer period, when the reservoir was strongly stratified, and times when a large mixing layer was present. In spring, when turbulence and nutrient input was high, species with small cell sizes and high growth rates (*r* strategists) dominated (Reynolds, 1984b; Sommer *et al.*, 1986; Gaedke, 1992). Silica users were also most abundant under well-mixed conditions such as in spring. This was probably because silica users have high sedimentation velocities due to their siliceous cell wall and were therefore favored by turbulence preventing them from sinking out of the photic zone (Sommer, 1984; Trimbee & Harris, 1984). The mixing of the water column and the poor light conditions in spring gave a competitive advantage to species with high light affinities (Yoshiyama *et al.*, 2009; Edwards, Litchman & Klausmeier, 2013a), i.e. the ability for more efficient utilization of low light, since they are better adapted to fluctuating light conditions. Phosphate affinity and alternative strategies for mineral nutrient acquisition, such as the traits nitrogen fixation and mixotrophy were less relevant in spring, since nutrient availability was high. Also the proportion of motile and buoyant species was lower in spring since cells were moved upwards towards the light by turbulence and hence investing in motility was not necessary (Jäger, Diehl & Schmidt, 2008; Visser *et al.*, 1996).

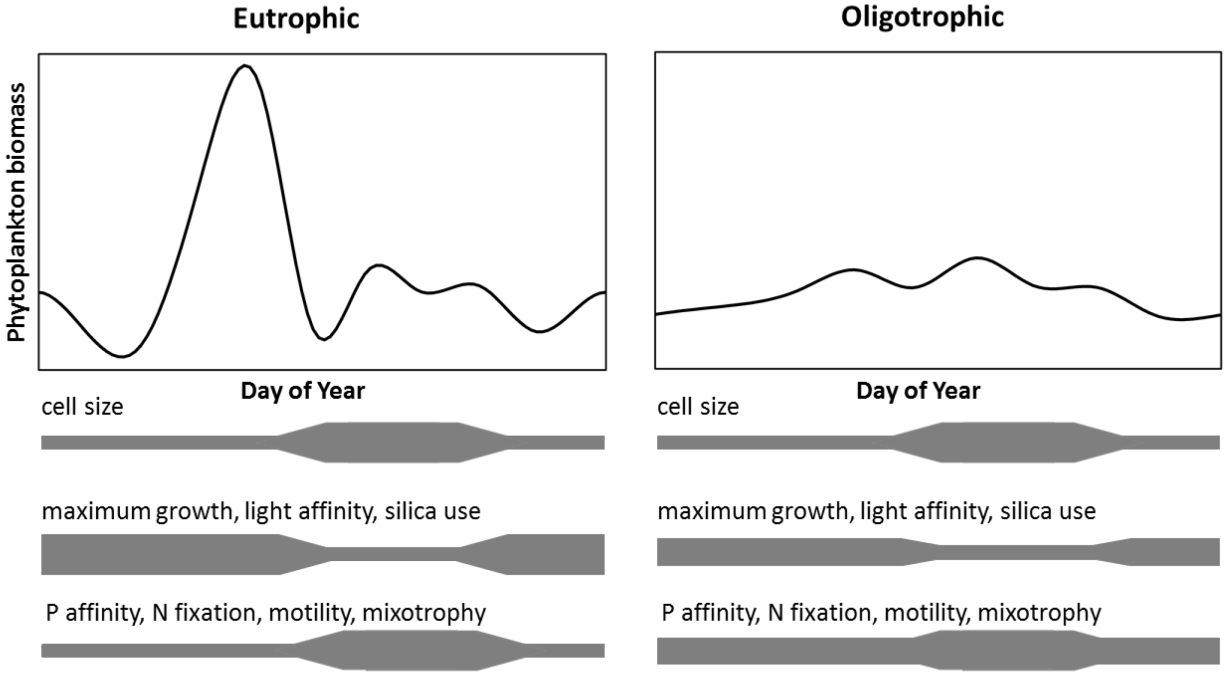


Fig. 3.5: Seasonal patterns of phytoplankton biomass and the importance of different phytoplankton traits during eutrophic (left) and oligotrophic (right) years. The thickness of the horizontal bars indicates the seasonal change in relative importance

of the phytoplankton traits cell size, maximum growth rate, light affinity, silica use, phosphate affinity, nitrogen fixation, motility and mixotrophy.

In contrast, turbulence and nutrients were low in summer and light penetrated deeper into the water column. In response to the changed environmental conditions phytoplankton developed different functional strategies to survive. In agreement with predictions from ecological theory (Margalef, 1978; Wirtz & Eckhardt, 1996; Litchman & Klausmeier, 2001), the summer community shifted towards slower growing species with larger cell sizes and higher tolerances towards periods of nutrient stress (*k* strategists). The nutrient limitation in summer provided opportunities for phosphate affine phytoplankton and the development of more complex nutrient acquisition strategies such as mixotrophy and nitrogen fixation. Organisms also invested in motility, which was either realized by the possession of flagella or by the regulation of buoyancy to overcome sedimentation losses and nutrient deficiency by migrating to deeper waters, which are important stressors during stratification in summer. This agrees with experiments, which observed a replacement of sinking taxa with buoyant and flagellated taxa with decreasing mixing depth (Jäger, Diehl & Schmidt, 2008; Reynolds *et al.*, 1983).

In summary, our results quantitatively show a shift from *r* strategists (small cell size, high maximum growth rate, low efficiency of nutrient use) in spring to *k* strategists (large cell size, slow growth rate, complex mechanisms of resource acquisition) in summer, which is in line with verbal descriptions of the typical successional sequence observed in temperate lakes (Reynolds, 1984a; Sommer *et al.*, 1986; Margalef, 1978). The major advancement of our analysis is to put these findings into a quantitative framework using functional traits. This allows not only to provide a quantitatively characterized functional template for trait-based succession patterns (Fig. 3.5) but moreover provides a testable framework that is prone to advanced statistical and experimental analysis.

3.5.2 Edibility trait shows unexpected seasonal pattern

The seasonal development of the edibility trait, i.e. the susceptibility towards grazing by *Daphnia*, as well as the ability of algae to form chains and colonies was surprising as it was contrary to expectations and widespread belief. Theories about plankton succession, observations from lakes as well as modelling studies, predict that the edibility of phytoplankton decreases after the clearwater phase towards summer and the algae composition responds to the increased grazing pressure by changing to less-edible, grazing resistant species (Lampert *et al.*, 1986; Sommer *et al.*, 1986; Vanni & Temte, 1990; Gaedke, 1998; Wirtz & Eckhardt, 1996) , which is e.g. attained by the ability to form chains, colonies or filaments (Gliwicz, 1977). We observed the opposite pattern with low edibility and high coloniality during spring and an increase in algae edible for *Daphnia* and low coloniality later in the year, when grazing pressure is expected to be high (Sommer *et al.*, 1986), both in nutrient rich and deficient years. In line with our observation, also studies from other lakes reported an increase of inedible algae in the absence of severe grazing and higher shares of edible algae when grazing pressure was high (Agrawal, 1998; Carpenter *et al.*, 1993), which contradicts predictions of defense theory (Coley, Bryant & Chapin, 1985; Fagerstrom, Larsson & Tenow, 1987; Porter, 1973). Agrawal (1998) hypothesizes that this paradox outcome might be explained by selective and size-specific grazing by zooplankton. As herbivores vary in their ability to consume the same phytoplankton species (Lundstedt & Brett, 1991), taxa that are edible to one grazer may be inedible to another. Hence edibility and resistance are specific to the particular grazer species, which can have opposing impacts on the phytoplankton composition (Sommer *et al.*, 2001; Knisely & Geller, 1986). In the present study edibility by *Daphnia* herbivores was considered. Possibly grazing pressure by other grazers, such as protozoans and calanoid or cyclopoid copepods, had an higher impact, resulting in algae being more edible towards *Daphnia* in summer. For example *Rhodomonas spp.* (130 µm^3 cell volume) and *Cryptomonas spp.* (1500 µm^3 cell volume) were characterized as rather edible to *Daphnia*, but have been shown to be spared by copepod grazing (Sommer *et al.*, 2001). Hence high grazing pressure by copepods in summer might have triggered an increase in those algae species, which were inedible to copepods, but edible to *Daphnia*.

This shows that the edibility of algae is predator-specific and thus difficult to define, making generalizations about the edibility of algae as proposed by the PEG model (Sommer *et al.*, 1986) difficult. Moreover, it is possible that the unexpected trends in the colony formation and edibility for *Daphnia* trait were due to reasons other than grazing pressure. E.g. the low abundance of colonial and filamentous organisms during summer stratification might be related to higher sinking velocities of colonies (Reynolds, 2006) rather than to grazing pressure. Since different traits are not completely independent from each other and therefore not freely combinable, the unexpected trends in coloniality and edibility might have been shaped by trends in other more important traits.

This would indicate that losses by grazing were not as important in shaping the phytoplankton communities (top-down) and that the seasonal phytoplankton dynamics in the Rappbode system were primarily regulated by resource availability (bottom-up).

3.5.3 Nutrient reductions affect biomass and functional traits mainly during spring

While the general succession patterns of functional traits were independent of nutrient regime, the extent of the seasonal changes of functional traits from spring to summer clearly differed with trophic status. Phytoplankton biomass and functional traits exhibited lower fluctuations along the season during oligotrophic years, as the differences between the traits in spring and summer were relatively small. In contrast, in eutrophic years seasonality of biomass and traits was more pronounced and the differences between spring and summer conditions were large. The increase in seasonal changes of traits with nutrient concentration was expected, as eutrophic systems usually show larger seasonal fluctuations in biomass and phytoplankton cell size spectra and more successional stages (Sommer *et al.*, 1986; Sommer, 1986a; Kalff, 2002; Gaedke, Seifried & Adrian, 2004).

Comparing the seasonal biomass development between the two trophic states, it became also evident that the strong phytoplankton spring bloom found in eutrophic years vanished with oligotrophication, while summer biomass changed little (or even became higher). This contradicts the PEG model which expects the disappearance of summer blooms with oligotrophication, while the magnitude of the spring bloom is less affected (Sommer *et al.*, 1986). Internal lake processes might be a reason for the differences in biomass patterns between the Rappbode Reservoir and, for example, Lake Constance, which was a major study site for the development of the PEG model. While in Lake Constance internal nutrient regeneration was rather important, allowing higher biomasses in summer in eutrophic years (Tilzer *et al.*, 1991; Gaedke & Straile, 1994), in the Rappbode Reservoir nutrient recycling was not as significant during eutrophic years, since strong diatom blooms in spring removed nutrients from the epilimnion preventing the recycling of nutrients and thus the development of strong summer blooms (Wentzky *et al.*, 2018). The loss of the pronounced spring bloom in the Rappbode Reservoir with oligotrophication was likely due to lower nutrient availability. In contrast, in Lake Constance the phytoplankton biomass during spring did not change significantly with decreasing nutrients, probably because zooplankton-grazer biomass has decreased (Tilzer *et al.*, 1991), which was not the case in Rappbode Reservoir (unpublished data).

A study from Lake Constance also showed that differences in the functional composition after nutrient reduction were most apparent during nutrient limitation in summer (Weithoff & Gaedke, 2016). In contrast, in the Rappbode Reservoir the largest changes with changing trophic status occurred in the spring community, while the traits in summer largely overlapped in the eutrophic and oligotrophic period. Hence, in oligotrophic years the functional composition of spring communities resembled summer communities, while in eutrophic years spring and summer communities were functionally very different. Intense nutrient limitation, which already occurred in spring during oligotrophic years, forced the community to adapt to low nutrient levels earlier in the year. This observation may provide an explanation for the relatively high summer biomasses observed in oligotrophic years in the Rappbode Reservoir (Wentzky *et al.*, 2018). Since species adapted to low nutrient availabilities were already present in significant amounts in spring, they had longer time to develop high abundances during summer. In summary, our study shows that trophic status strongly affected biomass and functional composition during spring, contradicting previous theories and observations that expect the largest changes in summer (Sommer *et al.*, 1986; Weithoff & Gaedke, 2016).

3.5.4 Traits as a unifying concept in ecology

Trait-based approaches allow for comparisons across different environments and habitats, despite different taxa, as taxonomic complexity is reduced to the unifying unit of functional traits. For example, comparing the results from this study with observations from marine habitats, the traits silica use, nitrogen fixation, buoyancy and mixotrophy (Klais *et al.*, 2017) as well as light affinity and maximum growth rate (Edwards, 2016) exhibited similar seasonal patterns in freshwater and marine systems. Cell size, however, showed opposing seasonal patterns in marine and freshwater habitats. While cells were smaller in spring and larger in summer in freshwater systems, the reverse trend for cell size was observed in marine and brackish habitats (Acevedo-Trejos *et al.*, 2015; Klais *et al.*, 2017). In this way trait-based approaches can serve as a unifying concept in plankton ecology. In line with Weithoff and Beisner (2019), we encourage researchers to take advantage of traits as a common currency to assess phytoplankton community structure across different gradients and systems, e.g. ranging from marine, over brackish to freshwater habitats.

3.6 Conclusions

The study provides a quantitatively supported functional template for phytoplankton succession in temperate lakes under different nutrient regimes (Fig. 3.5). In line with conceptual models (Margalef, 1978; Sommer *et al.*, 1986), we quantitatively showed that succession patterns of plankton communities were mainly driven by a trade-off between small celled, fast growing species that are able to incorporate existing resources at a reasonable short time (*r*-strategists) and large celled species with more complex and efficient mechanisms to exploit scarce mineral nutrients or acquire previously unexploited nutrient pools (*k*-strategists). Moreover, the seasonal development of functional traits mirrored environmental pressures over the year. For example phosphate affinity and mixotrophy peaked during phosphorous limitation in summer, while maximum growth rate and light affinity were high during the mixing season when light was limiting but nutrients were highly available. Noteworthy, the main features of functional trait succession were independent of nutrient regime and the seasonal development of functional properties of the community was similar during oligotrophic and eutrophic conditions. Distinct changes in functional composition occurred however, and seasonal differences during oligotrophic years were generally less pronounced over the year. Spring communities in the oligotrophic state moreover showed clear sign of nutrient limitation and therefore showed more functional resemblance with summer communities than under eutrophic conditions. In summary, translating species into functional traits by assigning trait values compiled from the literature provides a powerful method towards a more predictive community ecology. Functional traits can be applied to translate information about taxonomic composition into ecologically interpretable functions and eco-physiological processes that can be linked to resource competition, succession, and ecosystem dynamics. It enables ecological interpretation of observed phytoplankton community dynamics by quantification of functional characteristics and improves the predictability of community shifts in response to changing environmental conditions. This should open also new perspective for predictive limnology using lake ecosystem models.

3.7 Acknowledgements

We thank the water supply works “Wasserwerk Wienrode” and “Talsperren Betrieb Sachsen-Anhalt” for sharing phytoplankton community and environmental data with us. We are thankful for the funding received by the grants JA 2146/2-1 and RI 2040/2-1 from the German Research Foundation (DFG) within the priority program 1704 “DynaTrait”. The research was also supported by TERENO (TERrestrial ENvironmental Observatories) funded by the Helmholtz Association and the Federal Ministry of Education and Research (BMBF).

4 Mechanisms preventing a decrease in phytoplankton biomass after phosphorus reductions in a German drinking water reservoir – results from more than 50 years of observation

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Freshwater Biology (2018), 63(9), 1063-1076

4.1 Summary

1. To counteract the severe consequences of eutrophication on water quality and ecosystem health, nutrient inputs have been reduced in many lakes and reservoirs during the last decades. Contrary to expectations, in some lakes phytoplankton biomass did not decrease in response to oligotrophication (nutrient reduction). The underlying mechanisms preventing a decrease in biomass in these lakes are the subject of ongoing discussion.
2. We used a hitherto unpublished long-term data set ranging from 1961 until 2016 from a German drinking water reservoir (Rappbode Reservoir) to investigate the underlying mechanisms preventing a decrease in biomass. Total phosphorus (TP) concentrations in the Rappbode Reservoir dropped abruptly in 1990 from 0.163 mg L^{-1} to 0.027 mg L^{-1} within three consecutive years, as a result of banning phosphate-containing detergents. Despite substantial reductions in TP, total annual phytoplankton biomass did not decline in the long-run and therefore the yield of total phytoplankton biomass per unit phosphorus largely increased.
3. Regression analysis revealed a positive association between the yield and potentially phagotrophic mixotrophs ($R^2=0.465$, $p<0.001$). We infer that by ingesting bacteria, mixotrophic species were capable of exploiting additional P sources that are not accessible to obligate autotrophic phytoplankton, eventually preventing a decrease in algal biomass after TP reductions.

4. Long-term epilimnetic phosphorus concentrations during the winter mixing period decreased to a greater degree than summer phosphorus concentrations. Apparently, TP losses over the season were less intense. Spring diatom biomass also markedly decreased after oligotrophication. In fact, spring diatom biomass was positively related to the TP loss over the season suggesting diatoms play an important role in P reduction. However, this intraannual P processing was not the primary factor when focusing on the average yearly yield, which remained to be fully explained by mixotrophs.
5. Our study demonstrates this ecosystem´s ability to compensate for changes in resource availability through changes in phytoplankton community composition and functional strategies. We conclude that an increase in mixotrophy and the ability to make bacterial phosphorus available for phytoplankters were the main factors that allowed the phytoplankton community of the Rappbode Reservoir to adapt to lower nutrient levels without a loss in total biomass.

4.2 Introduction

The growth and production of phytoplankton in lakes and reservoirs is known to be limited by inorganic nutrients, most importantly phosphorus (P), causing eutrophication when overly supplied (Correll, 1998; Schindler, 2012). Therefore the implicit assumption of many scientific and regulatory frameworks is that aquatic ecosystems impacted by eutrophication can be reverted to its original condition by a reduction in phosphorus concentration, which hereafter we refer to as "oligotrophication", although an oligotrophic status is not achieved at the end of our observations. A decrease in nutrients towards a lower trophic state can be generally called oligotrophication (Jeppesen *et al.*, 2005). In fact, the majority of case studies about oligotrophication show a decline in phytoplankton biomass after phosphorus reductions, supporting the assumption that the trajectory of an ecosystem is reversible (Jeppesen *et al.*, 2005; Cooke *et al.*, 2016; Edmondson, 1994; Jeppesen, Jensen & Søndergaard, 2002; Schindler, 2012). However, there are also a substantial number of exceptions where a reduction in nutrients was not followed by a drop in biomass. A review by Jeppesen *et al.* (2005) revealed that 25% of the studied lakes did not show the expected response. Phytoplankton biomass showed no response to reduced nutrient loadings in four of the study lakes (Damhussøen, Denmark; Bryrup, Denmark; Maggiore, Italy; Vättern, Sweden), while an increase in biomass was even observed in three lakes (Võrtsjärv, Estonia; Peipsi, Estonia; Tystrup, Denmark). In addition, an increase in algal biomass after oligotrophication was evident in Lake Geneva, Switzerland (Anneville & Pelletier, 2000; Tadonleke *et al.*, 2009), in Sweden´s largest lake, Lake Vänern (Weyhenmeyer & Broberg, 2014), and in Germany´s Saidenbach Reservoir (Horn *et al.*, 2015). These findings are in conflict with the assumption that biomass forms a linear relationship with phosphorus, i.e. the yield of biomass per unit phosphorus should be constant (representing the slope of this relationship) and independent of the absolute nutrient concentrations (Vollenweider, 1971).

The relationship between phosphorus concentration, e.g. as given by the total phosphorus concentration during spring overturn, and summer chlorophyll concentration has been intensively studied (Dillon & Rigler, 1974; Jones & Bachmann, 1976; Canfield Jr & Bachmann, 1981). Several authors showed linear relationships on a double-log scale with relatively high coefficients of determination (around 0.9). Back-transformed to the original scale, however, a substantial variation of chlorophyll content is notable at a given nutrient concentration. In the work of Dillon and Rigler (1974), for example, a phosphorus concentration of 50 µg L^{-1} is associated with chlorophyll content in a confidence interval from 7.5 to 60 µg Chl L^{-1}. Based on these statistical findings we argue that there is a considerable plasticity of algal abundance at a given nutrient content. Accordingly, ecological properties and trophic interactions of the plankton community can be expected to shape the algal biomass yield per unit of phosphorus. This was already demonstrated by Mazumder and Havens (1998) who showed chlorophyll-TP relationships to be dependent on the presence or absence of large herbivores. Microbial food web architectures are another influential component affecting nutrient and carbon fluxes in pelagic environments (Mitra *et al.*, 2014).

While a weak (or no) responsiveness of phytoplankton biomass to decreasing nutrient concentrations has been observed in several lakes, the underlying mechanisms are often not known. In fact limnologists are puzzled by the phenomenon that some lake ecosystems are highly resilient against changes in nutrient conditions while others react promptly (Jeppesen *et al.*, 2005; Carpenter & Cottingham, 1997). However, understanding the driving factors for the disconnection between nutrient reductions and phytoplankton biomass is of great importance for setting reliable restoration targets in water management in such systems. This study investigates the mechanisms that could prevent a drop in biomass after P reductions.

We use an exceptionally long data set ranging from 1961 until today from Germany´s largest drinking-water reservoir, the Rappbode Reservoir. This deep reservoir is located in a mountain range in former East Germany. The data were collected by the local water supply works and have never been used for scientific purposes. It has been documented that phosphorus concentrations in the Rappbode Reservoir decreased strongly within a few years after the reunification of East and West Germany in 1990, mainly due to the reduced use of phosphate-containing detergents (Skibba & Matthes, 2005; Umweltbundesamt, 1994; Germanus, Krings & Stelter, 1995). Contrary to expectations, phytoplankton biomass did not significantly decline in the long-run and the yield of phosphorus per unit phytoplankton biomass even increased after oligotrophication. Since the nutrient reduction took place in a rather sudden shift, i.e. the time scale of this change was very short, this data set offers an excellent opportunity to study the resistance, adaptability and regulatory mechanisms of ecosystems. Long-term case histories of lake recovery are very important, because they provide the only reliable evidence about the response to reduced nutrient concentrations (Schindler, 2012). Here we explore the long-term trends in the main nutrients, water temperature, light conditions and phytoplankton biomass in the Rappbode Reservoir. Moreover, we analyze which mechanisms could have prevented the decrease in biomass after phosphorus reductions and enabled the increase in phytoplankton yield after 1990.

In this study we first present the long-term trends observed in the monitoring data for the Rappbode Reservoir. We found a strong decline in phosphorus concentrations in 1990. While total annual phytoplankton biomass did not decrease in response to this nutrient reduction, phytoplankton community composition did change. Based on these findings we examine the following observations and explore possible reasons leading to the unexpected response of algal biomass to phosphorus concentrations:

1. **More mixotrophs:** The phytoplankton community changed towards phagotrophic mixotrophs, which have a competitive advantage under low nutrient conditions, since they can use bacteria as an alternative energy and nutrient source (Nygaard & Tobiesen, 1993; Isaksson *et al.*, 1999). By exploiting additional P sources, they make nutrient sources available which

are otherwise not accessible to phytoplankton. Therefore the overall yield of phytoplankton biomass per unit phosphorus can increase (Mitra *et al.*, 2014; Bird & Kalff, 1987).

2. **More motile species:** The phytoplankton community changed towards species that are motile and can thus optimize light and nutrient limitation. Using flagella or the ability to adjust the position in the water colomn (buoyancy), motile species can migrate to the hypolimnion to overcome nutrient limitation or to the surface to overcome light limitation (Klausmeier & Litchman, 2001; Jäger, Diehl & Schmidt, 2008). By transporting nutrients from the nutrient-rich hypolimnion to the epilimnion, they increase the yield in the epilimnion.

3. **Less nutrient losses in spring due to declining diatom biomass:** Lower nutrient concentrations during mixing lead to reduced diatom blooms in spring. Diatoms suffer from higher sinking velocities compared to other taxa and are therefore very efficient in removing nutrients from the photic zone (Sommer, 1984; Reynolds, 2006). Hence, when diatom biomass decreases, less nutrients are removed from the epilimnion by sedimenting algal cells and consequently more nutrients stay available for the summer period (Horn *et al.*, 2015; Benndorf, 1968; Frassl, Rothhaupt & Rinke, 2014). A more even distribution of resources over the entire growing season results in biomass also being more evenly distributed. This affects the seasonal variability of phytoplankton biomass. Increasing biomass in summer compensates in part for decreasing biomass in spring and consequently the overall annual phytoplankton biomass does not drop after P reductions.

4.3 Methods

4.3.1 Study site

The Rappbode Reservoir is Germany´s largest drinking water reservoir (in terms of volume) and supplies drinking water for over one million people. It is located in central northern Germany in the small mid-mountain reach of the Harz Mountains, at a crest elevation of 423.6 m a.s.l. (Fig. 4.1). The annual precipitation in the mainly forested Harz region is higher than in the surrounding areas and at some places exceeds 1000 mm yr^{-1} (Rinke *et al.*, 2013). The Rappbode Reservoir was constructed between 1952 and 1959 and has the tallest dam wall (106 m) in Germany. It is fed by three pre-dams (Königshütte Reservoir, Hassel pre-reservoir and Rappbode pre-reservoir) (Tittel *et al.*, 2015), which were constructed for the purpose of sediment and nutrient trapping. The water of the Rappbode Reservoir discharges into the Wendefurth Reservoir, where it is used for energy production and energy storage by a pump-storage-station (Friese *et al.*, 2014). The Rappbode Reservoir is elongated in shape with a length of 8 km, a maximum surface area of 3.95 km^2, an inflow volume of 120×10^6 m^3 a^{-1} and a residence time of 344 days. The mean depth is 28.6 m and the maximum depth is 89 m. As a dimictic water body it stratifies in summer, in some years freezes in winter and completely mixes in spring and autumn. The water level fluctuates during the year by about 15 m (Bocaniov *et al.*, 2014). After its construction, it took until 1964 to completely fill the reservoir to its full storage capacity.

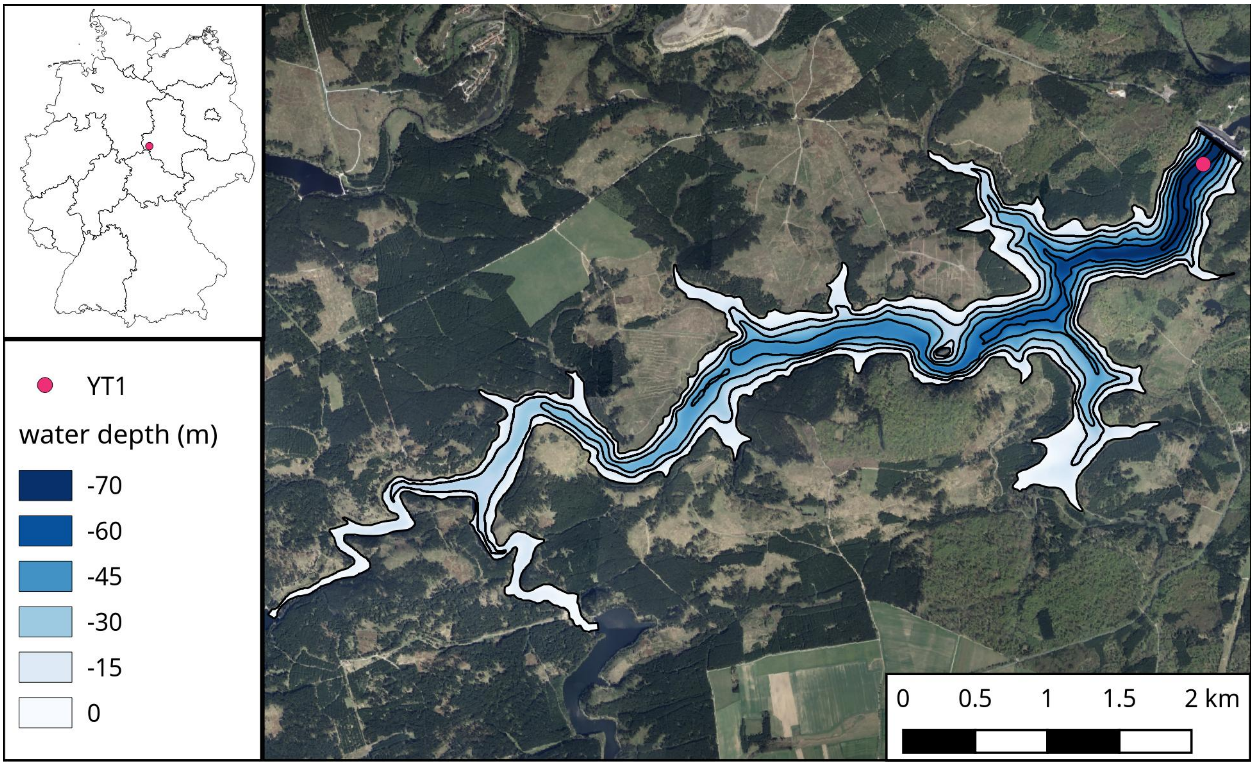


Figure 4.1: Map of Germany with its federal states (top left). The red point indicates the location of the Rappbode Reservoir within Germany. Bathymetric map of the Rappbode Reservoir (right). The red point indicates the sampling site (N51.73891 E10.89147). Geologic data are taken from © GeoBasis-DE / LVermGeo LSA (2016).

4.3.2 Sampling and sample analysis

The data presented here cover the period from 1961 to 2016 and were collected and analyzed by the local water supply works Wasserwerk Wienrode (today belonging to the company Fernwasserversorgung Elbaue-Ostharz GmbH) for the purpose of drinking water quality control. Over the whole investigation period, measurements were conducted at the deepest point of the basin close to the dam wall (N51.73891° E10.89147°, Fig. 4.1). Data were collected approximately six times a year from 1961-2016, usually between March and October in monthly intervals, at up to 11 different depths (0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80 m), depending on the water level. Samples were collected at the desired depth with an open cylinder sampler (2 L standard water sampler according to Ruttner from Hydro-Bios GmbH). In addition to this base sampling program, from 1980 to 2016 samples were taken once a week in the upper water column as a mixed sample from 0-10 m, except for phytoplankton, of which the time series ends in 2008. Samples were taken using a pipe, which was lowered to a depth of 10 m and then closed at the bottom, in order to get a representative integral sample of the upper water column. The total number of sampling dates was 1538 for plankton and 1942 for water chemistry data. In addition to these sampling dates, the surface water temperature was monitored on a daily basis since 1980 at a sampling point close to the shore. Water transparency was measured with a Secchi disc from 1972 onwards. Air temperature measurements were conducted by the German weather service (DWD) at the station Harzgerode.

The following variables were used for data analysis: Total phosphorus (TP), soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), silica (Si), light availability (Secchi depth), water temperature, phytoplankton biomass and community composition. Nutrients (total phosphorus, soluble reactive phosphorus, silica, ammonia, nitrate and nitrite) were analyzed by accredited methods according to German standards (see Legler, 1988). DIN was calculated as the sum of ammonia, nitrate and nitrite concentrations. Water temperature at different depth was measured with a thermometer inside the sampler before 2009 and with a multiparameter probe from 2009 onwards (both methods were compared and gave similar results). Daily surface temperature was measured using a manual thermometer.

Algae samples were preserved with Lugol´s solution for microscopic cell counting. For concentrating the plankton sample, the sedimentation technique developed by Utermöhl (1958) was used from 2002 onwards. Before 2002, a first phytoplankton sub-sample was either directly counted (if > 1000 cells per mL) or concentrated by sedimentation and subsequent decantation (from 10 mL to 5 mL). A second sub-sample for the quantification of larger microplankton organisms was concentrated using gauze with a mesh opening of 55 µm (von Tümpling & Friedrich, 1999; Breitig, 1982). From 1961-2000 taxonomic composition and species abundance were determined under a conventional microscope using Kolkwitz counting chambers. At least four chambers (two chambers from the first sub-sample and two chambers from the second sub-sample) were counted with a minimum of 15 fields of view each. After 2000 phytoplankton was counted under an inverted light microscope, using the common plankton sedimentation and counting chambers (Hydro-Bios GmbH) according to Utermöhl (1958). In 1998 a methodological study including several other laboratories revealed that results from the method used before 2000 were comparable to the results obtained by the Utermöhl method, except for the smallest algae. Algae in the size range between 1-3 µm were underrepresented in the method used before 2000. However, for the data analysis in this study, algae smaller than 3 µm were not relevant. The cell number of filamentous and colonial algae was estimated by measuring the dimensions of one filament or colony and dividing by an average number of cells per unit. The specific cell volumes of each taxonomic unit (mostly at species level) were derived from average cell dimension measurements and simple geometric approximations (Hillebrand *et al.*, 1999). Using the specific cell volumes, cell numbers could be converted to phytoplankton biovolume or wet-weight biomass respectively, assuming a specific density of 1.0.

4.3.3 Data preparation and statistical analysis

For data analysis, data collected at depths of 0, 5 and 10 m were combined to average epilimnion values (depth-weighted) in order to allow comparison with the mixed water samples taken from 0-10 m. Since the measurements of the depth integrated and depth resolved data-series fit well together, merging them appeared reasonable. Values exceeding four times the inter quartile range were considered as extreme outliers (e.g. typing error) and were removed from the TP and DIN data. The extreme outliers accounted for less than 0.5 % of the data. Data were aggregated to either annual means, spring means, summer means, or mean values during the mixing period. Annual means were calculated using data from March until October, because this period is the growing season for phytoplankton and because the winter period was not always (in 19 out of 56 years) sampled. Including the winter months (when present) in the annual mean would have lowered the phytoplankton biomass by 13 % on average. Spring was defined as the period from March until May, summer from July until October and the mixing period from January until April (spring mixing typically finished in the end of April). The onset of stratification was calculated from daily temperature data and was defined as the day when the surface water temperature exceeds 7 °C and the water body remains stratified. In the Rappbode Reservoir, the hypolimnion temperature remains close to 4 °C while surface water temperature rises steeply from the onset of stratification onwards. In addition, we visually assessed the temperature development over the season for each year, to ensure that the calculation was not affected by outliers (for examples see supporting information Fig. S9.3.2). The day of stratification onset could be calculated only from 1980 onwards, since no daily surface temperature data were available prior to that. To assess the relative importance of phosphorus and nitrogen as limiting nutrients, the N:P ratio was used. As an indicator for discriminating between N and P limitation, we used the DIN:TP ratio, which has been identified as the best predictor for phytoplankton nutrient limitation (Bergström, 2010; Ptacnik, Andersen & Tamminen, 2010; Dolman, Mischke & Wiedner, 2016). Above a ratio of 3.4, phytoplankton usually shifts from nitrogen to phosphorus limitation. DIN:TP mass ratios were evaluated for all sampling dates to allow the detection of short-term nutrient limitation. The yield of phytoplankton biomass per unit phosphorus was calculated by dividing annual phytoplankton biomass by TP_{mix}.

For assessing changes in community composition, species were grouped into chlorophytes, chrysophytes, cryptophytes, cyanobacteria, dinoflagellates and diatoms. Moreover, we classified algal species according to the functional trait mixotrophy. We define mixotrophy as the ability to perform phototrophy and phagotrophy within a single cell, but we did not consider osmotrophy, i.e. the uptake of dissolved organics, here. The biomass of mixotrophs was calculated as the sum of all species considered potentially mixotrophic. We only included species that have been proven to be able to ingest bacteria in laboratory experiments. Species of the following genera were characterized as mixotrophs: *Cryptomonas*, *Dinobryon*, *Gymnodinium*, *Peridinium*, *Pseudopedinella* and *Uroglena* (Sanders, 1991; Tranvik, Porter & Sieburth, 1989; Gerea *et al.*, 2016). Even though food vacuoles and feeding have been reported in *Ceratium hirundinella*, this species was not categorized as a phagotroph, since existing reports are questionable (Stoecker, 1999). Moreover we classified algal species according to their motility. We characterized species as motile that are able to actively adjust their position in the water column. Therefore species that either possess flagella or can regulate their buoyancy were included in the biomass of motile organisms.

For time series analysis generalized additive models (GAM) were fitted to the data using the method gam from the R-package mgcv (Wood, 2017). GAMs are a common tool used for analyzing environmental and plankton time series, allowing nonlinear relationships by fitting smoothing functions (Thackeray, Jones & Maberly, 2008; Jochimsen, Kümmerlin & Straile, 2013). The smooth terms fitted to the data as well as their confidence intervals are visualized in Fig. 4.2. For further statistical testing only data after 1970 were included in the analysis in order to ensure that a stable phytoplankton community had established after the reservoir was filled to full capacity. The significance of long-term trends in the studied variables was investigated using Kendall´s τ test. As a non-parametric test, Kendall´s τ test does not depend on any assumptions regarding the distribution between dependent and independent variables. The test gives a rank correlation coefficient (Kendall´s τ, ranging from -1 to 1), which is expected to be approximately 0 when there is no change over time. Moreover, the time series of TP during spring mixing was statistically analyzed for significant shifts using the breakpoints-method from the package strucchange (Zeileis *et al.*, 2003; Zeileis *et al.*, 2001). Number and timing of significant shifts were identified using the Bayesian Information Criteria (BIC). The structural change was tested by the method sctest from the same package, using a linear model approach based on F-statistics as outlined in Zeileis *et al.* (2001). To identify single factors and processes controlling phytoplankton biomass, linear regression analysis was carried out. For analyzing the combined effect of different processes in controlling phytoplankton biomass, the relevant factors (mixotrophs, diatoms in spring and air temperature as a climate signal) were used as explanatory variables in a multiple regression model with the yield of phytoplankton biomass per unit phosphorus as a response variable. Competing models were compared in a standard model selection procedure by selecting the most informative model based on the Bayesian information criterion (BIC). The lower the BIC value, the better the model. All data analysis and graphics were performed using the R statistics program version 3.3.2 (R Core Team, 2016) with a significance level of $\alpha=0.05$.

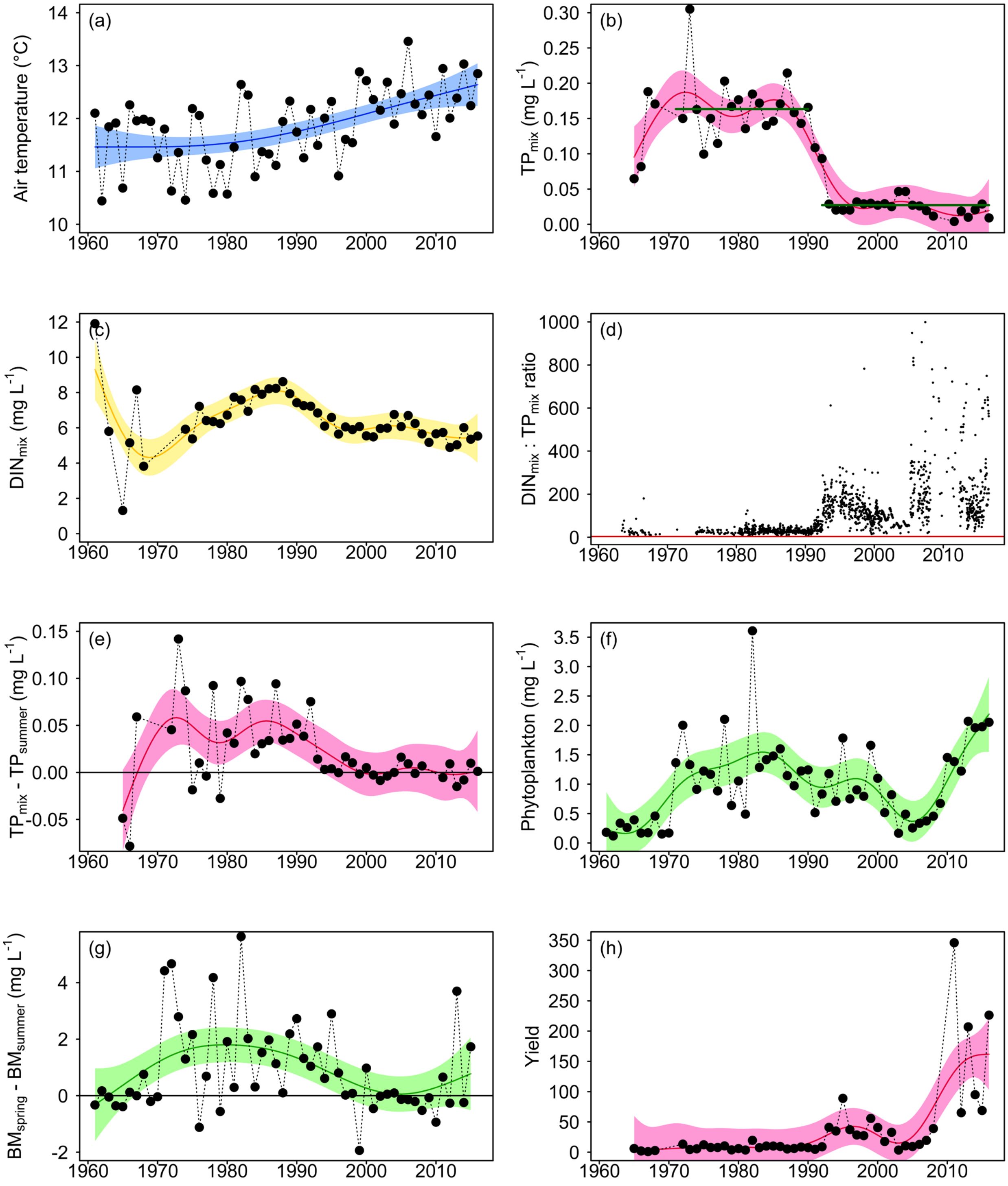
4.4 Results

4.4.1 Long-term trends

A significant increase was detected for air temperatures (Fig. 4.2a) as well as for surface water temperatures (see Fig. S9.3.1b supporting information) in the Rappbode Reservoir, especially in summer, pointing to significant climatic warming over the past decades (Kendall´s τ test, Table 4.1). As a direct consequence of this warming, the statistical analysis furthermore revealed an earlier onset of stratification (day 141 in 1980 to day 96 in 2016, Fig. S9.3.1f). In contrast to summer surface water temperatures, the hypolimnion temperatures in summer (measured at a depth of 50 m) showed a slightly decreasing trend (Table 4.1, Figure S9.3.1e). This suggests an increase in stratification stability in summer. The light conditions in the surface layer, as indicated by the Secchi depth, showed decreasing water clarity from 1972 until 1985 and stayed at a rather constant level thereafter (see Fig. S9.3.1a supporting information).

The results of Kendall´s τ test revealed a strongly decreasing trend in total phosphorus concentrations during the spring mixing period (Table 4.1, Fig. 4.2b). The development of TP_{mix} over time was characterized by a significant shift between 1991 and 1992 (breakpoint analysis: BIC = -159.2163, sctest: $p < 0.001$), no other shifts were detected before or after this major change. Until 1991, TP_{mix} remained at a high concentration with an average concentration of 0.163 mg L^{-1}. From 1992 onwards, TP_{mix} dropped to an average concentration of 0.027 mg L^{-1} and remained at this lower level until the end of the observation period. The maximum observed TP_{mix} value was found in 1973 (0.305 mg L^{-1}) and the minimum value in 2011 (0.004 mg L^{-1}). Similar to TP_{mix}, SRP_{mix} values decreased strongly after 1990 (Fig. S9.3.1k supporting information). Total dissolved inorganic nitrogen concentrations during mixing slowly increased until 1988 and showed a moderately decreasing trend thereafter (Fig. 4.2c). The DIN:TP mass ratio was above the critical DIN:TP ratio of 3.4 for all sampling dates (Fig. 4.2d), suggesting phosphorus as the main limiting nutrient in the Rappbode Reservoir. Also silicate was usually not limiting the growth of diatoms, as in 99.8 % of the sampling dates silicate was above the critical value of 0.109 mg L^{-1} (Reynolds, 2006). In conclusion, we focused on phosphorus as the primary limiting nutrient for phytoplankton growth. Similar to TP_{mix} and SRP_{mix}, the TP and SRP concentrations during summer (TP_{summer}, SRP_{summer}) also strongly decreased since 1990, but to a lesser extent, as indicated by a τ value closer to zero (Table 4.1). Hence, the difference between TP_{mix} and TP_{summer} (SRP_{mix} and SRP_{summer} respectively) decreased over time, indicating a higher availability of phosphorus in summer relative to spring after oligotrophication (Fig. 4.2e, Fig. S9.3.1l supporting information). Similar to surface phosphorus concentrations, TP and SRP concentrations in the hypolimnion in summer also strongly decreased after 1990 (Fig. S9.3.1m-n supporting information). Moreover the hypolimnion never went anoxic in summer during the investigation period (Fig. S9.3.1o supporting information). Both variables suggest that internal loading of phosphorus from the sediment was not significant in the Rappbode Reservoir.

Despite the substantial reductions in phosphorus, total annual phytoplankton biomass did not decrease in the long-term and even increased during the last decade (Fig. 4.2f, Table 4.1). Algal biomass showed no significant relationship with TP_{mix} (Table 4.2). While biomass during spring slightly decreased, biomass in summer significantly increased (Table 4.1, see Fig. S9.3.1g supporting information). Hence, also the difference between spring and summer algal biomass exhibited a significant decline over time (Fig. 4.2g, Table 4.1). While phytoplankton biomass was more equally distributed over the entire growing season after 2000, the maximum algal biomass slightly decreased (Fig. S9.3.1p supporting information). As a consequence of strongly reducing TP and not changing total annual phytoplankton biomass, the yield of biomass per phosphorus dramatically increased after 1990, both when excluding and including mixotrophs in the calculation of total biomass (Fig. 4.2h, Table 4.1). Diatoms during spring markedly decreased after oligotrophication from 1995-2005. In the past decade, however, spring diatoms steadily increased in biomass (Fig. 4.2i). This changing trend was associated with species replacements within the diatom community (Table S9.3.1 supporting information). While *Tabellaria fenestrata* and *Urosolenia longiseta*, which are rather tolerant towards nutrient deficiency (Reynolds *et al.*, 2002), became more dominant during the last two decades, *Asterionella formosa* and *Stephanodiscus hantzschii*, which are diatoms typical for eutrophic lakes, got less important. Mixotrophs and motile organisms significantly increased after nutrient reductions (Fig. 4.2j, for information on species composition see Table S9.3.1). However, when mixotrophs were excluded from the biomass of motile organisms, no detectable long term trend for motile species remained (Table 4.1, see Figure S9.3.1i). Cyanobacteria were never a dominant algae group in the Rappbode Reservoir (they usually accounted for less than 10% of the total phytoplankton biomass), even during the period with high nutrient concentrations.


(a)
Air temperature (°C)
14
13
12
11
10
1960
1970
1980
1990
2000
2010
(b)
TP_{mix} (mg L^{-1})
0.30
0.25
0.20
0.15
0.10
0.05
0.00
(c)
DIN_{mix} (mg L^{-1})
12
10
8
6
4
2
0
(d)
DIN_{mix} : TP_{mix} ratio
1000
800
600
400
200
0
(e)
TP_{mix} - TP_{summer} (mg L^{-1})
0.15
0.10
0.05
0.00
-0.05
(f)
Phytoplankton (mg L^{-1})
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(g)
BM_{spring} - BM_{summer} (mg L^{-1})
4
2
0
-2
(h)
Yield
350
300
250
200
150
100
50
0

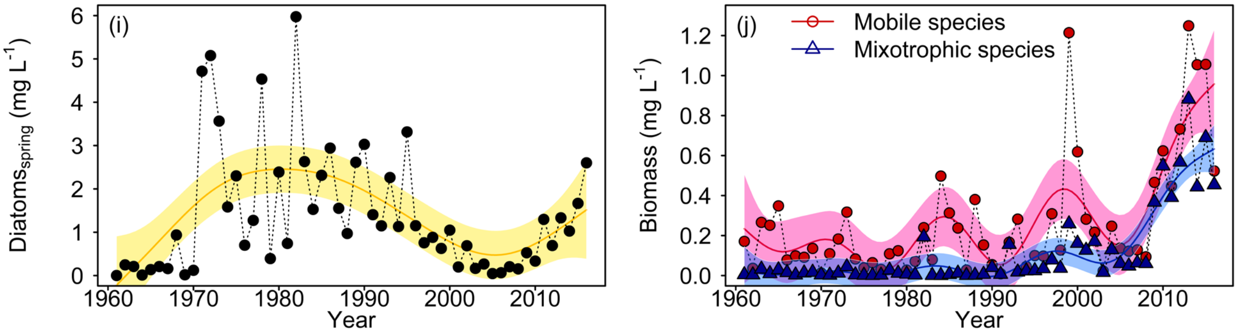


Figure 4.2: Long-term development of air temperature, mineral nutrients and phytoplankton at the Rappbode Reservoir. The solid, colored lines are the smoothers from the generalized additive models fitted to the data and the shades are the confidence intervals of these fits. (a) Mean air temperatures. (b) Mean concentrations of total phosphorus during mixing (TP_{mix}). The horizontal lines show the average value of the two periods identified by breakpoint analysis. (c) Mean concentrations of dissolved inorganic nitrogen during mixing (DIN_{mix}). (d) DIN:TP ratio of all surface samples. The red line indicates a ratio of 3.4, above which no nitrogen limitation is expected (Bergström, 2010). (e) Difference in total phosphorus concentrations between the mixing and the summer period. (f) Annual mean phytoplankton biomass. (g) Difference in phytoplankton biomass between spring and summer period. (h) Yield of phytoplankton biomass per unit phosphorus. (i) Diatom biomass in spring. (j) Annual mean biomass of motile species (red) and potentially mixotrophic species (blue).

Table 4.1: Means (± standard deviation) of the variables over the period 1971-2016, results of the Kendall´s τ statistics for detection of long-term trends (Kendall´s τ) and probability of the Kendall´s test (p-value). Results of generalized additive models (GAM) from 1961-2016, giving the deviance explained (Dev. exp.) in percent, the estimated degrees of freedom (edf) and statistical sigificance judged by F-tests (p-value).

		Kendall´s τ		**GAM**		
Variables	**Overall mean (SD)**	**Kendall´s τ**	**p-value**	**Dev. exp.**	**edf**	**p-value**
Air temperature (°C)	11.9 (0.7)	0.436	<0.001	33.4	2.05	<0.001
Surface water temperature (°C)	12.4 (1.9)	0.382	<0.001	29.8	2.55	<0.001
Surface water temperature$_{spring}$ (°C)	7.0 (1.4)	0.292	0.004	26.3	2.57	<0.001
Surface water temperature$_{summer}$ (°C)	16.59 (1.5)	0.581	<0.001	62.7	2.63	<0.001
Hypolimnion water temperature$_{summer}$ (°C)	5.5 (0.7)	-0.264	0.010	13.7	2.00	<0.001
Stratification onset (days)	119.7 (11.03)	-0.564	<0.001	57.3	1	<0.001
Secchi depth (m)	3.690 (0.596)	-0.266	0.010	48	6.33	<0.001
TP$_{mix}$ (mg L^{-1})	0.090 (0.076)	-0.626	<0.001	83.4	7.50	<0.001
SRP$_{mix}$ (mg L^{-1})	0.013 (0.010)	-0.591	<0.001	74.1	7.43	<0.001
Dissolved inorganic nitrogen (mg L^{-1})	6.476 (0.976)	-0.417	<0.001	56.1	7.57	<0.001
TP$_{summer}$ (mg L^{-1})	0.065 (0.053)	-0.565	<0.001	84.3	7.08	<0.001
SRP$_{summer}$ (mg L^{-1})	0.006 (0.005)	-0.540	<0.001	54.0	3.78	<0.001
TP$_{mix}$-TP$_{summer}$ (mg L^{-1})	0.024 (0.037)	-0.393	<0.001	44.1	7.12	<0.001
SRP$_{mix}$-SRP$_{summer}$ (mg L^{-1})	0.007 (0.007)	-0.477	<0.001	53.1	4.22	<0.001
Phytoplankton biomass (mg L^{-1})	1.164 (0.643)	-0.109	0.291	55.9	7.90	<0.001
Phytoplankton biomass$_{spring}$ (mg L^{-1})	1.869 (1.50)	-0.270	0.008	35	4.44	<0.001

Phytoplankton biomass$_{summer}$ (mg L^{-1})	0.757 (0.537)	0.228	0.027	47.7	7.95	<0.001
Phytoplankton$_{spring}$– Phytoplankton$_{summer}$ (mg L^{-1})	1.092 (1.647)	-0.388	<0.001	25.5	3.66	<0.001
Max. phytoplankton biomass (mg L^{-1})	5.470 (4.667)	-0.230	0.023	30.1	2.67	0.001
Yield (Biomass : TP$_{mix}$)	39.08 (67.26)	0.462	<0.001	59.0	6.98	<0.001
Yield excluding mixotrophs (Biomass$_{excluding\ mixotrophs}$: TP$_{mix}$)	30.59 (47.78)	0.406	<0.001	50.3	5.01	<0.001
Diatoms$_{spring}$ (mg L^{-1})	1.648 (1.426)	-0.386	<0.001	38.2	4.15	<0.001
Mixotrophic species (mg L^{-1})	0.135 (0.210)	0.602	<0.001	81.6	8.20	<0.001
Motile species (mg L^{-1})	0.294 (0.320)	0.347	<0.001	61.5	8.49	<0.001
Motile species excluding mixotrophs (mg L^{-1})	0.159 (0.187)	0.059	0.572	2.14	1	<0.001

4.4.2 Potential mechanisms preventing a decrease in total biomass

4.4.2.1 Observations 1 and 2: More mixotrophic and motile species

Regression analysis revealed a significant negative relationship between phosphorus concentrations and the biomass of mixotrophs (Table 4.2). In line with our observation, the ratio of total phytoplankton biomass to total phosphorus (yield) was positively related to the biomass of potentially mixotrophic species (R^2=0.465, p<0.001, Table 4.2). In contrast, there is no evidence for any influence of mobility on the yield, since the influence of mobility disappeared when mixotrophic species were excluded from this group (Table 4.2). We also found no evidence that motile, non-mixotrophic species increased over time, indicating that mixotrophy, and not motility, is the relevant trait here (compare Table 4.1).

4.4.2.2 Observation 3: Less nutrient losses in spring due to declining diatom biomass

Reduced phosphorus concentrations during mixing were associated with lower diatom biomass in spring (R^2=0.306, p<0.001, Table 4.2). As mentioned above, the difference between TP$_{mix}$ and TP$_{summer}$ decreased after oligotrophication (Fig.4. 2e). Obviously, less nutrients could be removed from the epilimnion by phosphorus sedimentation in spring and hence more nutrients remained available for the summer period. Differences between TP_{mix} and TP_{summer} were positively related to diatom biomass in spring ($R^2=0.354$, $p<0.001$, Table 4.2). Hence cellular P uptake by diatoms mediates not only the transfer of dissolved P into particulate P but also facilitates a subsequent, fast P sedimentation. The more efficient P recycling over the season in years with lower phosphorus concentrations might therefore be explained by the lower diatom abundances during these years. It is interesting to note that the difference between spring and summer phytoplankton biomass was also associated with differences between TP_{mix} and TP_{summer} ($R^2=0.288$, $p<0.001$, Table 4.2) and likewise with diatoms in spring ($R^2=0.846$, $p<0.001$, Table 4.2). According to these findings, we conclude that lowered TP losses in spring due to sedimenting diatom cells resulted in higher TP availability in summer, promoting more intense phytoplankton growth during the summer period. Increasing algal biomass in summer compensated for decreasing biomass in spring and consequently the overall annual phytoplankton biomass did not drop after P reductions (Table 4.1).

Table 4.2: Results of linear regressions, testing the predictions of observations. Reported are the equation of the linear models (Estimate ± standard error), the proportion of the variability in the dependent variable explained by the independent variable (R^2) as well as a significance statement, including the degrees of freedom (DF) and the significance level (p-value).

Dependent variable	**Explanatory variable**	**Estimate (±SE)**	**DF**	**R^2**	**p-value**
Phytoplankton biomass	TP_{mix}	2.008 (1.318)	41	0.054	0.135
Mixotrophs	TP_{mix}	-1.228 (0.370)	41	0.212	0.002
Yield	Mixotrophs	225.920 (37.873)	41	0.465	<0.001
Yield	Motile species	102.448 (28.088)	41	0.245	<0.001
Yield	Motile species excluding mixotrophs	40.93 (54.24)	41	0.014	0.455
$Diatoms_{spring}$	TP_{mix}	9.987 (2.348)	41	0.306	<0.001
TP_{mix}-TP_{summer}	$Diatoms_{spring}$	0.016 (0.003)	41	0.354	<0.001

$\text{Phytoplankton}_{\text{spring}}$– $\text{Phytoplankton}_{\text{summer}}$	TP_{mix} - $\text{TP}_{\text{summer}}$	22.844 (5.674)	40	0.288	<0.001
$\text{Phytoplankton}_{\text{spring}}$– $\text{Phytoplankton}_{\text{summer}}$	$\text{Diatoms}_{\text{spring}}$	-0.627 (0.149)	43	0.846	<0.001

4.4.2.3 Analysis of combined effects

Regression analysis revealed a significant positive effect of the biomass of potentially mixotrophic species on the ratio of total phytoplankton biomass to total phosphorus (yield) (Table 4.2). In a multiple linear regression model, we tested whether the addition of diatom biomass in spring and air temperature as a climate signal, would contribute to explaining the variation in yield (Table 4.3). This turned out not to be the case and they were not significantly related to the yield. Using the difference between TP_{mix} and $\text{TP}_{\text{summer}}$ or the difference between the spring and summer biovolume instead of $\text{diatoms}_{\text{spring}}$ as a factor in the model did not give significant results either. Therefore, as indicated by BIC the variability in yield was best explained by mixotrophs alone. $\text{Diatoms}_{\text{spring}}$ and air temperature added no further information to the model.

Table 4.3: Results of multiple linear regressions, testing the combined effect of different processes on the ratio of total phytoplankton biomass to total phosphorus (yield). Reported are the equation of the models (Estimate ± standard error), the proportion of the variability in the dependent variable explained by the independent variable (R^2) as well as a significance statement, including the degrees of freedom (DF) and the significance level (p-value). Different models are compared using the Bayesian information criterion (BIC).

Dependent variable	**Explanatory variables**	**Estimate (±SE)**	**Single p-values**	**Overall p-value**	**Overall DF**	**Overall R^2**	**Overall BIC**
Yield	Mixotrophs	201.916 (42.104)	<0.001	<0.001	39	0.492	472.7
	$\text{Diatoms}_{\text{spring}}$	4.384 (5.840)	0.457				
	Air temperature	16.601 (11.935)	0.172				
Yield	Mixotrophs	203.25 (41.84)	<0.001	<0.001	40	0.4844	469.5
	Air temperature	14.16 (11.42)	0.222				
Yield	Mixotrophs	225.920 (37.873)	<0.001	<0.001	41	0.465	467.4

4.5 Discussion

4.5.1 Response of phytoplankton biomass to phosphorus reductions

The hitherto unstudied long-term data set on phytoplankton dynamics in the Rappbode Reservoir adds a valuable case study to the research on the responsiveness of algal communities to nutrient reductions because of its unusual response. The Rappbode Reservoir underwent a strong and abrupt shift in phosphorus concentrations from approximately 0.163 mg L^{-1} to 0.027 mg L^{-1} after 1990, as a result of banning phosphate-containing detergents after the reunification of East and West Germany. The construction of a wastewater treatment plant in 2000 led to even slightly lower phosphorus concentrations during the last two decades, sometimes with TP concentrations below 0.01 mg L^{-1} during mixing. These P concentrations seem sufficient to reduce phytoplankton biomass. Substantial declines in chlorophyll were observed in several lakes with intermediately high P concentrations (Jeppesen, Jensen & Søndergaard, 2002; Köhler, Behrendt & Hoeg, 2000). Also in Lake Constance (Germany) a response of phytoplankton biomass was found at TP concentrations below 0.04 mg L^{-1} (Jochimsen, Kümmerlin & Straile, 2013). The TP concentrations in the Rappbode Reservoir fell below this threshold already from 1991 onwards. However, in the Rappbode Reservoir the phytoplankton biomass does not follow the expected patterns after nutrient concentrations were reduced. Despite reductions in P by a factor of 5 within 3 years, total annual biomass did not decline in the long-run and even increased during the last decade. Annual mean biomass was unrelated to phosphorus concentrations during mixing. This is in contrast to classical eutrophication models and loading concepts (Vollenweider, 1971) and to most previous studies of oligotrophication in lakes, which found a decline in phytoplankton biomass usually within 10 years after P reductions (Jeppesen *et al.*, 2005). Our study sought to reveal the mechanisms preventing such a reduction in phytoplankton biomass in response to declining phosphorus.

Limnologists differentiate between external and internal P loading and numerous studies documented that a reduction in external phosphorus load is ineffective on algal standing stocks when internal loading is intense (e.g. Søndergaard, Jensen & Jeppesen, 1999; Cymbola, Ogdahl & Steinman, 2008). Internal loading is usually associated with critically low redox potentials at the sediment-water interface, e.g. during anoxia in summer. This can be excluded as a possible explanation in case of the Rappbode Reservoir since the hypolimnion never went anoxic during summer. Moreover, our data refer to P concentrations. Any internal loading from the sediment would have become immediately detectable in hypolimnetic P concentrations. In the Rappbode Reservoir, summer phosphorus concentrations moreover decreased in the hypolimnion after 1990, indicating that internal loading did not play a significant role.

4.5.2 Mixotrophs increase yield

In line with ourobservation, we found that the higher yield of biomass per phosphorus after oligotrophication could be partly explained by an increase of potentially mixotrophic species. Mixotrophs make use of nutrient resources that would not be accessible otherwise, by ingesting bacteria as an additional P source. Predation of bacteria by phytoplankton seems to be a powerful strategy for gaining nutrients under P-depleted conditions, since bacteria are very phosphorus rich particles (Nygaard & Tobiesen, 1993; Vadstein *et al.*, 1993). Besides their ability to use bacteria as a supplementary resource, mixotrophs also have the competitive advantage of possessing flagella. By actively moving along the opposing vertical gradient of nutrient and light availability, motile species can adjust their position to a depth with optimized growth conditions and can select the appropriate environment in the water column (Klausmeier & Litchman, 2001; Jäger, Diehl & Schmidt, 2008). Hence they are able to increase the yield by transporting nutrients from the hypolimnion to the surface. However, since the biomass of other, non-mixotrophic motile species did not change over time we infer that the trait of mobility was less important than phagotrophy in providing additional P sources for phytoplankton under nutrient-depleted conditions.

Our results match well with other studies showing the relation between trophic state and the importance of mixotrophic species in the phytoplankton community. The competitive advantage of phagotrophic species when dissolved nutrients are low has been shown in experimental studies (Isaksson *et al.*, 1999; Katechakis & Stibor, 2006; Palsson, 2004) as well as in studies comparing aquatic systems of differing trophic status (Saad *et al.*, 2016; Stoecker *et al.*, 2017). In accordance with the data from the Rappbode Reservoir, an increase in potentially mixotrophic species (mainly cryptophytes, dinophytes and chrysophytes) was also observed in other lakes after oligotrophication, including those where no decrease in total biomass was found after nutrient reductions (Jeppesen *et al.*, 2005; Jeppesen, Jensen & Søndergaard, 2002; Kamjunke, Henrichs

& Gaedke, 2006; Weyhenmeyer & Broberg, 2014; Gaedke, 1998; Anneville, Gammeter & Straile, 2005; Anneville, Ginot & Angeli, 2002; Findlay *et al.*, 2001). While these studies clearly show the relation between trophic state and importance of the mixotrophic strategy, the mechanism by which mixotrophs increase with oligotrophication remains partly unclear: Due to their ability to prey on bacteria, mixotrophs are relieved from direct competition for inorganic phosphorus with obligate phototrophs. However, the reason why they do not build up high abundances in eutrophic waters remains open and to be tested experimentally.

Even though our results show that mixotrophs significantly explain the observed increased yield after oligotrophication, they are not the only explanation for the missing response of biomass to nutrient reductions. When calculating the yield while excluding mixotrophic species from the total biomass, the yield showed an increasing trend too, indicating that obligate autotrophic species also contributed to the increase in yield. Possibly the presence of mixotrophs stimulated the growth of other species by making nutrients available to the autrotrophs. One possible mechanism could be their low sinking velocity, keeping P in the photic zone for a longer time (Ptacnik, Diehl & Berger, 2003; Reynolds, 2006; Findlay *et al.*, 2001). Moreover it has been shown that mixotrophs can either retain or release phosphorus (Rothhaupt, 1996). By releasing nutrients into the environment, they could facilitate the growth of phototrophs. Some evidence for a positive effect of mixotrophs on the standing stock of non-mixotrophic algae is provided by the increasing phytoplankton biomass in the Rappbode Reservoir in the past decade. The continuous increase in total phytoplankton biomass since 2005 (about 2 mg L^{-1} in magnitude) is not entirely realized by the mixotrophic community. In fact, about half of the biomass increase can be directly assigned to increasing biomass of mixotrophs and the other half is attributable to changing non-mixotrophic algal groups, e.g. diatoms that increased in spring biomass (Fig. 4.2i). This increase in non mixotrophs that goes parallel to increasing mixotroph biomass may be taken as an indication for nutrient recycling by mixotrophs. In this respect, the access of mixotrophs on bacterial phosphorus has enhanced the P-flux towards mixotrophs and the non-mixotrophs can partly profit from this phosphorus by nutrient recycling by the mixotrophs. In addition, increasing protozoan biomass in the past years could also have contributed to P recycling towards obligate autotrophs (see below). However, whether phototrophs benefit from P recycling by mixotrophs is controversial in the literature. In experiments, Sanders *et al.* (2001) observed that phosphate and ammonia were rapidly released by the mixotrophic flagellate *Ochromonas* grown on bacteria in the light and in the dark.

They concluded that *Ochromonas* was unable to store or utilize N and P in excess of the quantities required for heterotrophic growth. Also Grover (2000) saw some evidence for an increase of phototrophs in response to recycling of bacteria nutrients because in four out of five cultures the net growth rate of the phototroph was higher in the presence than in the absence of *Ochromonas*. In contrast to this, Rothhaupt (1997) found that under nutrient limited situations, *Ochromonas* retained P for its own photosynthesis and did not stimulate other phytoplankton species.

4.5.3 Less nutrient losses in spring due to declining diatom biomass

In agreement with our observation we identified a positive relationship between diatom abundance and removal rates of phosphorus during summer. During the oligotrophication phase in the Rappbode Reservoir we observed a decrease in diatom biomass in spring, probably caused by lower TP concentrations during spring turnover and to some extent also an earlier onset of stratification (possible effects of climate change are discussed below). Diatoms have high sinking velocities due to their siliceous frustules (Sommer, 1984; Reynolds, 2006; Trimbee & Harris, 1984) but are relatively slowly re-mineralized (Elster, 1963; Krause, 1964). As a result of both traits, diatom cells sink out of the photic zone before they can be re-mineralized and therefore lead to high nutrient losses by sedimentation. Empirical evidence for the effect of sinking algae on phosphorus concentrations is, for example, also given by Benndorf (1968) and Horn *et al.* (2015). Due to the decreasing dominance of diatoms during oligotrophication the P removal by sedimentation in the Rappbode Reservoir has most probably diminished.

In a modelling study, Frassl, Rothhaupt and Rinke (2014) demonstrated that the uptake of phosphorus by sedimenting algae has an effect on phosphate depletion in Lake Constance. They showed that the depletion of phosphate in the surface layer was highest during the season when phytoplankton species with high phosphorus storage capacities and settling velocities, mainly belonging to the group of diatoms, dominated. In line with their modelling results, the reduced diatom biomass in spring in the Rappbode Reservoir may explain the lower removal of nutrients from the productive zone by sedimentation processes and hence more P resources stayed available for the summer period. As a result, higher phytoplankton biomass was realized in summer during the oligotrophic phase compensating for biomass losses in spring. As a consequence, phytoplankton biomass was more equally distributed over the season. This documents the importance of interactions between phytoplankton traits, phytoplankton community dynamics and biogeochemical processing.

The reduction in seasonal variability of biomass in nutrient poor waters is in line with general observations from lakes of different trophic status (Smith, 1990) as well as with the classical model of seasonal succession of plankton in fresh waters, the PEG (Plankton Ecology Group) model. The PEG model expects that under oligotrophic conditions, i.e. when resources for a pronounced spring bloom formation are lacking, the resources are more evenly available over the season, leading to a more even distribution of biomass over the year (Sommer *et al.*, 1986).

Moreover the PEG model predicts more successive stages in eutrophic lakes compared to oligotrophic ones, since nutrient limitation in eutrophic lakes occurs at shorter intervals in the season resulting in a higher heterogeneity of nutrient conditions and thus ecological niches (Sommer *et al.*, 1986). A changing seasonal development of phytoplankton species has also been observed in Lake Geneva and the Saidenbach Reservoir (Anneville, Ginot & Angeli, 2002; Horn *et al.*, 2015). A feature they have in common with the Rappbode Reservoir is that phytoplankton biomass resisted nutrient reductions and even increased after nutrient concentrations dropped. While this study demonstrated that the seasonal development of biomass has changed during oligotrophication, future studies should investigate the change in seasonal dynamics of algal functional traits (Weithoff & Gaedke, 2016) as well as potential self-stabilizing mechanisms (Jochimsen, Kümmerlin & Straile, 2013) in more detail.

Although we clearly identified associations between diatom biomass in spring, P recycling over the season and a changed seasonal development of biomass, it is important to note that these observations were not significantly related to the yield (Table 4.3). This suggests that, in contrast to the effect of mixotrophs, the effect of P sedimentation by diatoms was not the major mechanism preventing a decrease in total annual phytoplankton biomass over the course of oligotrophication.

4.5.4 The role of zooplankton

So far, we focused our analysis of the mechanisms preventing a phytoplankton biomass decline on bottom-up effects. However, top-down effects by higher trophic levels on the structure and productivity of aquatic ecosystems must also be taken into consideration (Mazumder & Havens, 1998; Kerimoglu, Straile & Peeters, 2013; Urabe, Nakanishi & Kawabata, 1995). In the Rappbode Reservoir, total zooplankton biomass and the most important grazer groups (*Daphnia* and other crustaceans) varied largely from year to year, but showed no significant trend over time (Wentzky, unpublished data). Since zooplankton biomass and hence grazing did not decrease since 1990, it appears unlikely that they prevented the decrease in phytoplankton biomass after P reductions or contributed to the increase in algal biomass during the last decade. However, a significant increase in protozoan biomass was detected since 2003 (Kendall´s τ=0.463, $p<0.001$). Protozoa efficiently graze on bacteria and have been shown to strongly increase the remineralization of mineral nutrients, particularly phosphorus, which is then available for phytoplankton growth (Bloem *et al.*, 1989; Hambright, Zohary & Güde, 2007). Increased excretion of nutrients by protozoa possibly contributed to the lower difference between TP_{mix} and TP_{summer} after 1990, in addition to reduced P losses from the productive zone due to sedimentation of diatoms. Thus higher protozoan biomass in the Rappbode Reservoir might have enhanced summer phytoplankton production through a more efficient P recycling. The start of the protozoan biomass increase, however, does not fit to the start of phytoplankton biomass increase making a major influence of protozoans rather unlikely.

4.5.5 Effects of climate change

Climate change has the potential to contribute to the resilience of phytoplankton biomass. It can alter primary productivity, phytoplankton taxonomic composition and seasonal dynamics in different ways (Winder & Sommer, 2012). Climate change can directly affect phytoplankton via physiology or indirectly by altering the physical structure of the water body (Winder & Sommer, 2012). As an indicator of climate change, an increase in air temperature was observed during the last decades at the Rappbode Reservoir. In agreement with observations from other lakes and reservoirs we found an earlier onset of thermal stratification and higher surface water temperatures, probably resulting in an increased stability of stratification (Adrian *et al.*, 2009; Jones & Brett, 2014). Besides the effects on the physical structure, climate warming has a strong effect on the duration of the stratified season, during which the phytoplankton biomass is generally higher than during the non-stratified period (Sommer *et al.*, 1986). In the case of the Rappbode Reservoir, the growing season – defined as the time between stratification onset and winter mixing – is about two months longer nowadays than in the 1970s. Therefore a prolongation of the growing seasons necessarily leads to higher annually averaged phytoplankton biomass.

By altering the position of algae cells in the water column relative to light and nutrients, vertical mixing has been shown to strongly affect the performance of phytoplankton species (Winder & Sommer, 2012). In the Rappbode Reservoir, the earlier onset of stratification might have negatively or positively affected diatom biomass in spring, resulting in reduced P sedimentation losses in spring (see paragraph above). Diatoms are well adapted to low light conditions and cold temperatures and accordingly may profit when the growing season starts earlier in the year. On the other hand, diatoms have high sinking velocities and therefore depend on turbulence to remain suspended in the photic zone (Ptacnik, Diehl & Berger, 2003; Huisman *et al.*, 2004; Jäger, Diehl & Schmidt, 2008). Intensified stratification strengths and earlier onset of stratification are associated with reduced mixing intensity. As a consequence, diatoms have a competitive disadvantage and can be outcompeted by algae better adapted to stratified water bodies. The diatom dynamics in Rappbode Reservoir in fact showed strong reductions and similarly strong increases in the times after nutrient reduction took place (see Fig. 4.2i). These dynamics are associated with species replacements in the diatom community (see above) and it remains to be analyzed which physiological traits are associated with these community changes and in which respect these trait dynamics may explain the observed dynamics in diatom biomass.

4.5.6 Combined effects and conclusions

An increase in potentially mixotrophic species capable of exploiting additional P sources was speculated to be the main mechanism explaining why overall phytoplankton biomass did not drop after P reductions. Furthermore we found that reduced P losses by diatoms sedimenting out of the photic zone, led to higher biomass in summer compared to spring biomass. Climate change, manifested as increased air and surface water temperature, an earlier onset of thermal stratification and stronger stratification stability might have supported these processes via effects on community composition. However, the combined analysis of the mentioned mechanisms on the yield of phytoplankton biomass per unit of phosphorus revealed that only mixotrophs significantly affected the yield. This suggests that losses in total biomass were primarily prevented by changes in the microbial food web including the mixotrophs and their ability to make bacterial phosphorus available for photosynthetic organisms (Vadstein *et al.*, 1993; Rothhaupt, 1992). Changes in seasonal biogeochemical cycling of nutrients within the pelagic zone as well as climate change are evident, but play a minor part in preventing losses in total biomass. In conclusion, the results show that the phytoplankton community in the Rappbode Reservoir was able to adapt to lower nutrient levels without a loss in total biomass. This study demonstrates the ecosystem´s ability to compensate for changes in resource availability through changes in internal processes and functional strategies.

4.6 Acknowledgements

We thank the water supply works Wasserwerk Wienrode and Talsperren Betrieb Sachsen-Anhalt for sharing their monitoring data with us. Special thanks go to Jan Donner and Wolf-Dieter Skibba (Wasserwerk Wienrode) for providing us with information about the analytical and biological methods used during the 50 years of monitoring. Thanks to Detlef Cöster (Talsperren Betrieb Sachsen-Anhalt) for providing daily water temperature data. We also thank Sabrina Eichler, Eva Meyer, Yuhan Xie and Thomas Plumbohm for digitalizing the monitoring data and Marieke Frassl, Karoline Morling, Katja Westphal, Bertram Böhrer and Martin Schultze (Helmholtz Centre for Environmental Research) for constructive discussions. Thanks to Philipp Keller (Helmholtz Centre for Environmental Research) for preparing the bathymetric map. This research was supported by grants JA 2146/2-1 and RI 2040/2-1 from the German Research Foundation (DFG) within the priority program 1704 "DynaTrait".

5 Metalimnetic oxygen minimum and the presence of *Planktothrix rubescens* in a low-nutrient drinking-water reservoir

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Water Research (2019), 148, 2008-218

5.1 Highlights

- A metalimnetic oxygen minimum (MOM) down to 40% saturation was observed in summer
- The MOM was not imported from the sediment at the side walls
- The MOM was a consequence of pelagic processes, such as respiration in the metalimnion
- We hypothesize that *Planktothrix rubescens* in the metalimnion caused the MOM

5.2 Abstract

Dissolved oxygen is a key player in water quality. Stratified water bodies show distinct vertical patterns of oxygen concentration, which can originate from physical, chemical or biological processes. We observed a pronounced metalimnetic oxygen minimum in the low-nutrient Rappbode Reservoir, Germany. Contrary to the situation in the hypolimnion, measurements of lateral gradients excluded the sediment contact zone from the major sources of oxygen depletion for the metalimnetic oxygen minimum. Instead, the minimum was the result of locally enhanced oxygen consumption in the open water body. A follow-up monitoring included multiple chlorophyll a fluorescence sensors with high temporal and vertical resolution to detect and document the evolution of phytoplankton. While chlorophyll fluorescence sensors with multiple channels detected a mass development of the phycoerythrin-rich cyanobacterium *Planktothrix*

rubescens in the metalimnion, this species was overlooked by the commonly used single-channel chlorophyll sensor. The survey indicated that the waning *P. rubescens* fluorescence was responsible for the oxygen minimum in the metalimnion. We hypothesize that pelagic processes, i.e., either oxygen use through decomposition of dead organic material originating from *P. rubescens* or *P. rubescens* extending its respiration beyond its photosynthetic activity, induced the metalimnetic oxygen minimum. The deeper understanding of the oxygen dynamics is mandatory for optimizing reservoir management.

5.3 Introduction

Dissolved oxygen is a key variable for nearly all organisms in the aquatic environment. Especially in stratified lakes, vertical transport of dissolved substances is limited, which can result in sharp vertical gradients of oxygen concentration. Usually in direct contact with the atmosphere, the epilimnion shows a gas pressure that is close to equilibrium with the atmosphere, while the hypolimnion has trapped a limited amount of oxygen, which is subjected to depletion over the summer months until the thermal stratification breaks and deep recirculation recharges the hypolimnion with oxygen (e.g. Boehrer & Schultze, 2008).

In many cases, however, a more complex picture is observed. Especially in the thermocline, where high density gradients restrict the vertical exchange, gradients of dissolved substances are formed and sustained due to small vertical transport. Both metalimnetic oxygen maxima and metalimnetic oxygen minima can be found. Though both features are commonly encountered, oxygen maxima have been dealt with in more detail in the literature (Wilkinson *et al.*, 2015). Here we concentrate on the case of metalimnetic oxygen minima, which often get attributed to eutrophic lakes (e.g. Lake Arendsee: Boehrer & Schultze, 2008; in general see also Wetzel, 2001) or reservoirs (Zhang *et al.*, 2015); however, also lakes of lower trophic state can show metalimnetic oxygen minima (e.g. Joehnk & Umlauf, 2001).

Metalimnetic maxima can appear as an artefact when trapped water warms and hence saturation levels increase due to lower solubility of oxygen at higher temperatures (e.g. Wilkinson *et al.*, 2015). Alternatively, oxygen maxima can originate from oxygen production by photosynthesising phytoplankton (Parker, Wenkert & Parson, 1991; Stefan *et al.*, 1995), which form a deep chlorophyll maximum in the metalimnion. Deep chlorophyll maxima can be observed in many lakes (Leach *et al.*, 2017). In contrast to metalimnetic oxygen maxima, metalimnetic oxygen minima cannot be the consequence of diffusive heating from the surface and hence must result from oxygen depletion. When a minimum forms, the shape of the oxygen profile depends on both: the oxygen depletion at the respective depth as well as vertical transport from the layers above and below (Kreling *et al.*, 2017).

Both, oxygen depletion in the open water and at the sediment surface can be responsible for the formation of a metalimnetic oxygen minimum. Modelling approaches include one oxygen depletion rate in the water column and one value for the oxygen depletion rate per sediment surface (e.g. Livingstone & Imboden, 1996; Weber *et al.*, 2017). Often oxygen consumption by the sediment is considered as the leading part. In addition, advective processes like inflows with high oxygen demand have been claimed to be important: flood waters with easily degradable organic material often find their way into the metalimnion of a stratified water body (as shown by Nix, 1981; DeGray Reservoir, Arkansans, USA). As oxygen depletion in a lake is usually attributed to the sediment contact zone, it has been reasoned that the oxygen minimum can be an effect of oxygen depletion at the side boundaries, which is advected into the main body of the lake on isopycnal (constant density) surfaces. The corresponding lake morphometry may be supportive of different depletion rates (Shapiro, 1960; Wetzel, 2001) due to variable ratios of sediment area to layer volume with depth and varying temperatures with depth.

Despite its low trophic status, a reoccurring metalimnetic oxygen minimum was observed in the Rappbode Reservoir (see Fig. 5.7). Hence we planned an investigation of the metalimnetic oxygen minimum, to find clues about possible reasons. Understanding oxygen depletion is essential for a proper management of reservoirs. Low oxygen conditions interfere with water quality and can thereby largely increase costs of drinking water treatment. Too low oxygen concentrations are also risky for biota within the lake depending on a sufficient oxygen supply, like fish (Rice *et al.*, 2013). The Rappbode Reservoir harbours a managed stock of lake trout (*Salmo trutta f. lacustris*), which are sensitive to low oxygen levels and, therefore, concentrations below 4 mg L^{-1} should be avoided.

To shed light on the causes of the metalimnetic oxygen minimum in the Rappbode Reservoir, we organised our investigation in three distinct steps, over two years:

(1) The horizontal and vertical variability of the oxygen minimum was studied on one single day in September 2015 to determine the location of the most intense oxygen depletion (possibly the sediment surface?).

(2) The temporal and spatial evolution of the oxygen in 2016 was documented by measurements of multiparameter profiles of high resolution.

(3) The ecological evolution was studied by multichannel fluorescence profiles with fine vertical resolution and water samples for identification of organisms with microscopy.

5.4 Field Site, Measurements and Methods

The Rappbode Reservoir is located in the Harz Mountains in Northern Germany (coordinates 51°44’N 10°54’E) and is the largest drinking water reservoir in Germany (in terms of volume), providing water to about 1 million people. The catchment is covered by forest and farmland (Friese *et al.*, 2014). The dam has been constructed in the 1950s forming a lake of a complex shape with an 8 km long main channel towards south-west and two side arms facing north and south (Fig. 5.1). Water enters via three pre-dams, which have been built for the purpose of sediment and nutrient retention (Rinke *et al.*, 2013). The reservoir has a maximum depth of 89 m and a mean depth of 28.6 m. As typical for deep temperate water bodies, the Rappbode Reservoir completely mixes in late autumn and spring. It always stratifies in summer, but only occasionally in winter during the last years, due to the lack of ice cover. Since 1991, phosphorus concentrations have been low enough to expect a mesotrophic to oligotrophic waterbody (on average 0.014 mg L-1 total phosphorus during the last 10 years, Wentzky *et al.*, 2018). More information about the major ion composition and physicochemical properties of Rappbode water can be found in Moreira *et al.* (2016). Details about dissolved organic carbon composition are given in Tittel *et al.* (2015) and Morling, Herzsprung and Kamjunke (2017).

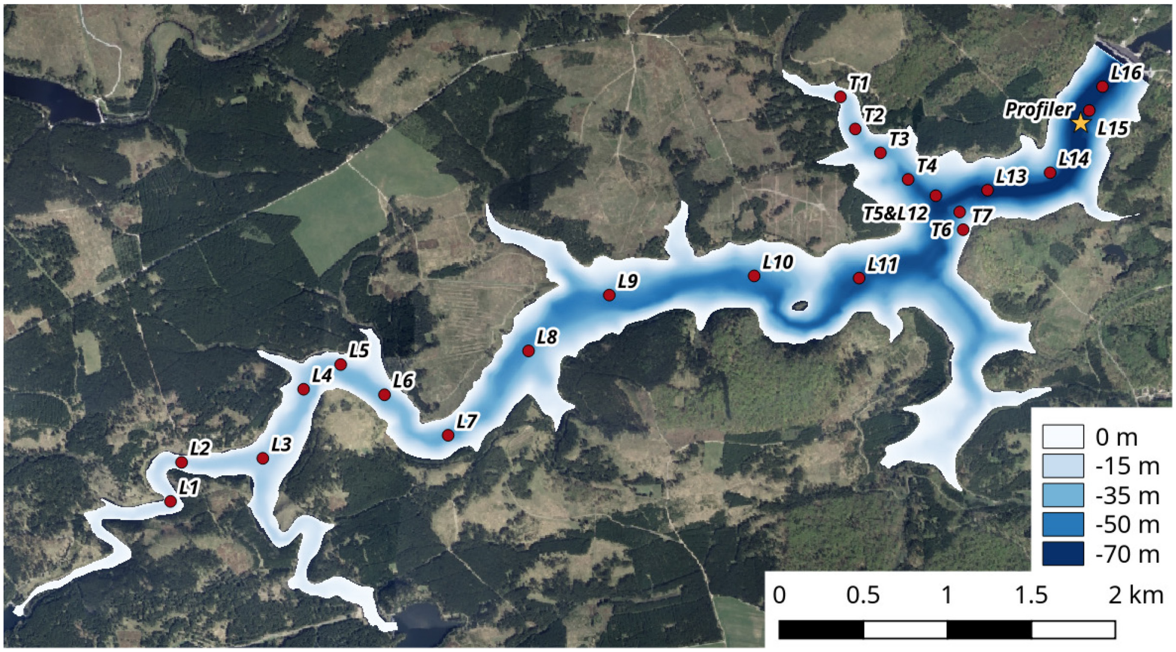


Figure 5.1: Bathymetric map of the Rappbode Reservoir with sampling stations. Measuring stations L1-L16 belong to the longitudinal transect, while measuring stations T1-T7 belong to the transversal transect. The location of the automatic profiler is indicated by the star. Geologic data were taken from © GeoBasis-DE / LVermGeo LSA (2016).

Taking advantage of the morphometric complexity of the reservoir, we measured two transects: longitudinally (L16-L1 in Fig. 5.1), following the thalweg as closely as possible from the dam wall to the beginning of the backwater, and laterally from one side arm across the main channel towards the steep opposite side wall (T1-T7 in Fig. 5.1). We used a multiparameter probe (CTD90M Sea and Sun Technology, Trappenkamp, Germany, serial number: 644) with sensors for chlorophyll a fluorescence (Cyclops 7, model number: 2100-000, excitation wavelength 460 nm), temperature (PT100), electrical conductivity, pressure (for depth) and dissolved oxygen (optical sensor, Rinko III) with a response time of about 2 s. The species *Planktothrix rubescens* is almost invisible for the Cyclops 7 sensor with blue excitation, since the fluorescence yield of *P. rubescens* for this sensor´s excitation wavelength (460 nm) is very low. The reasons for this low fluorescence yield is low chlorophyll a content in photosystem II and the lack of alternative pigments that absorb light at the applied short wavelengths. The investigation was conducted in late summer (10th September 2015) when the metalimnetic oxygen minimum had enough time to develop, but clearly before any large-scale cooling driven recirculation set in.

We further made use of a regular monitoring programme, in which multiparameter probe profiles (as in Fig. 5.2) were measured at fortnightly intervals during the entire year 2016 (from 19th January 2016 to 19th December 2016). The multiparameter probe mentioned above was used. Additionally, a multi-channel fluorescence probe (FluoroProbe, bbe moldaenke GmbH, Germany, serial number: 2101) was used to detect phytoplankton and to characterise the algal community. The FluoroProbe measured fluorescence emitted from chlorophyll a in the PS II (at wavelength 685 nm), which was triggered through the excitation at different wavelengths (370, 470, 525, 570, 590 and 610 nm). The signal at 370 nm excitation wavelength was used to correct for the fluorescence of dissolved organic matter. The other wavelengths referred to different accessory pigments present in phytoplankton, which allowed discrimination into four algal groups: (1) Green algae (rich in chlorophyll a/b), (2) diatoms/dinoflagellates (containing xanthophyll and chlorophyll c), (3) phycocyanin-rich cyanobacteria and (4) phycoerythrin-rich cyanobacteria and cryptophytes (for more information on the measurement principle we refer to Beutler *et al.*, 2002a; Beutler *et al.*, 2002b). We used the probe measurements, and more precisely the signal of the red group (cryptophytes and phycoerythrin-rich cyanobacteria, excitation wavelength 570 nm), for assessing the vertical distribution of the phycoerythrin-rich cyanobacterium *Planktothrix rubescens* in the water column (for more information on occurrence of cyanobacteria in general see Carey *et al.*, 2012; and specifically for stratified lakes see Cuypers *et al.*, 2011).

To get a clearer picture of the short-term dynamics and spatio-temporal distribution patterns, in the following year (2016), we installed an automatic profiler system (for location of profiler see Fig. 5.1) with a multiparameter probe (YSI 6820 V2-2 O) including sensors for dissolved oxygen (YSI 6150), temperature (YSI 6560), and two sensors for chlorophyll fluorescence: one sensor measuring chlorophyll a fluorescence directly (YSI 6025, excitation wavelength 470 nm) and one measuring chlorophyll fluorescence via exciting phycocyanin (YSI 6131, excitation wavelengths 565-605 nm). Besides phycoerythrin, *P. rubescens* also contains phycocyanin and hence could also be detected by the phycocyanin sensor. The profiler covered the upper 26 m of the water column with depth-intervals of 0.25 m between 0-20 m and 0.5 m from 20-26 m. Profiles were measured at intervals of three hours. Operation began on 4th August 2016 and ran until 9th November 2016.

For microscopic species determination, phytoplankton samples were preserved with Lugol´s solution, concentrated using the sedimentation technique developed by Utermöhl (1958) and counted under an inverted light microscope. Most phytoplankton taxa were counted as cells. An exception was filamentous phytoplankton, such as *P. rubescens*, which was counted by measuring the filament length. Biovolumes for each species were derived from average cell dimension measurements and simple geometric shapes (Hillebrand *et al.*, 1999). Phytoplankton biovolume was converted to biomass assuming a specific density of 1.0.

To assess the long-term development of oxygen in the Rappbode Reservoir, weekly oxygen profiles were taken between 2009 and 2016. Oxygen concentrations and temperatures were measured with the DS5 multiparameter probe from Hydrolab. (An overview about all probes and sensors used in this study, including information about their characteristics, can be found in Table S9.4.1 in the supporting information.)

As a stability quantity of stratification, the square buoyancy frequency (N^2) was calculated from CTD90M profiles, using the formula

$$N^2 = -\frac{g}{\rho} \cdot \frac{\mathrm{d}\rho}{\mathrm{d}z}$$

where density ρ was calculated using a specific formula for the Rappbode Reservoir from Moreira *et al.* (2016), g was the earth acceleration (9.81 m/s²) and z was the vertical coordinate. In discrete steps,

$$N^2 = -\frac{g}{\rho(z)} \cdot \frac{\rho(z+a) - \rho(z-a)}{(z+a) - (z-a)}$$

$$= -\frac{g}{\rho(z)} \cdot \frac{\rho(z+a) - \rho(z-a)}{2a}$$

where a~1m was the vertical resolution of the calculation. For other quantities on stability see MacIntyre *et al.* (2009), Boehrer and Schultze (2009) and Read *et al.* (2011).

Chlorophyll a fluorescence values obtained by the FluoroProbe and the multi-parameter CTD90M probe were supplied as µg L^{-1} of chlorophyll by the manufacturer. However, before using the chlorophyll a fluorescence data quantitatively, they were compared with high pressure liquid chromatography (HPLC) measurements to evaluate the accuracy of the probe measurements. Samples for HPLC analysis were taken biweekly at the same sampling dates as the vertical probe profiles. Samples were collected at discrete depths (0, 2.5, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 m) using an open cylinder water sampler (4.2 L standard water sampler from Limnos). The total number of water samples used for analysis was 229. For chlorophyll a quantification, 1.5 L of sample water was filtered using glass fibre filters and extracted with 5 mL ethanol and several freezing/thawing cycles. Subsequently 20 µL of extract was measured by HPLC (for details on method see Van Pinxteren *et al.*, 2017). For comparison, the probe data were aggregated to the same discrete depth levels as the HPLC samples. To test for relationships between chlorophyll a data obtained by fluorescence sensors (CTD90M and FluoroProbe) and data from HPLC measurements, linear regression analysis was performed: The coefficients of determination (R^2) described the relationship between both variables and indicated the goodness of fit ($R^2=0$: no relationship; $R^2=1$: perfect relationship), while the p-values for the coefficients quantified the statistical significance of this relationship.

For a quantitative estimate of possible oxygen depletion through the deep chlorophyll maximum, we converted the maximum observed chlorophyll a concentration from the FluoroProbe into carbon, using a specific *Planktothrix* C:Chl-a conversion factor of 90 mg C/mg Chl-a (Copetti *et al.*, 2006). Secondly, we calculated the concentration of possible oxygen depletion by assuming 1 mol C (12 g C) of carbon biomass requires 1 mol O_2 (32 g O_2) for oxidation.

5.5 Results

5.5.1 Local extension of the metalimnetic oxygen minimum

To localize places of particularly high oxygen depletion, we firstly investigated the horizontal and vertical distribution of the metalimnetic oxygen minimum throughout the Rappbode Reservoir. During our campaign in September 2015, the Rappbode Reservoir showed vertical gradients typical for temperate stratified water bodies (Fig. 5.2). Below the 10 m thick epilimnion, a density gradient stabilized the water column limiting vertical exchange between the epilimnion and deeper layers. The reservoir showed relatively low conductivity, neutral pH, a chlorophyll a maximum in the epilimnion and a pronounced metalimnetic oxygen minimum.

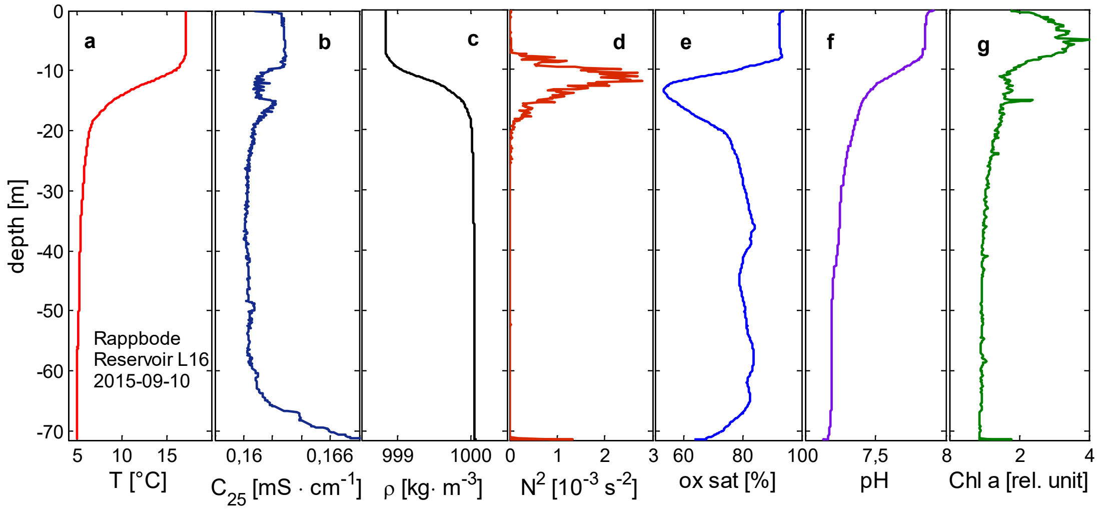


Figure 5.2: Profiles of (a) temperature, (b) electrical conductivity C25, (c) density, (d) squared buoyancy frequency (calculated), (e) oxygen saturation, (f) pH and (g) fluorescence of chlorophyll a in the Rappbode Reservoir on 10th September 2015 measured at the deep sampling location L16 close to the dam wall (see Fig. 5.1).

Surface waters showed oxygen concentrations close to the equilibrium with the atmosphere. On the transversal transect (Fig. 5.3a), the oxygen curve below 10 m was similar for all profiles. Differences between profiles occurred at deep sites at depths below 20 m. In the longitudinal transect, vertical oxygen profiles varied between different stations (Fig. 5.3b). The upper edge of the gradient varied by about four meters over the entire length of the reservoir, which could be attributed to uplift and internal waves due to wind stress along the lake (see Bocaniov *et al.*, 2014). All profiles captured a metalimnetic oxygen minimum at depths between 11 and 13 m, oxygen levels of about 55% saturation and documented higher oxygen concentrations in the deep water. Only at station T1, the water depth was too shallow to show the complete structure. Hence the metalimnetic oxygen minimum can be verified throughout the water body at the depth of the maximum density gradient (see Figs. 5.2c and d).

Below the metalimnetic oxygen minimum, i.e. below 20 to 30 m depth, we found differences of oxygen concentration between profiles: shallower water depths (see shorter profiles) corresponded with lower concentrations of oxygen in the hypolimnion. This fact was indicative of higher oxygen depletion due to a higher sediment area to water volume ratio at the shallow sites, i.e. oxygen depletion imported from the side walls (e.g. Müller *et al.*, 2012; Dadi *et al.*, 2016). This observation agreed with the approach taken in numerical models, which implement oxygen uptake at the sediment surface as an important contribution for oxygen depletion. Horizontal mixing in the waterbody was obviously not sufficient to remove these oxygen gradients within the hypolimnion. The observation of lower oxygen saturation at stations with a higher sediment influence was visible in both the longitudinal and the lateral transect (Fig. 5.3).

The depth and extent of the metalimnetic oxygen minimum was identical in all profiles of the lateral transect and also nearly identical in the longitudinal transect from the dam wall (L16) up to at least station L8. Further above, along the thalweg towards the inflow, the water was very shallow and differences between the profiles became visible. In conclusion, there was no indication that oxygen depletion in the metalimnion would vary between locations throughout most of the water body and no horizontal gradients were observable as seen in the hypolimnetic oxygen levels. Hence, the prevailing oxygen depleting process in the metalimnion was the same along the horizontal axes of the water body. As the sediment contact area in the side arm was larger than in the main channel or at the rock wall, the leading process for oxygen uptake could not significantly be connected to the sediment. In conclusion, the measurements indicated that the metalimnetic oxygen minimum in the Rappbode Reservoir was the consequence of pelagic processes.

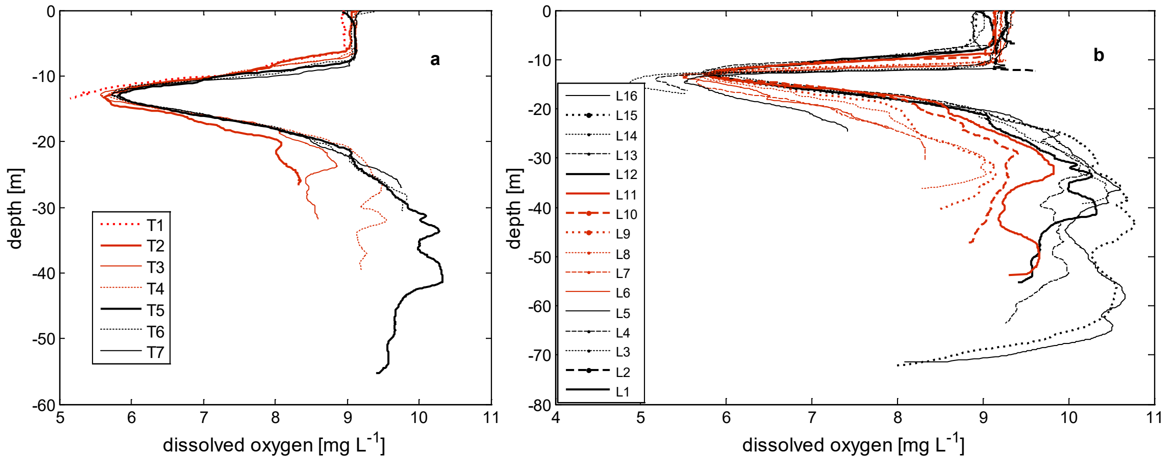
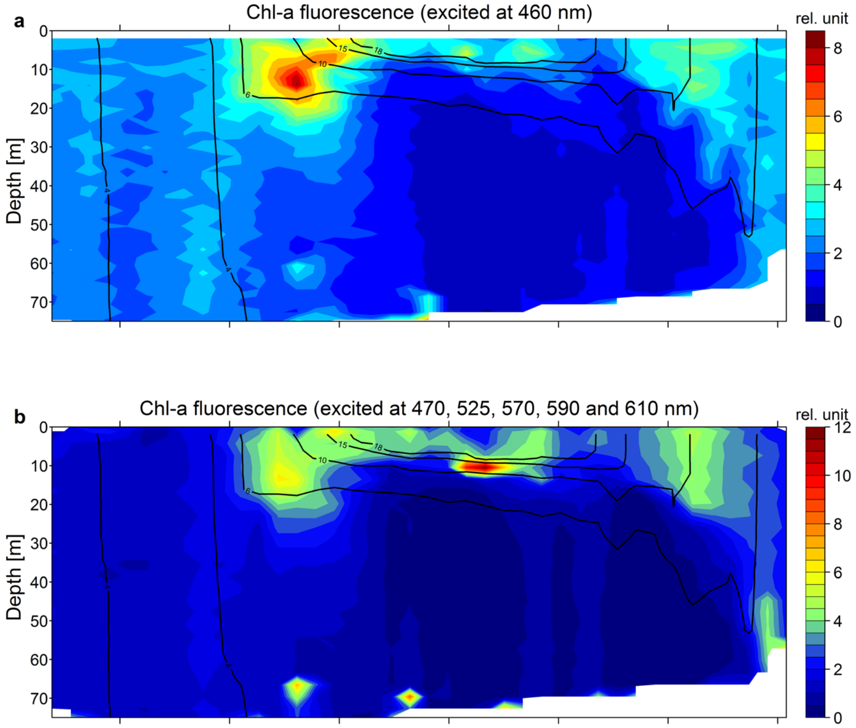


Figure 5.3: Profiles of oxygen on a transversal (a) and a longitudinal (b) transect measured on 10th September 2015. See Fig. 5.1 for locations of measuring stations.

5.5.2 Temporal evolution of the metalimnetic oxygen minimum

The results of the transect measurements clearly showed that no horizontal gradient was visible in the metalimnetic oxygen minimum. Accordingly, measurements at one single pelagic site were sufficient to investigate the temporal evolution.

Temperature stratification in 2016 was typical for the location (Wentzky *et al.*, 2018). The summer stagnation period started in April after day of the year (DOY) 100 (see contour lines Figs. 5.4a-c) and lasted until December. During summer, the major temperature gradient was located at around 10 m depth, which deepened slightly over time. Phytoplankton (chlorophyll fluorescence, Figs. 5.4a and b) increased after thermal stratification had started. Later in summer, a strong phytoplankton peak occurred in the metalimnion, as indicated by the chlorophyll fluorescence measured by the multi-channel FluoroProbe (Fig. 5.4b). This deep phytoplankton peak was not visible in the chlorophyll a profile measured by the Cyclops 7 sensor of the CTD90M probe, which used only one single excitation wavelength and cannot detect phycoerythrin-rich cyanobacteria like *Planktothrix* (Fig. 5.4a).



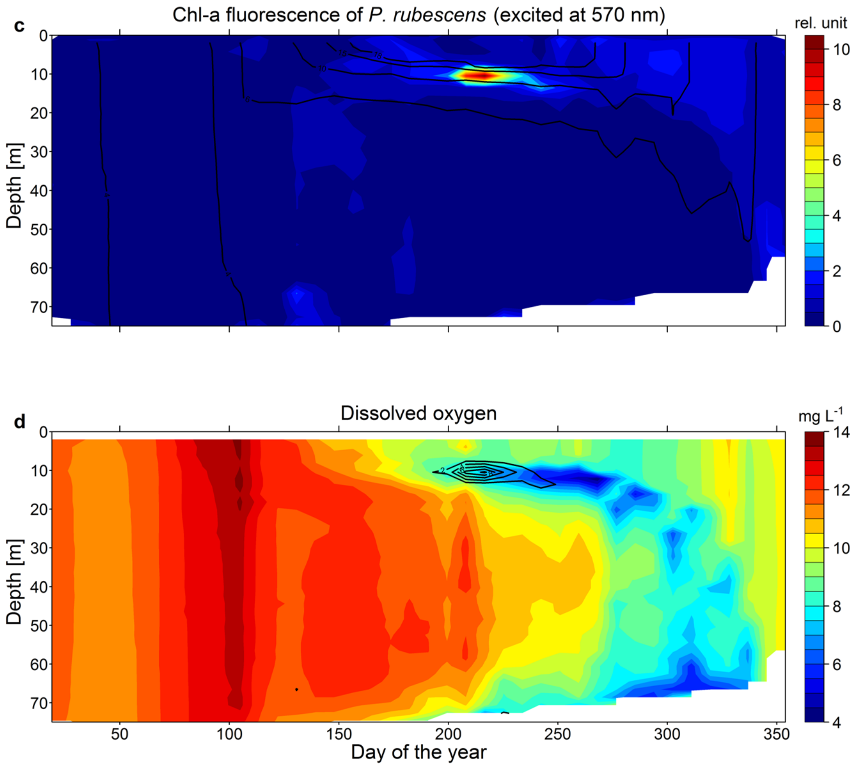


Figure 5.4: Seasonal and vertical development (depth vs. time) during the year 2016. Color contours of fortnightly profiles of (a) chlorophyll a fluorescence measured by CTD90M probe at excitation wavelength 460 nm, (b) summed chlorophyll a fluorescence measured by FluoroProbe at excitation wavelengths 470, 525, 570, 590 and 610 nm, (c) chlorophyll a fluorescence measured by FluoroProbe at excitation wavelength 570 nm (proxy for phycoerythrin-rich cyanobacteria and cryptophytes), (d) dissolved oxygen (mg L^{-1}). Contour lines indicate water temperature (°C) (in Fig. 5.4a-c) or chlorophyll a fluorescence measured by FluoroProbe at excitation wavelength 570 nm (proxy for phycoerythrin-rich cyanobacteria and cryptophytes, in Fig. 5.4d).

Specific *in situ* fluorescence measurements of the "red channel" of the FluoroProbe (representing phycoerythrin-rich cyanobacteria and cryptophytes) indicated the development of the phycoerythrin-rich cyanobacterium *P. rubescens* in the metalimnion (Fig. 5.4c). This fluorescence signal was present from DOY 200 (18th July) to DOY 230 (17th August) and faded out over further 40 days (until DOY 270). Likewise, phycocyanin fluorescence was recorded at the thermocline depth and hardly ever at other depths or other times (Fig. 5.5c). Microscopic inspection of the phytoplankton confirmed the occurrence and dominance of *P. rubescens* at the respective depths

(Fig. S9.4.1 supporting information). In addition, the cyanobacteria *Oscillatoria limnetica* and *Limnothrix redekei*, which contain phycocyanin but not phycoerythrin, were observed in the metalimnion, but at very low abundances. In conclusion, the signal of the "red channel" of the FluoroProbe and the phycocyanin fluorescence measured by the automatic profiler system could mainly be attributed to *P. rubescens*.

Oxygen concentrations were high during deep recirculation in winter and early spring due to cold temperatures and mixis of re-oxygenated water from the surface (Fig. 5.4d). In the epilimnion, oxygen concentrations during summer dropped due to higher temperatures and hence lower solubility. In the hypolimnion, oxygen was confined and subject to gradual depletion. As in the previous year, a clear minimum appeared at the thermocline after DOY 230 (17th August) and ended when the recirculation included the oxygen depleted layers into the surface mixed layer. Minimal oxygen levels in the metalimnion reached down to 4 mg L^{-1} around DOY 270 (26th September). Towards the end of the stratification period, low oxygen concentrations were found at the lake bed until the deep recirculation also removed this minimum. In some summer profiles, we observed small distinct local oxygen maxima of up to 0.5 m thickness at the upper edge of the metalimnion, directly above the oxygen minimum (Fig. S9.4.2 supporting information). These peaks were an indicator for oxygen production by photosynthetic organisms. Due to the small extent of the peaks, they were not visible in the contour plots.

The formation of the metalimnetic oxygen minimum coincided with the breakdown of the metalimnetic *P. rubescens* population (Fig. 5.4d and Fig. S9.4.1 in supporting information). Moreover, the layer thickness of the oxygen minimum resembled the thickness of the *Planktothrix* layer before and looked like the continuation of the previous feature.

The high-resolution data from the automatic profiler confirmed the upper edge of the metalimnetic oxygen minimum as the lower boundary of the epilimnion (Figs. 5.5a and d). The oxygen minimum was clearly terminated by the gradual inclusion of the oxygen depleted layer into the epilimnion. The high resolution data also revealed additional features, such as elevated chlorophyll a fluorescence at the time and depth where phycocyanin occurred (Figs. 5.5b and c).

The disappearance of phycocyanin (mainly representing *P. rubescens*) coincided with the strong oxygen depletion, which stopped at a saturation level of about 40% (or 4 mg O_2 L^{-1}) (Figs. 5.5c and d). Since the stratification persisted for longer, oxygen concentrations could have dropped further, if oxygen depletion had continued. A downward track of phycocyanin indicated that layers were drawn down due to shrinking hypolimnetic volume as a consequence of withdrawal through the bottom outlet. Notably, the oxygen minimum followed this downward trend.

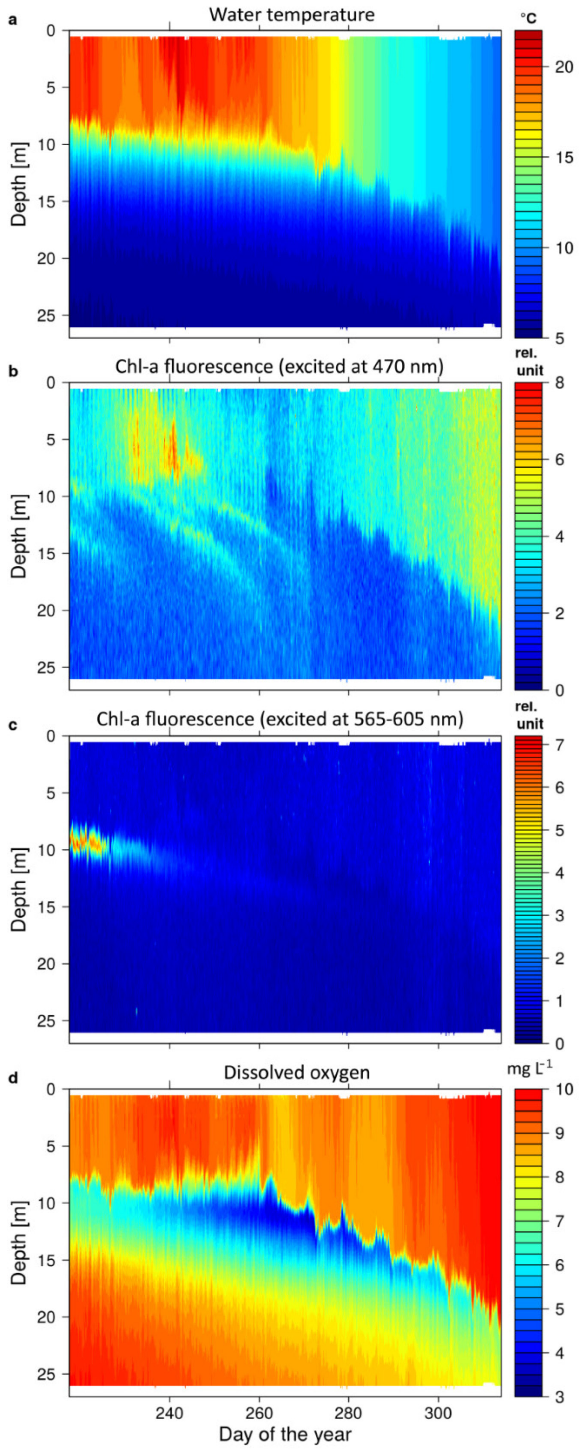


Figure 5.5: Seasonal and vertical development (depth vs. time) during the year 2016. Color contours display 3-hourly profiles of (a) water temperature (b) chlorophyll a fluorescence excited at 470 nm (c) chlorophyll a fluorescence excited through phycocyanin at 565-605 nm (d) dissolved oxygen.

5.5.3 Comparison of chlorophyll a quantification methods based on fluorescence with HPLC

Chlorophyll a values obtained by the CTD90M probe and FluoroProbe during the monitoring in 2016 were in good agreement with HPLC measurements from discrete sampling (Fig. 5.6). The fit of the correlation with HPLC was higher for the FluoroProbe (R^2=0.78, $p<0.001$) than for the CTD90M (R^2=0.63, $p<0.001$). The FluoroProbe and the CTD90M probe were also significantly correlated (R^2=0.62, $p<0.001$). As a result of the good correlation between chlorophyll a fluorescence of the probes and chlorophyll a concentrations measured by HPLC, the values of the FluoroProbe were later used for a quantitative estimate on phytoplankton carbon biomass.

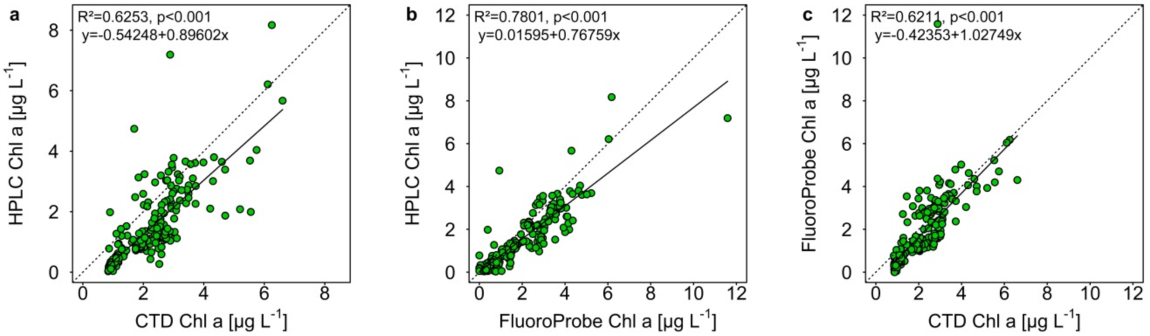


Figure 5.6: Relationship of different chlorophyll a quantification methods (n=229). Correlation between (a) HPLC and CTD90M probe, (b) HPLC and FluoroProbe, (c) FluoroProbe and CTD90M probe. The black line shows the regression line. The dashed line corresponds to the 1:1 relationship.

5.5.4 Calculation of potential oxygen depletion from available phytoplankton carbon biomass

Our calculations showed that 3.1 mg O_2 L^{-1} oxygen could be depleted from the available phytoplankton carbon biomass in the metalimnion (12.79 µg chl-a L^{-1} or respectively 1.15 mg C

L^{-1}). In 2016, we observed an oxygen depletion of about 5 mg O_2 L^{-1} in the Rappbode Reservoir (Fig. 5.4a). Since the calculated and the observed oxygen depletion were in a similar range, the *Planktothrix* biomass in the metalimnion could be connected to the metalimnetic oxygen minimum formation.

5.6 Discussion

A pronounced metalimnetic oxygen minimum, which reached down to only 40% saturation level, was documented in the meso- to oligotrophic Rappbode Reservoir. The distinctiveness of the metalimnetic minimum was surprising, given the low trophic state of the reservoir and the low phytoplankton abundance. The oxygen depleted water volume showed a sharp upper edge to the epilimnion and extended about 5 m into the stratified waterbody below. The oxygen minimum endured from the middle of August, when a population of *P. rubescens* disappeared at the same depth, to the end of October, when deep recirculation removed the metalimnion by inclusion into the epilimnion.

The small horizontal variability observed in September 2015 (Fig. 5.3) indicated that the forming process was not significantly connected to side wall effects, such as oxygen depletion at the sediment surface, inflows or side wall mixing providing water that could have intruded the metalimnion. The oxygen depletion had to be mainly attributed to depletion in the open water body. Hence the further investigation focused on finding reasons for oxygen depletion in the pelagial.

There was evidence for a connection of the metalimnetic oxygen minimum to the phycocyanin fluorescence maximum measured by the automatic profiler (as well as to the signal of the “red channel” of the FluoroProbe) and thus organisms related to this fluorescence (Figs. 5.4d and 5.5c-d): firstly the oxygen minimum formed in the same water layers, secondly the oxygen depletion started with the disappearance of the phycocyanin (and the signal of the “red channel” of the FluoroProbe respectively) and thirdly the depletion stopped at 40% of oxygen saturation. Hence there seemed to be a limited reservoir of oxygen demand. Phycocyanin fluorescence and the signal detected by the “red channel” of the FluoroProbe could be related to a *P. rubescens* mass development, which was microscopically confirmed in the Rappbode Reservoir at the respective depths. Most likely, the oxygen depletion was related to the discontinuation of photosynthetic activity of the *Planktothrix* population.

A metalimnetic *P. rubescens* maximum has also been observed in other lakes (Walsby & Schanz, 2002; Cuypers *et al.*, 2011). *P. rubescens* populations commonly form at this depth, since gas vesicles allow regulating their position within the depth of optimal growth conditions (Walsby *et al.*, 2004). Their preference for the metalimnion is due to physiological traits that differ from those of other bloom forming species: *P. rubescens* favours low temperatures (Dokulil & Teubner, 2000; Holland & Walsby, 2008) and is adapted to low light conditions (Walsby & Schanz, 2002; Walsby & Juttner, 2006). Its ability to use organic compounds as a carbon source under extremely low irradiances (photoheterotrophy) allows for survival and even slow growth in the metalimnion (Zotina, Köster & Jüttner, 2003). As in the Rappbode Reservoir, *P. rubescens* has been found to be a dominant species in the metalimnion even under low nutrient conditions (Steinberg & Hartmann, 1988; Posch *et al.*, 2012).

While the *P. rubescens* peak was detected by the multi-channel FluoroProbe (*excitation wavelengths 470, 525, 570, 590 and 610 nm,* Fig. 5.4b), it was not captured by the single-channel chlorophyll a fluorescence measurements of the Cyclops-7 sensor of the CTD90M probe (excitation wavelength 460 nm, Fig. 5.4a) and chlorophyll a fluorescence YSI sensor of the automatic profiler (excitation wavelength 470 nm, Fig. 5.5b), which are sensor types commonly used for lake monitoring to estimate phytoplankton biomass (Brentrup *et al.*, 2016). Given the importance of phycoerythrin-rich species such as *P. rubescens* for biogeochemical processes, important information might be missed by using chlorophyll a fluorescence sensors with only one single excitation wavelength to quantify chlorophyll a or phytoplankton biomass. This finding agrees well with other studies showing that chlorophyll a concentration and phytoplankton biovolume were better estimated by the FluoroProbe than by chlorophyll sensors measuring only at a single wavelength, especially in case of cyanobacterial blooms (e.g. Gregor & Marsalek, 2004; Catherine *et al.*, 2012). Given the fact that *P. rubescens* is almost invisible for chlorophyll fluorescence sensors based on blue excitation implies that the occurrence of this highly relevant cyanobacteria can be overlooked in monitoring campaigns. Hence, for a more complete picture of the organisms the use of fluorescence sensors based on multiple wavelengths instead of single-wavelength sensors is recommended. This is particularly important when microscopic cell counts are not conducted and phytoplankton monitoring is entirely based on fluorescence measurements.

Moreover the FluoroProbe had been shown to strongly correlate with the standard ISO method for chlorophyll a quantification (r2 = 0.97, $p < 0.05$, Gregor & Marsalek, 2004) as well as with phytoplankton biovolume obtained by microscopic analysis (r2 = 0.89, p-value < 0.001, Catherine *et al.*, 2012). This was valid also for cyanobacteria dominated waters: for instance, Leboulanger *et al.* (2002) found that *P. rubescens* cell counts from discrete sampling were closely correlated with data obtained by the FluoroProbe (r2 = 0.89, $p < 0.01$, Leboulanger *et al.*, 2002). Also during the monitoring in 2016 in the Rappbode Reservoir chlorophyll a fluorescence measured by the FluoroProbe compared well with chlorophyll a concentrations determined by the HPLC method (R^2=0.78, $p<0.001$, Fig. 5.6b). In conclusion, we used chlorophyll a fluorescence measurements from the FluoroProbe to get a rough quantitative estimate of phytoplankton carbon biomass. Using this approximation of phytoplankton carbon biomass, our calculations showed that it could deplete approximately 3.1 mg O_2 L^{-1}. Considering that this value was just a rough estimate, it compared rather well with the observed oxygen depletion in the metalimnion of 5 mg O_2 L^{-1}. Nevertheless, a reason for the discrepancy between calculated and observed value might lie in the fact that the peak of the *Planktothrix* biomass was not recorded by our biweekly monitoring programme.

The oxygen minimum in the metalimnion in late summer was a reoccurring feature in the Rappbode Reservoir (Fig. 5.7). Phytoplankton count data verified the presence of *P. rubescens* during summer at the thermocline since 2009, usually with a peak at a depth around 10 m. The occurrence of *P. rubescens* in previous years and the simultaneous observation of a metalimnetic oxygen minimum supported our hypothesis, that both features were connected.

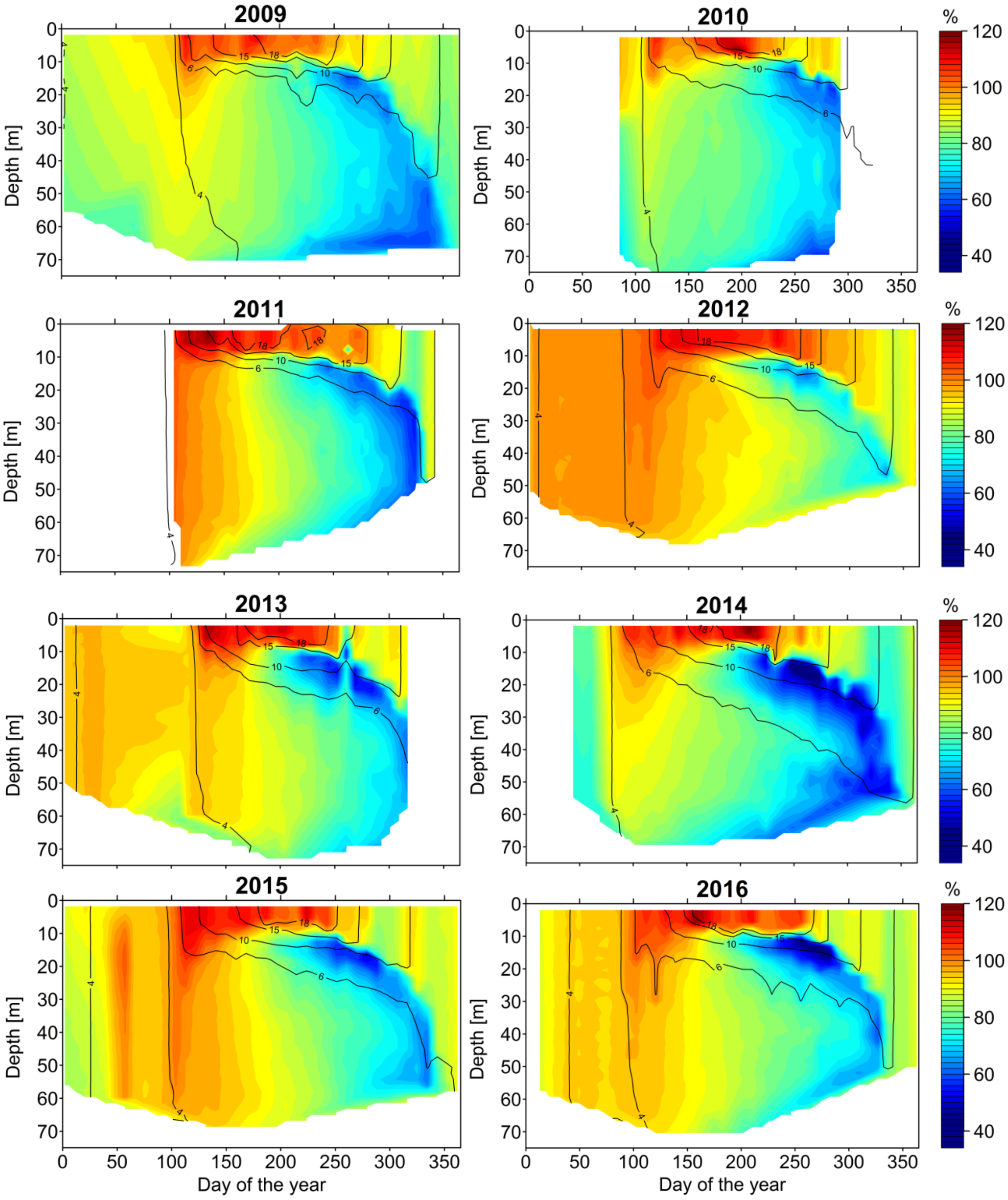


Fig. 5.7: Seasonal and vertical (depth vs. time) oxygen levels from 2009 to 2016. Color contours display oxygen profiles (oxygen saturation in %) and contour lines indicate water temperature (°C).

Both features, the colonization by *P. rubescens* and the persistence of a metalimnetic oxygen minimum were connected to the presence of density stratification: a) for *P. rubescens* to allow for depth control by buoyancy, b) for the metalimnetic oxygen minimum to limit the diffusive exchange with neighbouring layers at higher oxygen concentration. Hence we displayed both features within the stratification (Fig. 5.8), where we calculated the square buoyancy frequency (N^2) from CTD90M profiles. Both, the *P. rubescens* fluorescence and the oxygen minimum followed the depths of high density gradients.

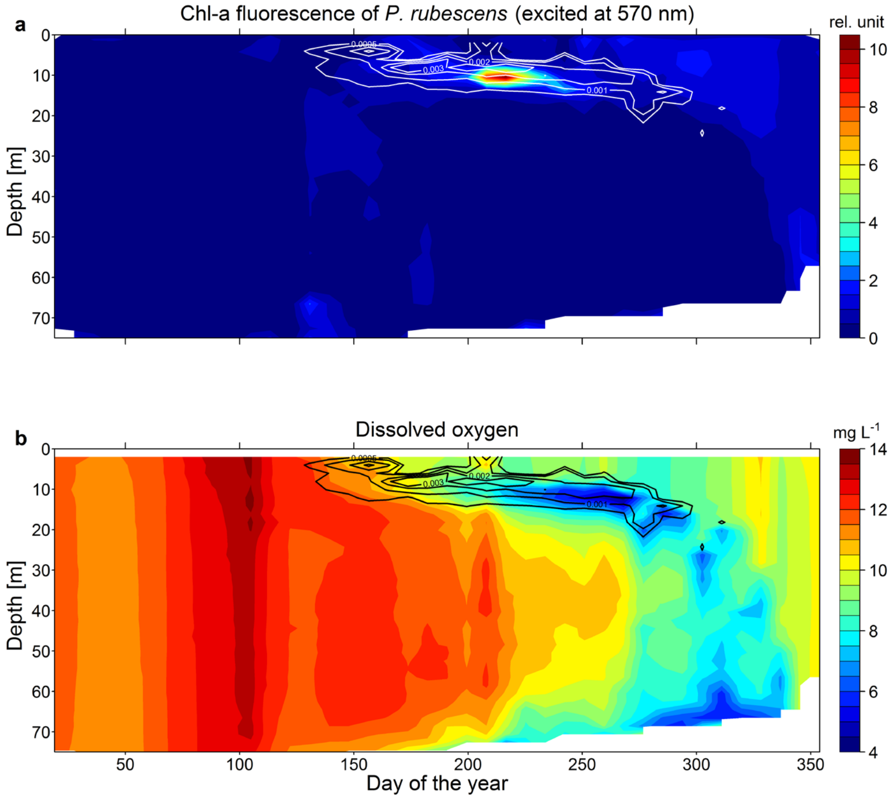


Fig. 5.8: Position within the stratification represented as contour lines of the squared buoyancy frequency N² [s⁻²] during 2016 of (a) chlorophyll a fluorescence measured by FluoroProbe at excitation wavelength 570 nm (proxy for phycoerythrin-rich cyanobacteria and cryptophytes) and (b) oxygen concentration.

Prior to this study, the connection between metalimnetic *P. rubescens* mass developments and metalimnetic oxygen depletion had not been studied nor documented in similar detail. However, we found hints in the literature that also in other lakes low metalimnetic oxygen levels occurred simultaneously with the predominance of *P. rubescens*, supporting our hypothesis of a causal relationship between both. For example, in Lake Zürich (Switzerland), known for its recurrent mass developments of *P. rubescens* in summer (Micheletti, Schanz & Walsby, 1998), a metalimnetic oxygen minimum developed subsequent to a *Planktothrix* bloom in the metalimnion (Van den Wyngaert *et al.*, 2011). Moreover, profile measurements conducted in Crooked Lake (USA) in the summer of 1979 showed a metalimnetic *Planktothrix* peak accompanied by a decrease in oxygen (Konopka, 1980). In Lake Ammersee (Germany) a gradual depletion of oxygen to very low levels was reported, slightly below a *Planktothrix* peak (Ernst, Hitzfeld & Dietrich, 2001; Hofmann & Peeters, 2013).

We could think of three reasonable cases that produced an oxygen depletion with the disappearance of photosynthetic activity in the metalimnion: (1) *Planktothrix rubescens* died and microbial degradation of its biomass required oxygen (2) *P. rubescens* perpetuated respiration longer than photosynthetic activity (3) heterotrophic organisms requiring oxygen for respiration (Shapiro, 1960; Raateoja *et al.*, 2010), which was disguised while *Planktothrix* was photosynthetically active in the metalimnion. However, firstly the quantitative estimate of possible oxygen depletion through *Planktothrix,* as well as secondly the local confinement of the oxygen depletion to the same depths as the deep chlorophyll maximum and thirdly the termination of oxygen depletion at 40% saturation indicate that process (3) is probably of subordinate importance.

Considering that mass developments in the metalimnion of *P. rubescens* were favoured by lake warming (Posch *et al.*, 2012; Yankova *et al.*, 2017), metalimnetic oxygen minima might increase simultaneously with *Planktothrix* blooms during the next decades, causing severe problems for water quality. Given the importance of oxygen for water quality, the connection between *P. rubescens* in the metalimnion and oxygen depletion should be studied further and analysed also in other lakes.

5.7 Conclusions

- A reoccurring metalimnetic oxygen minimum was observed in the low nutrient Rappbode Reservoir (Germany) during late summer. It was characterized by a sharp edge towards the epilimnion and a thickness of about 5 m.
- Oxygen depletion in the metalimnion was a consequence of processes occurring in the pelagic water and was not imported from the sediment on the side walls nor by inflows.

- The emergence of the metalimnetic oxygen minimum was connected to the disappearance of a *Planktothrix rubescens* population in the metalimnion.
- The available phytoplankton carbon biomass could suffice to deplete oxygen in the observed range. This suggested that biological activity induced by the end of a *Planktothrix* mass development was an essential factor in forming the oxygen minimum in the Rappbode Reservoir.
- The presence of *P. rubescens* was responsible for the later appearance of the metalimnetic oxygen minimum either through bacterial decomposition of dead *P. rubescens* cells, or respiration of *P. rubescens* beyond their photosynthetic activity. The presented data showed no evidence for significant oxygen consumption in the metalimnion from sources other than the deep chlorophyll maximum.

5.8 Acknowledgements

Many thanks to Karsten Rahn and Martin Wieprecht for preparing field equipment and acquiring the fortnightly profiles, many thanks to Burkhard Kuehn for deploying the automatic profiling station on the Rappbode Reservoir, thanks to Kerstin Lerche for analysing chlorophyll a via HPLC, thanks to Philipp Keller for preparing the bathymetric map, and thanks to the participants of a teaching excursion on “physical limnology” of the Heidelberg University for the longitudinal and lateral transect. We also thank the water supply works Wasserwerk Wienrode (especially Jan Donner) for sharing phytoplankton counts and profile data with us. This research was supported by grants JA 2146/2-1 and RI 2040/2-1 from the German Research Foundation (DFG) within the priority program 1704 “DynaTrait” as well as the project “Managing Water Resources for Urban Catchments” (grant number 02WCL1337A) by the German Federal Ministry of Education and Research (BMBF).

6 Overall discussion and conclusions

The following sections pick up the main findings of this thesis in order to illustrate, how they contribute to a better understanding of phytoplankton dynamics and their interactions with the abiotic environment. I moreover discuss which implications can be derived from my results for trait-based research and for lake management practices. I refer to the questions raised in chapter "2 Motivation and objectives" and the respective numbers of the corresponding questions are added in brackets. Finally, further research needs are discussed.

6.1 Seasonal dynamics in phytoplankton functional traits

The results of the thesis add a quantitative mechanistic understanding of seasonal succession of phytoplankton communities. Even though the study of phytoplankton seasonal succession has a long tradition in freshwater sciences, phytoplankton community dynamics along the seasonal gradient are complex and still difficult to predict (Reynolds, 2000). One reason for this might be the focus on phylogeny, e.g. on species or higher taxonomic units (e.g. Padisak, 1992; Tallberg *et al.*, 1999; Kalff, 2002; Rychtecký & Znachor, 2010), which impede ecological interpretation and general statements. Trait-based approaches are seen as a way towards higher generality, predictability and a better ecological interpretation of phytoplankton dynamics (Litchman & Klausmeier, 2008).

In this thesis, the taxonomic composition of the phytoplankton community was combined with morphological, behavioral and physiological trait measurements collected from the literature in order to translate them into community-weighted mean trait values. These values provide a quantification of the aggregated trait attributes of the community and were used to analyze the seasonal succession of phytoplankton. As the use of functional traits facilitates ecological interpretation of the observed patterns, they are a major advancement compared to taxonomic approaches. From a functional perspective, the seasonal succession patterns in temperate lakes have been described verbally by the r- versus K-strategy continuum (Margalef, 1978; Reynolds, 1984a) and the PEG model (Sommer *et al.*, 1986). The findings from this thesis about the seasonal development of functional traits mainly agree with their verbal models. However, as the approach used in this thesis allows for quantification of functional characteristics of the phytoplankton community, the results obtained in this thesis are a major advancement in contrast to verbal descriptions, since they allow for quantitative statements and statistical analysis. Also ecosystem models can benefit from this trait-based approach, since the aggregation of species information into trait values reduces the complexity and overcomes the problem of models being limited in the number of modelled species. Traits also facilitate parametrization and scaling-up of models and, compared with species-based models, usually produce more generalizable results that can be projected to other ecosystems, since traits function as a unifying currency across scales (Zakharova, Meyer & Seifan, 2019).

As species cannot invest into all ecological strategies at the same time, trade-offs exist between different functional traits, resulting in different species being characterized by different trait combinations. A quantification of traits, as done in this thesis, can reveal these trade-offs. The data showed that small cell size was associated with high maximum growth rates (Banse, 1976; Finkel, 2001; Tang, 1995), light affinity (O'Farrell, de Tezanos Pinto & Izaguirre, 2007; Edwards *et al.*, 2015), susceptibility to grazing (Thingstad *et al.*, 2005) and the ability to form chains and colonies. In contrast, phytoplankton species with larger cell sizes were related to higher phosphate affinity, mixotrophy and mobility – traits that give a competitive advantage under low nutrient conditions. In line with literature, trade-offs were identified between nutrient and light competitive abilities as well as between nutrient competitive abilities and maximum growth rate (Leibold, 1997; Huisman & Weissing, 1995; Grover, 1991; Litchman & Klausmeier, 2001; Sommer, 1986b). These trade-offs between traits were reflected in the seasonal succesion of phytoplankton communities in Rappbode Reservoir. In agreement with the verbal model by Margalef (1978), the seasonal development was mainly shaped by a trade-off between on the one hand fast growing species with small cells and the ability to rapidly incorporate existing resources (r-strategy) and, on the other hand, species with more efficient and complex mechanisms to exploit scarce mineral nutrients or acquire previously unexploited nutrient pools (K-strategy). While the exact species composition may vary from year to year, the functional characteristics of phytoplankton communities showed distinct reoccurring seasonal patterns (**Q1.1**).

With gradually changing environmental conditions along the season, different traits become important and hence species and trait replacements took place. In spring, when turbulence and nutrient concentrations were high, r-strategists dominated. They are characterized by high maximum growth rates, high light affinities and a high proportion of silica users. The dominance of these traits can easily be ecologically interpreted: Due to a high surface to volume ratio, small cells can quickly take up the available nutrients, allowing them to grow very fast in spring. High light affinities were also an important trait in spring, since the ability to efficiently use light is beneficial during fluctuating light conditions as they exist during high turbulence (Yoshiyama *et al.*, 2009; Edwards, Litchman & Klausmeier, 2013a). Turbulence prevents the silica users from sinking out of the photic zone and hence favors them under well-mixed conditions, e.g. in spring (Reynolds, 2006). The dominance of silica users finishes when stratification is becoming more stable and they are fast removed from the photic zone through their high sinking velocities due to their heavy siliceous cell wall (Trimbee & Harris, 1984; Sommer, 1984) (**Q1.1**).

In contrast to this, in summer turbulence and nutrients are low and K-strategists dominated, characterized by high phosphate affinity, the capacity to fix atmospheric nitrogen, motility, buoyancy control and the ability to ingest bacteria (mixotrophy). For example, from spring to summer the phosphorus affinity of the phytoplankton community increased from around 100 to 240 L μmol^{-1} d^{-1} and motility from less than 10 % to more than 30 %. At the same time, maximum growth rate decreased from around 0.86 to 0.73 d^{-1} and the share of silica users from on average 90 % to 45 %. This change in functional strategy can be interpreted as a reaction to the changed environmental conditions. As a response to the low nutrient concentrations during summer species with higher tolerances towards nutrient stress are favored, while fast growth was no advantage. Phosphate affine algae with the ability to efficiently take up phosphorus even under low nutrient concentrations dominate the community. More complex nutrient acquisition strategies such as nitrogen fixation and phagotrophic mixotrophy also become ecologically important traits to overcome the scarcity in mineral nutrients. Another ecologically meaningful strategy to survive nutrient limitation in the epilimnion during stratification in summer was motility: The possession of flagella and the regulation of buoyancy reduces sedimentation losses and allows the algae to migrate to deeper water layers below the thermocline to take up nutrients (Visser *et al.*, 1996; Jäger, Diehl & Schmidt, 2008) (**Q1.1**).

These results show that the occurrence and dominance of specific trait combinations along the season can be ecologically interpreted and that intra-annual changes in trait composition clearly mirror changes in environmental pressures (**Q1.2**). For example, light affinity was highest during light limitation, maximum growth rates were highest when nutrients were abundant and phosphate affinity peaked during phosphorus limitation in summer. While the seasonal development of all mentioned traits agreed well with conceptual models about plankton succession (Margalef, 1978; Sommer, 1985; Sommer *et al.*, 2012), the edibility traits (ability to form chains/colonies and edibility towards *Daphnia*) showed unexpected seasonal patterns, as they did not reflect grazing pressure as proposed by theory. In contrast to theory and previous observations, the edibility traits were highest during summer, when grazing is expected to be high (Lampert *et al.*, 1986; Sommer *et al.*, 1986; Vanni & Temte, 1990; Wirtz & Eckhardt, 1996). This unexpected pattern was possibly caused by other reasons than grazing pressure, since traits are not completely independent from each other. It can be speculated that grazing losses were not the most important factor shaping the phytoplankton community of the Rappbode Reservoir, but instead resource availability was the primary regulator of phytoplankton dynamics. This would indicate that the Rappbode Reservoir is a primarily bottom-up regulated system, while top-down effects are of subordinate importance.

6.2 Impact of nutrient reductions on seasonal and long-term trajectories of phytoplankton traits

Phytoplankton community composition does not only change along the season, but also inter-annually, particularly if environmental conditions undergo major changes. For instance, the increase and reduction of nutrients (eutrophication and oligotrophication) can cause changes in total biomass (Jeppesen *et al.*, 2005) as well as in species composition of the communities (Sommer, Gaedke & Schweizer, 1993; Kamjunke, Straile & Gaedke, 2009; Anneville, Gammeter & Straile, 2005). Previous studies mainly focused on changes in phytoplankton structure on a yearly-aggregated basis, while changes in the seasonal cycle in response to changed nutrient loads were rarely looked at (but see Anneville *et al.*, 2002a). This thesis, for the first time, analyzes the impact of nutrient reductions on the long-term development as well as on the seasonal succession of phytoplankton communities, using functional traits. The 50-year long, seasonally resolved data set from Rappbode Reservoir provided a unique study site for this research topic, since it underwent a strong and abrupt shift in trophic conditions in the nineties (average TP concentration was 0.12 mg/L for eutrophic years and 0.021 mg/L for oligotrophic years). In contrast to comparisons across lakes (e.g. Edwards, Litchman & Klausmeier, 2013b), using long-term data-series from one single study site allow studying the response to altering nutrient regimes without the confounding effects of other factors, such as morphometry, geology or geographical setting.

The thesis shows that the general seasonal patterns of phytoplankton functional traits, such as the shift from r-strategists in spring to K-strategists towards summer, were the same for nutrient-rich and nutrient-deficient years and were thus independent of trophic status (**Q1.3**). Given the large differences in nutrient concentrations between the two trophic periods, the high similarity in seasonal succession is remarkable. It indicates that the recurrent character of the succession of seasonal events is a highly conserved process, which is maintained not only by seasonal nutrient limitation, but also by other physical factors (e.g. mixing regime and light availability) and biotic interactions.

While the general succession patterns in functional traits were largely retained despite differing nutrient status, eutrophic and oligotrophic years also showed clear differences. First, nutrient reductions modified the extent of the seasonal succession of functional traits. In nutrient-rich years the differences in functional traits between spring and summer were very pronounced, while in nutrient-deficient years, traits exhibited lower fluctuations and were more evenly distributed along the season (**Q1.3**). For example, under eutrophic conditions P-affinity increased from around 60 L µmol^{-1} d^{-1} in spring to 240 L µmol^{-1} d^{-1} in summer, while in nutrient-poor years the difference between spring and summer was less strong and increased only from 140 to 240 L µmol^{-1} d^{-1}. The weaker seasonal changes of functional traits after nutrient reductions agreed well with previous studies showing a decrease in seasonal fluctuations in biomass, cell size spectra and the number of successional stages with oligotrophication (Sommer *et al.*, 1986; Sommer, 1986a; Kalff, 2002; Gaedke, Seifried & Adrian, 2004).

Second, nutrient reductions affected functional traits mainly during spring, while the traits in summer largely matched in eutrophic and oligotrophic years (**Q1.3**). For example, the share of silica users was around 45 % in summer, both in eutrophic and in oligotrophic years. In summer, however, the share of silica users was almost 100 % in nutrient-rich years, while it was only around 75 % in nutrient-deficient years. Hence, during the oligotrophic period the functional properties of the spring community resembled the summer community, while the functional properties of spring and summer communities during the eutrophic period were very different. An ecological interpretation of this observation might be that the phytoplankton community had to adapt to low nutrient concentrations already early in the year during nutrient-deficient years, since nutrient limitation already occurred in spring after oligotrophication. Moreover, due to higher phosphorus removal from the epilimnion from spring to summer in eutrophic years (see chapter 6.3 for details), nutrient concentrations were rather low in the summer of eutrophic years. Hence the nutrient conditions of eutrophic summers were similar to the ones in nutrient-poor years, providing a further hypothesis why the functional traits of the phytoplankton community in eutrophic summers were similar to those in nutrient-deficient years. However, these results opposed previous theories and observations from Lake Constance, which found the largest changes after nutrient reductions during nutrient limitation in summer (Sommer *et al.*, 1986; Weithoff & Gaedke, 2016). Whether nutrient reductions mostly affected phytoplankton composition in spring or in summer probably depends on the specific lake ecosystem.

Examples of traits which changed in spring, but not in summer after oligotrophication, were cell size, light affinity, maximum growth rate, P-affinity, mixotrophy and motility. In oligotrophic years the spring community consisted of phytoplankton with larger cell size, higher P-affinity and motility, while the maximum growth rate and light affinity were lower. The competitive advantage of algae with high nutrient affinities and larger cell size at the expense of species with high maximum growth rate and good light competition abilities under nutrient poor conditions agreed with experiments and observations from other lakes (Reynolds, 1984b; Reynolds, 2006; Yoshiyama *et al.*, 2009; Gaedke, 1992; Margalef, 1978).

While for most traits, only the spring community was affected by reduced nutrient concentrations, some traits changed during all parts of the season (**Q1.4**). The most eye-catching change with oligotrophication was the increased share of potentially mixotrophic species, which was much higher throughout the year during oligotrophic years. The increase in mixotrophic species with decreased nutrient concentrations has been reported before for other lakes (Gaedke, 1998; Jeppesen *et al.*, 2005; Kamjunke, Henrichs & Gaedke, 2006; Anneville, Ginot & Angeli, 2002) and seems to be a key feature of oligotrophication. The success of phagotrophic mixotrophs in nutrient poor environments can be explained by their ability to ingest bacteria as an alternative mineral nutrient source (Bird & Kalff, 1987; Nygaard & Tobiesen, 1993).

Based on these findings about seasonal phytoplankton trait dynamics under different nutrient regimes, I could generalize the existing patterns and provide a quantitatively supported functional template for seasonal succession patterns under eutrophic and oligotrophic conditions in temperate lake ecosystems (see Fig.6.1). The conceptual model developed in this thesis largely extends the verbally formulated PEG model (Sommer *et al.*, 1986; Sommer *et al.*, 2012), since it is based on quantitative data and can therefore be statistically tested and used for predictions using lake-ecosystem models.

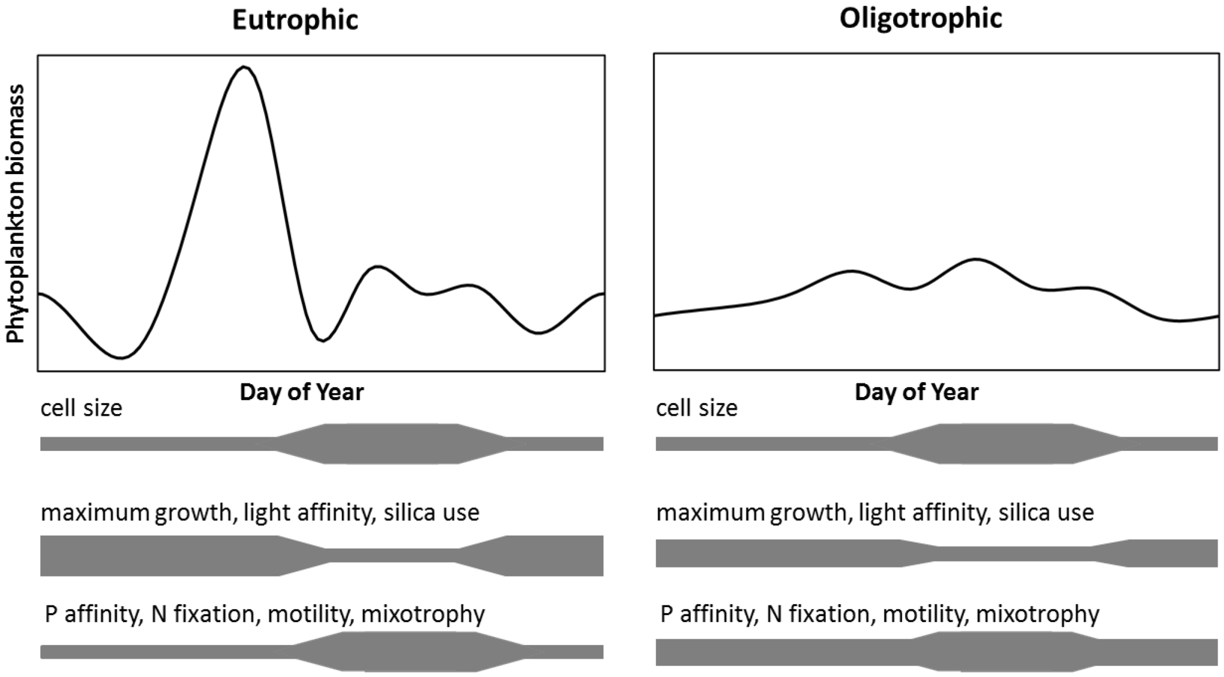


Figure 6.1: Conceptualization of seasonal patterns of phytoplankton biomass and the importance of different phytoplankton traits during eutrophic (left) and oligotrophic (right) years. The thickness of the horizontal bars indicates the seasonal change in relative importance of the phytoplankton traits cell size, maximum growth rate, light affinity, silica use, phosphate affinity, nitrogen fixation, motility and mixotrophy.

6.3 Identifying causes for the resistance of phytoplankton biomass to react to nutrient reductions using functional traits

As a next step, the thesis investigates whether functional traits are useful to identify causal relationships and mechanisms leading to a decoupling of phytoplankton biomass from nutrient concentrations. Understanding the oligotrophication process is a highly relevant topic, especially for lake management, since much effort and money has been invested into reducing nutrient inputs to aquatic ecosystems during the last decades in order to mitigate the damaging effects of eutrophication on water quality and ecosystem health. The expected response to reduced phosphorus loads is a decrease in algal biomass, since phosphorus is the main limiting element in freshwater systems (Hecky & Kilham, 1988) and the linearity between phosphorus and biomass is widely accepted (Vollenweider, 1971). Many scientific and regulatory frameworks are based upon this assumption that an ecosystem impacted by eutrophication can be reverted to its original condition by phosphorus reductions (e.g. EU-WFD, 2000/60/EC). Also a number of oligotrophication studies support this idea, showing a decline in algal biomass after phosphorus has been reduced (Edmondson, 1994; Jeppesen, Jensen & Søndergaard, 2002; Jeppesen *et al.*, 2005; Schindler, 2012; Cooke *et al.*, 2016). However, in this thesis, I show that the trajectory of an ecosystem is not always reversible: In case of the Rappbode Reservoir, where phosphorus concentrations were reduced by a factor of six within a very short time period, annual mean phytoplankton biomass did not respond in the long run and there was no linear relationship between biomass and phosphorus (Fig. 6.2) (**Q2.1**).

Actually, the Rappbode Reservoir is no exception, as the resistance against nutrient reductions has been shown in many other aquatic ecosystems, such as lakes (Anneville & Pelletier, 2000; Jeppesen *et al.*, 2005; Tadonleke *et al.*, 2009; Weyhenmeyer & Broberg, 2014), reservoirs (Horn *et al.*, 2015), rivers (Jarvie *et al.*, 2013; Westphal *et al.*, 2019) and estuaries (Duarte *et al.*, 2008). Already in the early work of Dillon and Rigler (1974), who studied the relationship between phosphorus concentration and chlorophyll-a concentrations, for example a phosphorus concentration of 50 mg m^{-3} was associated with chlorophyll-a values in a 90% confidence interval from 7.5 to 60 mg m^{-3} and 100 mg TP m^{-3} with a confidence interval of 20 – 162 mg chl-a m^{-3}. Their findings illustrated that there was a substantial variability of algal abundance at a given nutrient concentration.

This thesis adds to the growing awareness that algal growth responses can decouple from phosphorus concentrations and hence their pressure-response trajectory is not always fully reversible (Duarte *et al.*, 2008; Jarvie *et al.*, 2013; Westphal *et al.*, 2019). Non-reversible trajectories, where the recovery differs from the degradation pathway, have also been observed for other ecosystem components, e.g. for the recovery of watersheds from nutrient loads (Goyette, Bennett & Maranger, 2018) and for the lacking recovery of submerged vegetation after increased water transparency (Jeppesen *et al.*, 2005; Kemp *et al.*, 2005; Hilt *et al.*, 2006; Sand-Jensen *et al.*, 2008). This suggests that flexible community patterns can exist at the same pressure level and that ecosystems may not return to an anticipated reference status after removing or lowering the pressure (**Q2.2**).

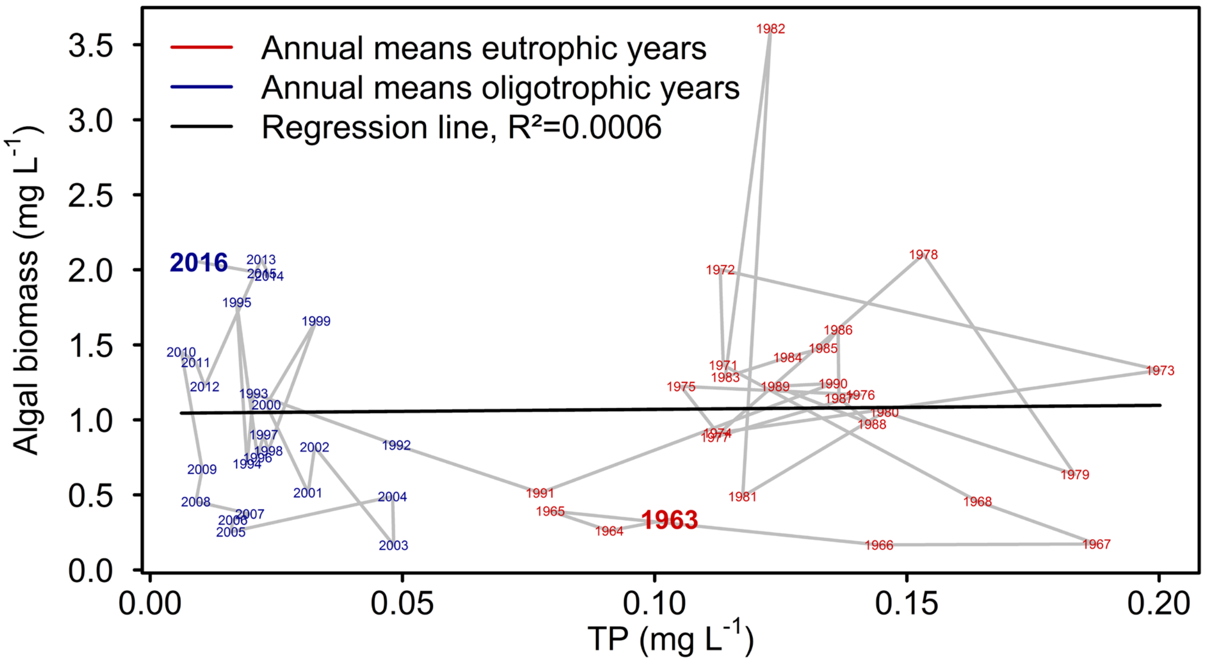


Figure 6.2: Long-term trajectory of annual mean phytoplankton biomass versus annual mean total phosphorus (TP) concentration in the Rappbode Reservoir from 1963 untill 2016. The nutrient-rich (eutrophic) years are highlighted in red, the nutrient-poor (oligotrophic) years in blue. The solid black line shows the linear regression between phytoplankton biomass and TP, indicating that there is no significant relationship between both parameters.

Despite the evidence for this obvious resistance of phytoplankton biomass against nutrient reduction and its relevance for setting realistic restoration targets and developing effective management strategies, the reasons for the decoupling of algal growth from its limiting nutrient resource are not well understood. Previous discussions and speculations about causes for nonlinearities in the chlorophyll-TP relationships in lakes include changes in trophic interactions, such as the presence or absence of herbivorous fish (Mazumder & Havens, 1998; Anneville *et al.*, 2019), effects of global warming, including altered mixing and stratification patterns, on community structure and dynamics (Anneville, Ginot & Angeli, 2002; Horn *et al.*, 2015), self-stabilizing mechanisms and compensatory dynamics of the phytoplankton community (Jochimsen, Kümmerlin & Straile, 2013) as well as changes in ecological properties of the community towards mobile species with low-light tolerances, which can migrate below the P-depleted layer (Anneville, Ginot & Angeli, 2002).

In case of the Rappbode Reservoir, losses in total biomass during oligotrophication were primarily prevented by internal changes in the functional structure of the community (Fig. 6.3). The thesis identified an increase in the functional group of phagotrophic mixotrophs as the most important mechanism, as the yield of biomass per unit phosphorus was significantly correlated with the biomass of mixotrophic species (**Q2.3**). By ingesting bacteria as a supplementary nutrient and energy resource, mixotrophs can make nutrient sources available that would not be accessible otherwise (Isaksson *et al.*, 1999; Nygaard & Tobiesen, 1993). Consequently, mixotrophy is a powerful functional strategy for gaining nutrients under P-depleted conditions, explaining high total algal biomasses despite P-reductions in the Rappbode Reservoir. Studies indicate that mixotrophs not only increase their own yield via bacterial supplementation, but can also stimulate the growth of non-mixotrophic species by releasing excess P to the environment (Grover, 2000; Sanders *et al.*, 2001). In addition, mixotrophs are characterized by low sinking velocities, keeping P in the photic zone for a longer time (Findlay *et al.*, 2001; Ptacnik, Diehl & Berger, 2003; Reynolds, 2006). Consequently, the access of mixotrophs on bacterial phosphorus has enhanced the P-flux towards mixotrophs and non-mixotrophs can partly profit from this phosphorus via nutrient recycling. This was probably also the case in the Rappbode Reservoir, since not only mixotrophs, but also obligate autotrophic species contributed to the increased yield of biomass per unit phosphorus over time. An increase in the taxonomic groups of cryptophytes, dinophytes and chrysophytes, which include mixotrophic species, has also been observed in other lakes, including those where no decrease in total biomass was found after nutrient concentrations decreased (Gaedke, 1998; Findlay *et al.*, 2001; Jeppesen, Jensen & Søndergaard, 2002; Anneville, Ginot & Angeli, 2002; Anneville, Gammeter & Straile, 2005; Jeppesen *et al.*, 2005; Kamjunke, Henrichs & Gaedke, 2006; Weyhenmeyer & Broberg, 2014). This indicates that also in other lakes the mixotrophy trait could be an important ecological mechanism explaining the resistance of total algal biomass to respond to nutrient reductions.

A second, but subordinate, mechanism leading to constant total algal biomasses after P reductions were changes in the seasonal biogeochemical cycling of nutrients within the pelagic zone, which were triggered by altered abundances of species with high sinking velocities (Fig. 6.3). I could show that the decrease in diatom blooms in spring with oligotrophication was related to less phosphorus losses over the season, allowing for higher biomasses in summer (**Q2.3**): Diatoms suffer from high sinking velocities due to their siliceous frustules (Trimbee & Harris, 1984; Reynolds, 2006), but are relatively slowly remineralized (Elster, 1963; Krause, 1964). As a result of both traits, diatoms lead to high sedimentation losses, since they sink out of the photic zone before their nutrients can be released by mineralization (empirical evidence is given by Benndorf, 1968; Horn *et al.*, 2015; Frassl, Rothhaupt & Rinke, 2014). Due to the decreasing dominance of diatoms in spring caused by nutrient reductions in the Rappbode Reservoir, less phosphorus was removed from the productive zone by sedimentation processes and hence more nutrients stayed available for the summer period. As a result, phytoplankton biomass was more evenly distributed over the season during the oligotrophic period and higher summer biomasses compensated biomass losses in spring. This illustrates the importance of the interplay between phytoplankton traits, community dynamics and biogeochemical processing for a better mechanistic understanding of the resistance of phytoplankton biomass to nutrient reductions.

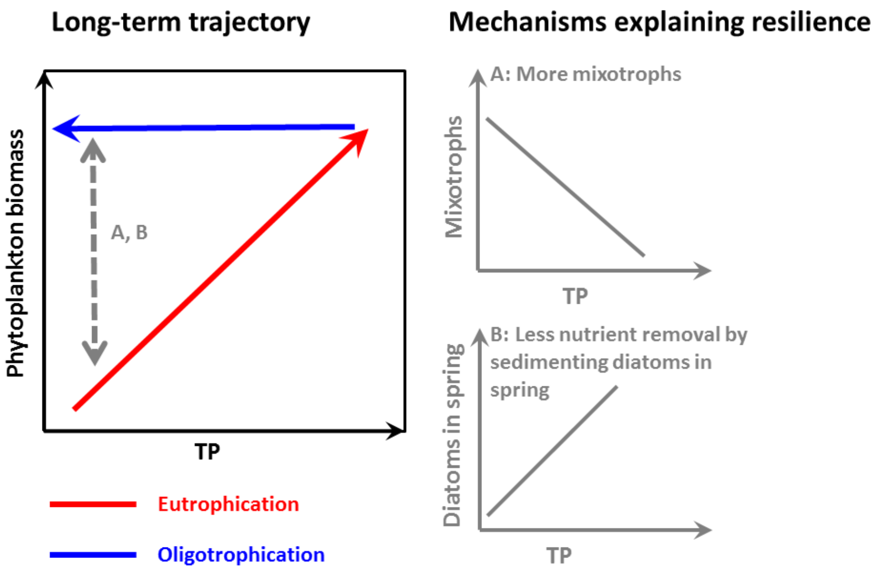


Figure 6.3: Conceptualization of the long-term trajectory of phytoplankton biomass during eutrophication and oligotrophication (left) and reasons for the missing response in biomass after total phosphorus (TP)

decreased (right) in Rappbode Reservoir. The reasons for the resistance of phytoplankton biomass to react to nutrient reductions are A) an increase in mixotrophs and B) less nutrient removal by sedimenting diatoms in spring, leaving more nutrients for phytoplankton in summer.

In conclusion, a shift in the functional structure allowed the phytoplankton community to adapt to lower nutrient levels without a loss in total biomass and explained the decoupling of inorganic nutrients from its ecological response. This case study demonstrates the ecosystem´s ability to compensate for changes in resource availability through changes in internal processes and functional strategies, leading to a non-reversible eutrophication-trajectory. This, however, does not mean that restoration efforts have no positive effect on eutrophication symptoms at all: For example, the undesired high biomass peaks during spring blooms diminished with oligotrophication in the Rappbode Reservoir. Also the summer peaks of nitrogen fixing cyanobacteria became less.

Moreover, the case study from Rappbode Reservoir exemplifies how functional traits can be used to identify casual relationships and mechanisms to gain a better understanding of ecosystem processes. Hence this thesis supports studies claiming that functional traits are a promising approach towards a higher generality and predictability in ecology (Weithoff, 2003; McGill *et al.*, 2006; Litchman & Klausmeier, 2008) (**Q2.3**).

6.4 Impact of functional traits on the geochemical environment

So far, the thesis mainly focused on the response of phytoplankton functional traits to changes in the abiotic environment. However, phytoplankton communities also shape their surrounding environment and therefore affect geochemical cycles. For example, the results from Rappbode Reservoir show an increase in pH during the growing season when phytoplankton biomass is high. This is most likely caused by higher primary production, since photosynthesis removes CO_2 and consequently the pH value increases (Kalff, 2002). The Rappbode Reservoir also shows lower nutrient concentrations (e.g. phosphorus, nitrogen and silica) in summer in the epilimnion as a result of nutrient uptake by algae, which require nutrients for their metabolism (Kalff, 2002), and consecutive nutrient removal by sinking of algae cells.

While these examples illustrate the impact of phytoplankton abundance on their geochemical environment, the functional composition of the community can also be of importance in controlling ecosystem processes. Functional traits, which simultaneously respond to environmental conditions (response traits) and at the same time directly affect biogeochemical processes (effect traits), tightly link community structure with their environment and hence may enhance predictability of ecosystem functioning (Lavorel & Garnier, 2002; Litchman, Edwards & Klausmeier, 2015). An example from Rappbode Reservoir of how phytoplankton traits affect biogeochemical cycling is the effect of high sedimentation velocities of diatoms (Trimbee & Harris, 1984) on the spatial distribution of nutrients. Since diatom cells sink very quickly out of the photic zone, they lead to high removal of nutrients from the upper water column and high burial in the sediment, as shown in previous studies for phosphorus, silica and carbon (Benndorf, 1968; Nelson *et al.*, 1995; Tréguer & De La Rocha, 2013; Frassl, Rothhaupt & Rinke, 2014; Horn *et al.*, 2015). This effect is also supported by the results from Rappbode Reservoir, showing a higher removal of silica and phosphorus during eutrophic years when diatom concentrations were high in spring (**Q3.1**). The removal rate of nutrients, in turn, affected summer biomasses (as discussed in section 6.3), illustrating the feedback effects between biology and geochemistry.

While the effects of functional composition on nutrient concentrations have been shown before in other studies, this thesis provides novel evidence about the importance of the functional composition of metalimnetic phytoplankton communities for the formation of metalimnetic oxygen minima and hence adds a completely new example to the research about the impact of functional traits on the geochemical environment (reviewed by Litchman *et al.*, 2015) (**Q3.1**). Metalimnetic taxa, i.e. the functional group of species forming a biomass maximum in the metalimnion or upper hypolimnion during the stratification period in summer, are equipped with a special set of traits to be able to grow under the low light environment in the thermocline (Reynolds *et al.*, 2002). As outlined in detail in section 1.3.2, metalimnetic taxa are characterized by low light requirements and the ability to utilize a wide range of the light spectrum by possessing phycobilin pigments (Vila & Abella, 2001; Camacho, 2006). Also their ability for vertical movement through flagella or buoyancy are important traits of metalimnetic species, allowing them to migrate between upper water layers with higher light availability and nutrient-rich deeper layers (Salonen, Jones & Arvola, 1984; Camacho, Vicente & Miracle, 2001). While it is known that the occurrence of the functional group of metalimnetic taxa highly depends on environmental conditions, e.g. a stratified water body (Camacho, 2006), light penetration into the metalimnion (Reynolds, 1992; Moll, Brache & Peterson, 1984) and the availability of nutrients in or below the thermocline (Gervais, 1997; Gong *et al.*, 2015), the effect of this functional group on the geochemical environment is not well studied.

For the first time, the thesis gives empirical evidence that metalimnetic taxa, in case of the Rappbode Reservoir mainly represented by the cyanobacterium *Planktothrix rubescens*, provide an important contribution for the emergence of oxygen minima, which are a phenomena described in many lakes and reservoirs worldwide (Wetzel, 2001; Joehnk & Umlauf, 2001; Boehrer & Schultze, 2008; Zhang *et al.*, 2015) (**Q3.2**). Using a temporally and vertically highly resolved monitoring system, including multiple chlorophyll-a fluorescence sensors with the ability to detect phycoerythrin-rich algae such as *P. rubescens*, I could show that the disappearance of *P. rubescens* florescence was connected to the emergence of a metalimnetic oxygen minimum in the Rappbode Reservoir (Fig. 6.4). Moreover, I showed that the available phytoplankton carbon biomass in the metalimnion was sufficient to cause the observed oxygen depletion in the metalimnion. This supports the assumption that biological activity induced by the end of the *P. rubescens* bloom was an essential factor for the formation of the metalimnetic oxygen minimum. While the thesis provides clear evidence that the waning of *P. rubescens* fluorescence was associated with the emergence of the oxygen minimum in the metalimnion, the exact processes inducing the oxygen depletion remain open to speculation: Both, oxygen use through bacterial decomposition of dead organic material originating from *P. rubescens* or *P. rubescens* extending its respiration beyond its photosynthetic activity are possible explanations for the formation of the metalimnetic oxygen minimum.

The connection between metalimnetic taxa, whose occurrence highly depends on the presence of a strong thermocline as well as other environmental conditions (Gervais, 1997; Camacho, 2006; Gong *et al.*, 2015), and oxygen depletion in the metalimnion illustrate that organisms and their abiotic environment are tightly coupled through functional traits. Moreover, it displays how important the functional composition of the community is for the effect of phytoplankton on the geochemical environment: Without the possession of a specific trait combination (e.g. mobility and ability for efficient light harvesting) the group of metalimnetic taxa could not survive and grow in the metalimnion and consequently no oxygen minimum could develop. Hence I conclude that functional trait composition is an important factor controlling ecosystem processes and functioning.

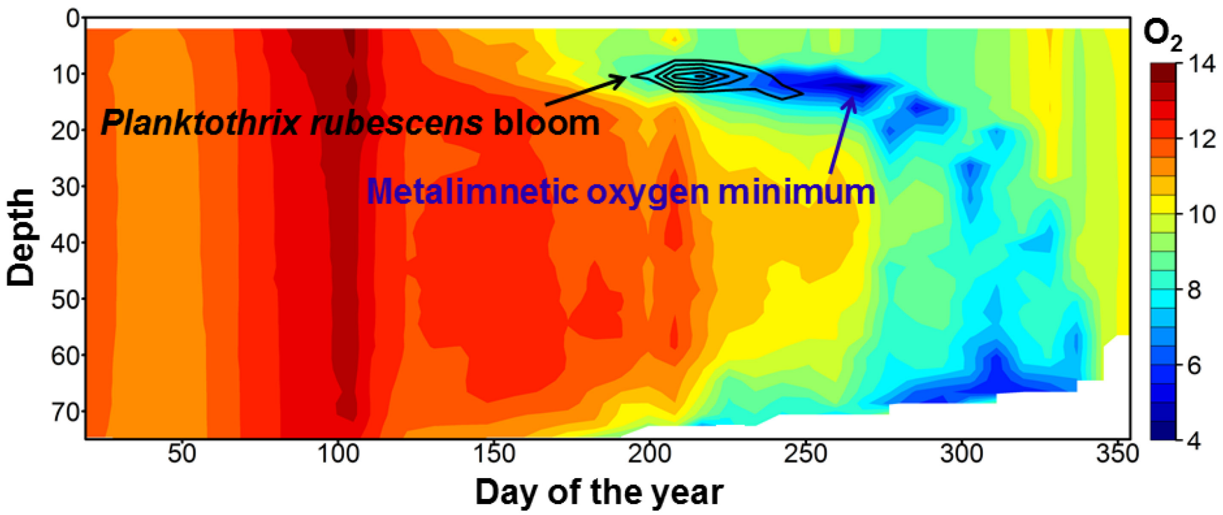


Figure 6.4: Oxygen distribution along the season and depth shows the development of a metalimnetic oxygen minimum after the disappearance of fluorescence from the Planktothrix rubescens bloom (black contour lines) in the Rappbode Reservoir.

However, the effect of the DCM forming algae on oxygen dynamics not only exemplifies how functional traits affect the geochemical environment, but also identifies a previously undiscovered mechanism for the development of metalimnetic oxygen minima. So far, four factors had been discussed in literature that may lead to enhanced oxygen depletion in the metalimnion (Fig. 6.5):

(1) Inflows carrying oxygen depleted water or water with easily degradable material with high oxygen demand that find their way into the metalimnion (Nix, 1981).

(2) Oxygen depletion at the sediment contact zone, which is advected into the metalimnion at the side boundaries, especially when the slope of the lake basin is very gentle (Shapiro, 1960; Wetzel, 2001).

(3) Respiration of dense populations of non-migrating copepods in the metalimnion (Shapiro, 1960; Raateoja *et al.*, 2010).

(4) Decomposition of organic algal material sinking from the epilimnion (Müller *et al.*, 2012).

This thesis identifies the end of the mass development of metalimnetic taxa as a fifth factor that can trigger the formation of metalimnetic oxygen minima (Fig. 6.5), either through bacterial decomposition of their dead material or through respiration beyond their photosynthetic activity.

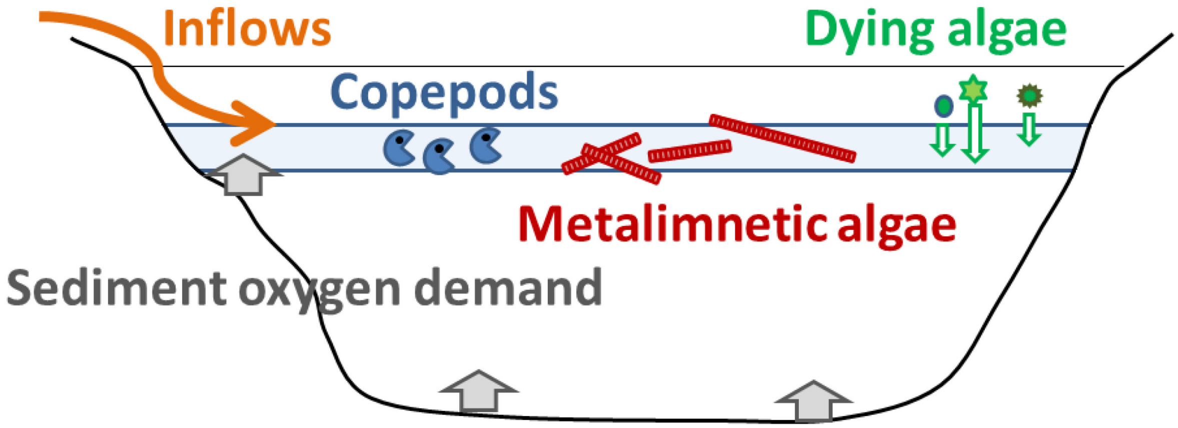


Figure 6.5: Schematic overview about possible mechanisms leading to the formation of metalimnetic oxygen minima.

The finding that oxygen deficits can be caused by mass developments of metalimnetic algae is highly relevant for lake management, as low oxygen concentrations can have severe consequences for ecosystem functioning (Magee *et al.*, 2019), biodiversity (Wetzel, 2001; Schindler, 2017), water quality and human health (North *et al.*, 2014; Harke *et al.*, 2016). Rice *et al.* (2013) for example showed that metalimnetic hypoxia can cause fish kills. The Rappbode Reservoir harbors a managed stock of lake trout, which is sensitive to low oxygen levels, and therefore very low oxygen levels in the metalimnion should be avoided. With climate warming oxygen concentrations in lakes and reservoirs are generally predicted to decline, due to reduced gas solubility (Benson & Krause, 1984) and increased thermal stability preventing the replenishment of oxygen from surface to deep-waters (Livingstone, 2003; Jankowski *et al.*, 2006; North *et al.*, 2014). Lake warming has also been shown to favor the development of *P. rubescens* in the metalimnion (Posch *et al.*, 2012; Yankova *et al.*, 2017). Consequently, metalimnetic oxygen minima might increase simultaneously with *P. rubescens* blooms in the future, adding upon the previously mentioned mechanisms of oxygen decline due to climate change.

Based on these findings, reservoir operators could optimize their management to avoid negative consequences for water quality. One possible option to counteract the effects of climate warming is the modification of water column stability via selective withdrawal depth to prevent a further increase in *P. rubescens* blooms in the metalimnion and the resulting oxygen depletion (see Mi *et al.* (2019) for possibilities to change stratification patterns via selective withdrawal). Another idea is withdrawal of water at the depth where the *P. rubescens* population peaks, resulting in a decrease of *P. rubescens* biomass and their negative effects on water quality.

6.5 Conclusions and implications for trait-based ecology

Since the dynamics of individual species are often complex and hard to predict (Reynolds, 2000), taxonomical ecology often tends to have restricted rules and special cases (Lawton, 1999). To discover general patterns, laws and rules in nature, the use of traits has been claimed to be a promising tool (McGill *et al.*, 2006; Litchman & Klausmeier, 2008), since they reduce the complexity and directly link changes of communities with changes in the environment via functions. The work conducted in this thesis strongly supports this assertion. I could show that during the 50 years of observation in the Rappbode Reservoir the studied functional traits mirrored environmental pressures over the year and showed consistent, reoccurring seasonal developments, which could be ecologically interpreted. Bottom-up effects, including nutrient and light availability, seemed to be the most important factors shaping the functional structure of the community along the season, while top-down effects of grazers were probably less important. While the main features of functional trait succession were independent of trophic status, the seasonal differences in trait composition were less pronounced after oligotrophication. Based on the observed trait patterns, this thesis provided a functional template for trait based succession patterns under eutrophic and oligotrophic conditions for temperate lakes. This trait based template largely extends the verbally formulated PEG model (Sommer *et al.*, 1986; Sommer *et al.*, 2012), since it is based on a quantification of functional properties and thus allows for statistical testing and modelling.

Similar seasonal dynamics were observed in all studied traits, even though they originated from different sources and belonged to different trait categories (morphological, behavioral and physiological traits, continuous vs. binary traits). This supports the reliability of the observed patterns and demonstrates that different trait types can detect functional shifts in phytoplankton communities (e.g. morphological traits used by Kruk et al. (2011), Stanca, Cellamare and Basset (2012) and Acevedo-Trejos et al. (2015), physiological traits used by Edwards, Litchman and Klausmeier (2013) or binary traits used by Klais et al. (2017)). These results show that translating species into functional traits by assigning trait values compiled from the literature is a powerful method to enable ecological interpretation of observed phytoplankton community dynamics by quantification of functional characteristics. This is remarkable as all traits (except cell size) were measured on laboratory cultures (e.g. phosphate affinity) or refer to the potential to express a specific trait (e.g. the potential for mixotrophy).

The quantitative approach based on traits presented in this thesis can open new perspectives for predictive ecology using lake ecosystem models. Models that can realistically represent the adaptive response of diverse plankton communities are urgently needed to understand how aquatic ecosystems and the biogeochemical cycles that they mediate are impacted by environmental changes, such as climate change and oligotrophication. However, most available phytoplankton models are limited in the number of modelled phytoplankton species or species groups (Mooij *et al.*, 2010). Formulating functional groups (e.g. Reynolds, Alex Elliott & Frassl, 2014) or aggregating species information by describing them by their traits (e.g. Wirtz & Eckhardt, 1996; Smith *et al.*, 2014; Acevedo-Trejos *et al.*, 2015; Berge *et al.*, 2017) overcomes this problem and also reduces complexity allowing for more generalized statements and higher predictability (McGill *et al.*, 2006; Litchman & Klausmeier, 2008).

In contrast to taxonomic approaches, where the presence of species largely differs from habitat to habitat, the trait-based approaches used in this study allow for comparisons across different environmental systems, since taxonomic complexity is reduced to the unifying unit of functional traits. Comparing the results of this thesis to the results of recent studies from brackish and marine habitats reveals that the seasonal succession patters for the traits silica use, nitrogen fixation, mixotrophy and buoyancy (Klais *et al.*, 2017) as well as maximum growth rate and light affinity (Edwards, 2016) are similar in freshwater and marine systems, while cell size shows opposing seasonal developments (Acevedo-Trejos *et al.*, 2015; Klais *et al.*, 2017). In this way, trait-based approaches can serve as a common currency in ecology and traits can be used to assess phytoplankton community structure across different gradients and environments, as also suggested by Kilham and Hecky (1988) and Weithoff and Beisner (2019).

The thesis indicates that functional traits link organisms with their bio-geochemical environment, allowing for an ecological interpretation of how phytoplankton community dynamics are shaped by their environment, but also how in turn community dynamics influence the geochemical environment (biogochemical impacts of phytoplankton traits are revievied by Litchman *et al.*, 2015). For instance, evidence is provided that the functional group of metalimnetic species can cause oxygen minima in the metalimnion, indicating the importance of the vertical distribution of phytoplankton traits for biogeochemical processes. Phytoplankton traits can also shape spatial resource distributions, as shown by the effect of diatoms with high sinking velocities on nutrient losses from the photic zone. The effects of the sedimentation velocity trait on nutrient distribution in turn contributed to the unexpected resistance of total phytoplankton biomass after nutrient reductions. Also mixotrophs and their ability to make bacterial phosphorus available provided an important explanation for the decoupling of inorganic nutrients from total biomass concentrations, demonstrating the usefulness of functional traits for a better mechanistic understanding of aquatic ecosystems. Obviously, functional composition is an important factor in controlling biogeochemical processes and ecosystem functioning and hence trait-based approaches could provide a framework to identify biotic mechanisms relevant for the delivery of ecosystem services.

In conclusion, the trait-based approaches presented in this thesis enhance ecological understanding of the observed phytoplankton community dynamics, their response to environmental gradients (intra-annual, inter-annual and vertical) as well as their impact on the geochemical environment, by focusing on the mechanisms that govern interactions between biota and the surrounding environment. I show that functional traits can be applied to translate information about taxonomic composition into ecologically meaningful functions and eco-physiological processes that can be used to understand e.g. seasonal succession patterns, long-term community changes in response to environmental pressures (e.g. nutrient loads) or previously unresolved phenomena in the abiotic (e.g. metalimnetic oxygen minima) and biotic environment (e.g. unexpected high biomasses). The quantitative, trait-based approaches used in this thesis give more generality and predictability than traditional taxonomic approaches and are hence a step forward towards a more predictive community ecology based on a mechanistic foundation. The trait-based approaches developed in aquatic ecology can also advance other fields of research, such as medicine, as shown by Guittar, Shade and Litchman (2019), who used trait-based approaches to understand the mechanisms of community assembly and succession of the infant gut microbiome.

In addition to the advances for trait-based approaches, the thesis for the first time studies phytoplankton-dynamics in the Rappbode Reservoir and hence provides detailed process-knowledge about this system. This gives the opportunity to further use the Rappbode Reservoir as a well-studied research site for future investigations. Moreover, the new insights gained through using the 50-year dataset from the Rappbode Reservoir illustrate the enormous value of conducting and maintaining long-term monitoring to assess the impact of changing environmental conditions on ecosystems. Many important aspects studied in this thesis, such as the recovery-response and resistance behavior of the ecosystem after nutrient-pressures diminished, cannot be studied experimentally or by cross-system comparisons. Facing future challenges such as climate change, I want to explicitly encourage the maintenance and further collection of long-term datasets, even though it is costly, time-consuming and does not immediately lead to publication success.

6.6 Future directions

The thesis provides new insights into phytoplankton dynamics and their interaction with geochemical processes by using functional traits and it exemplifies how functional traits can be used to gain a better mechanistic understanding of aquatic ecosystems. However, there are still many open questions remaining and further research needs to be conducted regarding the usage of functional traits. Here I give a small selection of topics that need further investigation.

This thesis provides a template for seasonal succession patterns of functional traits under eutrophic and oligotrophic conditions. This template should also be applied in other lakes in order to evaluate if the patterns are similar in specific groups of lakes or if there are deviations. In case of differences, possible reasons should be identified with the final goal to develop functional templates for seasonal succession for all different lake types (e.g. eutrophic vs. oligotrophic as provided by this thesis, deep vs. shallow lakes, temperate vs. tropical lakes, brown vs. transparent lakes). Research should also further exploit the advantages of traits as a common currency in ecology to assess community structure across different habitats, gradients and ecosystems. Traits as a unifying concept in ecology not only allow for comparisons between different aquatic habitat types, such as marine, brackish and freshwater habitats, but also between aquatic and terrestrial ecosystems.

The thesis illustrates that the translation of taxonomic information into functional information by using trait values obtained from physiological measurements of laboratory cultures is a useful method to increase ecosystem understanding. However, for many species no trait values are available, since obtaining them is very labor intensive. Given the potential of these trait data, more studies should anyway collect trait measurements to finally create a complete species-trait table.

Also methods for inferring missing trait data could be further developed (e.g. as shown by Bruggeman, Heringa & Brandt, 2009; Edwards, 2016). An important aspect is intraspecific trait variability, which is not considered in this thesis as I assigned static trait values to each algal species. However, traits can be plastic and differ depending on environmental conditions and the presence of grazers (Rhee, 1974; Van Donk & Hessen, 1993; Yoshida, Hairston & Ellner, 2004; Malerba *et al.*, 2016), time of the year (Morabito *et al.*, 2007) or between different isolates of the same species (Bolius, Wiedner & Weithoff, 2017). Malerba *et al.* (2016) showed that representing a species by their mean trait value could underestimate their physiological performance by one order of magnitude due to within-species trait variability. Given these results, further research should focus on incorporating intraspecific trait plasticity into trait-based approaches.

While this thesis focused on the effect of nutrient reductions on phytoplankton, other trophic levels are also important parts of lake ecosystems. It remains open whether and how the changes in phytoplankton community composition after oligotrophication affected higher trophic levels. It is well known that abundance and composition of zooplankton is largely influenced by phytoplankton community structure, since algae differ in their grazing resistance (Van Donk, Ianora & Vos, 2010) as well as in their nutritional value and fatty acid composition (Arnold, 1971; Spijkerman *et al.*, 2012; Taipale *et al.*, 2013; Galloway *et al.*, 2014). To investigate the effect of oligotrophication on the phytoplankton food quality and changes in energy transfer to higher trophic levels, the fatty-acid composition of single algal species could be translated into a community-averaged food quality trait value. This food quality trait could link changes in the phytoplankton community directly with changes in higher trophic levels and could be used to analyze the effect of nutrient reductions on the zooplankton and fish community.

Based on the findings about the connection between metalimnetic algal blooms and the development of oxygen minima in the metalimnion, a logical next step would be to investigate the mechanisms causing this connection. The thesis provides several hypotheses how the disappearance of *P. rubescens* fluorescence could lead to the observed oxygen depletion (see section 6.4), but they still remain to be proven. Further investigations should also focus on disentangling the effect of different mechanisms on the formation of metalimnetic oxygen depletion (for mechanisms see section 6.4), as the contribution of each process to the metalimnetic oxygen depletion is probably lake-specific. While I excluded a major contribution of sediment oxygen demand and inflows to the metalimnetic oxygen minimum in the Rappbode Reservoir, the effect of algae sedimenting from the epilimnion should be further evaluated.

The results from Rappbode Reservoir as well as observations from other lakes (Gaedke, 1998; Findlay *et al.*, 2001; Jeppesen, Jensen & Søndergaard, 2002; Anneville, Ginot & Angeli, 2002; Anneville, Gammeter & Straile, 2005; Jeppesen *et al.*, 2005; Kamjunke, Henrichs & Gaedke, 2006; Weyhenmeyer & Broberg, 2014) show that mixotrophs increase with oligotrophication. However, the mechanisms by which mixotrophs increase with decreasing nutrient concentrations remains partly unclear: As mixotrophs are relieved from direct competition for inorganic nutrients due to their ability to feed on bacteria, they should be able to grow under all nutrient conditions. Therefore the reasons why they do not build up high biomasses under eutrophic conditions remain open and should be experimentally tested.

Further, the observations from Rappbode Reservoir indicate that P-recycling by mixotrophs makes phosphorus accessible for the whole phytoplankton community and thus stimulates the growth of phototrophs. This mechanism is important as it might contribute to the resistance of phytoplankton biomass to react to nutrient reductions. However, if phototrophs actually profit from P-recycling by mixotrophs is controversial. While some experiments indicate that phototrophs benefit from the access of mixotrophs on bacterial phosphorous (Grover, 2000; Sanders *et al.*, 2001), other experiments come to opposing conclusions (Rothhaupt, 1997). Therefore further experiments on P-recycling by mixotrophs and their effect on non-mixotrophs are required for clarification.

Since the good or very good ecological status, as required by the EU Water Framework Directive, is currently only achieved in 53.6% of Europe´s and in 26,1% of Germany´s lakes (European Environment Agency, 2018), future research should also focus on transferring ecological knowledge to water management by providing ideas how to enhance restoration success or how to set more realistic targets. For example, this thesis provides evidence that the resistance of phytoplankton biomass to react to reduced phosphorus loads was related to nutrient supplementation by mixotrophs ingesting bacteria. Further research could take this as a starting point to first investigate if a reduction in bacterial biomass would lead to less mixotrophs and then how the results could be translated into management practice. For instance, as bacteria can use DOC as a carbon source, DOC promotes bacterial growth (Tranvik, 1988). Hence DOC-rich lakes can support higher bacterial biomasses than DOC-poor lakes (Jones, 1992; Lennon & Pfaff, 2005;

Berggren *et al.*, 2010; Roiha *et al.*, 2011). Possibly increased DOC loads during the recent decades promoted bacterial growth and thus mixotrophy in the Rappbode Reservoir. In that case, measures could focus on reducing DOC inputs to the reservoir to prevent the transfer of bacterial phosphorus into algal biomass.

Moreover, modelling studies could investigate the possibility of changing stratification patterns via selective withdrawal depth in reservoirs (e.g. see Mi *et al.*, 2019) as a possible mechanism to reduce 1) the growth of mixotrophs and thus total algal biomass and 2) the growth of metalimnetic algae (e.g. *P. rubescens*) to prevent the development of metalimnetic oxygen minima and harmful cyanotoxins. As both functional groups, mixotrophs as well as metalimnetic species, profit from stratified conditions (Jäger, Diehl & Schmidt, 2008; Walsby *et al.*, 2004), an increased mixing regime could prevent their growth. However, negative effects of increased turbulence should be carefully evaluated, e.g. increased nutrient resuspension from the sediment and deeper layers into the photic zone (Pierson & Weyhenmeyer, 1994; Song *et al.*, 2013) and higher productivity of diatoms which are favoured by destratification (Jäger, Diehl & Schmidt, 2008). In addition to changes in stratification patterns, selective withdrawal of algal peaks from the metalimnion is an option to prevent metalimnetic oxygen minima and their negative consequences for water quality. Lake ecosystem models could be used to evaluate this option and to quantitatively determine to which extent selective withdrawal could help to reduce metalimnetic algal blooms and the development of oxygen deficits.

While knowledge gained in this dissertation through the use of functional traits provides an important step towards a better understanding of phytoplankton community dynamics and their responses to and impact on the surrounding environment, much more research needs to be done to achieve a more complete picture of lake ecosystem dynamics and develop general rules to be able to predict how lake ecosystems behave under changing environmental conditions.

7 Literature

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8 Acknowledgements

First of all, I want to thank Karsten Rinke, Jörg Tittel, Christoph Jäger, Bertram Boehrer and Dietrich Borchardt for your awesome supervision! You were a great combination of advisers with different skills, all very experienced, sparkled with ideas, enthusiastic as well as kind, helpful and open minded.

Karsten, you have been a great main PhD supervisor and your door was always open for questions of all kind. You provided me with a perfect combination of guidance and freedom to develop and follow own ideas. You also made it possible for me to travel to many conferences and workshops and build up a network. You have been always very inspiring for me, with your skill to explain concepts and ideas, cleverness, efficiency and with an impressive quickness of perception and thinking. You are not only a superior scientist and likeable person, but also a great teacher and guide – you are what I would call a “Eierlegende Wollmilchsau”.

Christoph, especially during the first time of my PhD in Magdeburg, you warmly welcomed me, introduced me to the laboratory facilities at UFZ, helped me to plan the experiments and always had time for questions and discussions. Despite the distance between Magdeburg and Rosenheim later on, you supported me as much as possible and always gave good advice.

Jörg, I really appreciate your guidance, clever ideas and thinking out of the box. The discussions with you have been very stimulating and they made me consider things I never thought about before. Your critical opinion was always very important for me and largely improved my manuscripts. Knowing that I can trust your critical judgement helped me to know when something is good enough. You always knew the point at which I should stop further data analysis or improvement of the manuscript and pushed me to finalize things. I also very much appreciate your quickness in giving feedback – getting a detailed feedback on a manuscript within very few days is very rare. Thanks for taking so much time for me!

Bertram, your moral support was so important for me. Your door was always more than open for me, almost on a daily basis we talked about scientific as well as everyday topics. I am very grateful that I ended up in the limnophysics working group, even though my topic is more biological. I very much enjoyed the weekly limnophysics meetings, the group dinners we had at your house and the great excursions – they were educational, teambuilding and fun. Writing the manuscript

with you was really fun too. Especially the scientific discussions we had while the idea for the paper emerged were really inspiring and motivating. It showed me how important discussions and interdisciplinary exchange (e.g. between physics and biology) is in developing novel ideas. Thanks for being there for me during my PhD time and for pushing me to finish this thesis!

Dietrich, thanks a lot for guiding me through the process of this dissertation and giving support whenever needed. I am very grateful that you believed in me and were always positively minded. You gave me many opportunities to develop myself further (e.g. the opportunity to visit conferences, giving talks at the IP meeting) and getting insights into the more political structures of scientific organizations (e.g. while participating in the UFZ evaluation).

A big “thank you” also goes to the technicians at UFZ, who helped in the lab and in the field. Karsten Rahn, Martin Wieprecht and Michael Herzog, I really admire your technical skills and efficiency. I learned a lot from you during the field work. Going to Rappbode Reservoir with you was always a lot of fun, no matter if it was sunny, rainy, snowy or if we had to break the ice. Thanks to Burkhard Kuehn for installing the autonomous vertical profiler at Rappbode Reservoir. I am also especially grateful for the work of Kerstin Lerche, who spend a lot of time analyzing my pigment samples on the HPLC.

Thanks to my colleagues and friends from UFZ for scientific exchange, but also for warmly welcoming me in Magdeburg and for quickly integrating me in their team, making me feel home and for the emotional support I received from you. You were all very inspiring for me and I learned a lot from you during my time at UFZ. You went with me through the challenging time of doing a PhD with all the ups and downs. Doing my PhD at UFZ and getting to know all of the great people there influenced my life a lot in a very positive way. I am very happy and grateful for this experience. I especially appreciated the nice coffee breaks, where we could discuss ideas and opinions, but could also share the challenges of a life as a PhD student. Special thanks to Marieke Frassl and Katja Westphal for all the detailed scientific discussions, for proofreading parts of my thesis and for telling me that I will finally finish my thesis. Also special thanks to Anders Ekman for proofreading my thesis and supporting me with solving R problems. Thanks for believing in me!

Thanks to the Wasserwerk Wienrode (especially Jan Donner and Wolf-Dieter Skibba) and the Talsperren Betrieb Sachsen-Anhalt for providing long-term data from the Rappbode Reservoir.

Without this great data set my PhD work would not have been possible. I also thank the DFG for funding this project (grants JA 2146/2-1 and RI 2040/2-1). The project was part of the priority program 1704 “DynaTrait” – in this network I met many people all over Germany, which gave me the opportunity to exchange knowledge about trait dynamics.

Finally I thank all my family members and friends for their moral support during my whole life, for listening to the challenges I was facing during the PhD time and for giving good advice. In addition to this, I thank Christoph Purrer for solving some technical problems and for introducing me to the logic behind programming, which helped me a lot with learning R. I especially thank my parents, who always had an open ear for me, listened for hours to my problems and complaints on the phone and also pushed me to finally finish my dissertation.

9 Supporting information

9.1 Supporting information 1 for chapter 3 (S9.1)

Phylogenetic inference of quantitative traits of freshwater phytoplankton

A phylogenetic method (Bruggeman, 2011; Bruggeman et al., 2009) was used to infer quantitative traits characterizing the competitive ability of all sampled species. This analysis was based on the Phyto-PhyloPars dataset of phytoplankton traits (Bruggeman, 2011), which includes cell volume, surface area, length and diameter, maximum growth rate, phosphate affinity and susceptibility to predation by *Daphnids*. To also capture the role of light in controlling seasonal succession, the dataset was complemented with values of light affinity (the initial slope of the growth-irradiance curve, α) taken from a recent reanalysis of light utilisation traits across freshwater phytoplankton species (Schwaderer et al., 2011). Maximum growth rates collected by this study were also used. Further, a new datasets with cell volume measurements from phytoplankton in the Rappbode Reservoir were included.

From the Schwaderer *et al.* (2011) compilation, all entries in appendix B were used except those marked with “Error in alpha too high”, those that could not be fit by either of the proposed models (“Too few data, linear fit”), and all values that were originally taken from Tuji et al. (2000), as that study focused on benthic rather than pelagic species. This left 67 values for maximum growth rate and 74 values for light affinity from a total of 29 studies. Each of these original studies was revisited to determine the temperature and light : day period under which experiments were conducted; trait values were subsequently adjusted for a standardized environment as described below.

As in Bruggeman (2011), maximum growth rate and phosphate affinity were standardized for a temperature of 20°C. Values from experiments conducted at different temperatures were corrected using a Q_{10} relationship with factor 1.88. Only results from experiments conducted at a temperature between 10 and 20 °C were used to ensure the positive relationship between rates and temperature implied by the Q_{10} relation is valid for most species. No temperature correction was applied to the initial slope of the growth-irradiance curve, as growth at low light is controlled by arrival of photons, which is independent of temperature.

Maximum growth rate and phosphate affinity were not corrected for light period. This differs from the approach adopted by Bruggeman (2011), who assumed growth rate was proportional to light period. Review of the light utilisation studies referenced by Schwaderer et al. (e.g., Foy and Gibson, 1993) suggested that this proportionality is not universally found: while there is a clear positive relation between light period and growth for shorter light periods (< 12 hours of light), growth saturates at longer light periods for nearly all (temperate) species surveyed. Most experiments are conducted under light periods of 12-24 h, likely because such conditions were found to be optimal for growth. To account for this, we include only observations from experiments with at least 12 h of light per 24 h. Maximum growth rates at these light periods are assumed to have saturated (i.e., longer light periods would not increase the population growth rate); thus, neither the maximum growth rate nor the phosphate affinity (the ratio of maximum growth rate to half saturation phosphate concentration) require correction. The consequence of this is that our values for maximum growth rate and phosphate affinity differ from Bruggeman (2011) – typically they are 42-50 % lower, as most experiments were conducted under 12 : 12 or 14 : 10 h L : D periods. We believe these new values better reflect the true daily mean maximum growth rate achievable.

Light affinities were corrected for light period. Daily mean growth at low light is determined by the daily integrated photon flux, which is the product of the light period and the instantaneous photon flux. Thus, the initial slopes of the growth-irradiance curves estimated by Schwaderer et al. reflect both true differences between species and differences in the light period they were subjected to. Light affinities were therefore multiplied by 24/(number of light hours) in order to make them representative for growth under continuous light exposure.

Trait values for all sampled phytoplankton species were derived following the methodology described by Bruggeman (2011), using the expanded trait compilation described above, and an updated phytoplankton taxonomy based on the World Register of Marine Species (http://marinespecies.org).

9.2 Supporting information 2 for chapter 3 (S9.2)

Table S9.2.1: Five most abundant genera or species in spring (March, April, May), clearwater (June), summer (July, August, September, October) and winter (November, December, January, February) season during the eutrophic (1970-1990) and oligotrophic (1996-2006) period.

Season	**Eutrophic period (1970-1990)**	**Oligotrophic period (1996-2016)**
Spring	*Asterionella formosa* *Aulacoseira sp. (italica/granulata)* *Stephanodiscus hantzschii* *Fragillaria sp.* *Diatoma sp.* *Cyclotella sp.*	*Asterionella formosa* *Tabellaria fenestrata* *Gymnodinium sp.* *Aulacoseira sp. (italica/granulata)* *Dinobryon sp.* *Cryptomonas sp.*
Clearwater	*Asterionella formosa* *Fragillaria sp.* *Aulacoseira sp. (italica/granulata)* *Nitzschia sp.* *Ceratium hirundinella* *Cryptomonas sp.*	*Asterionella formosa* *Dinobryon sp.* *Tabellaria fenestrata* *Cryptomonas sp.* *Rhizosolena longiseta* *Peridinium sp.*
Summer	*Fragilaria sp.* *Asterionella formosa* *Aulacoseira sp. (italica/granulata)* *Stephanoon wallichii* *Ceratium hirundinella* *Staurastrum sp.*	*Fragillaria sp.* *Asterionella formosa* *Tabellaria fenestrata* *Cryptomonas sp.* *Peridinium sp.* *Dinobryon sp.*
Winter	*Asterionella formosa*	*Asterionella formosa* *Tabellaria fenestrata*

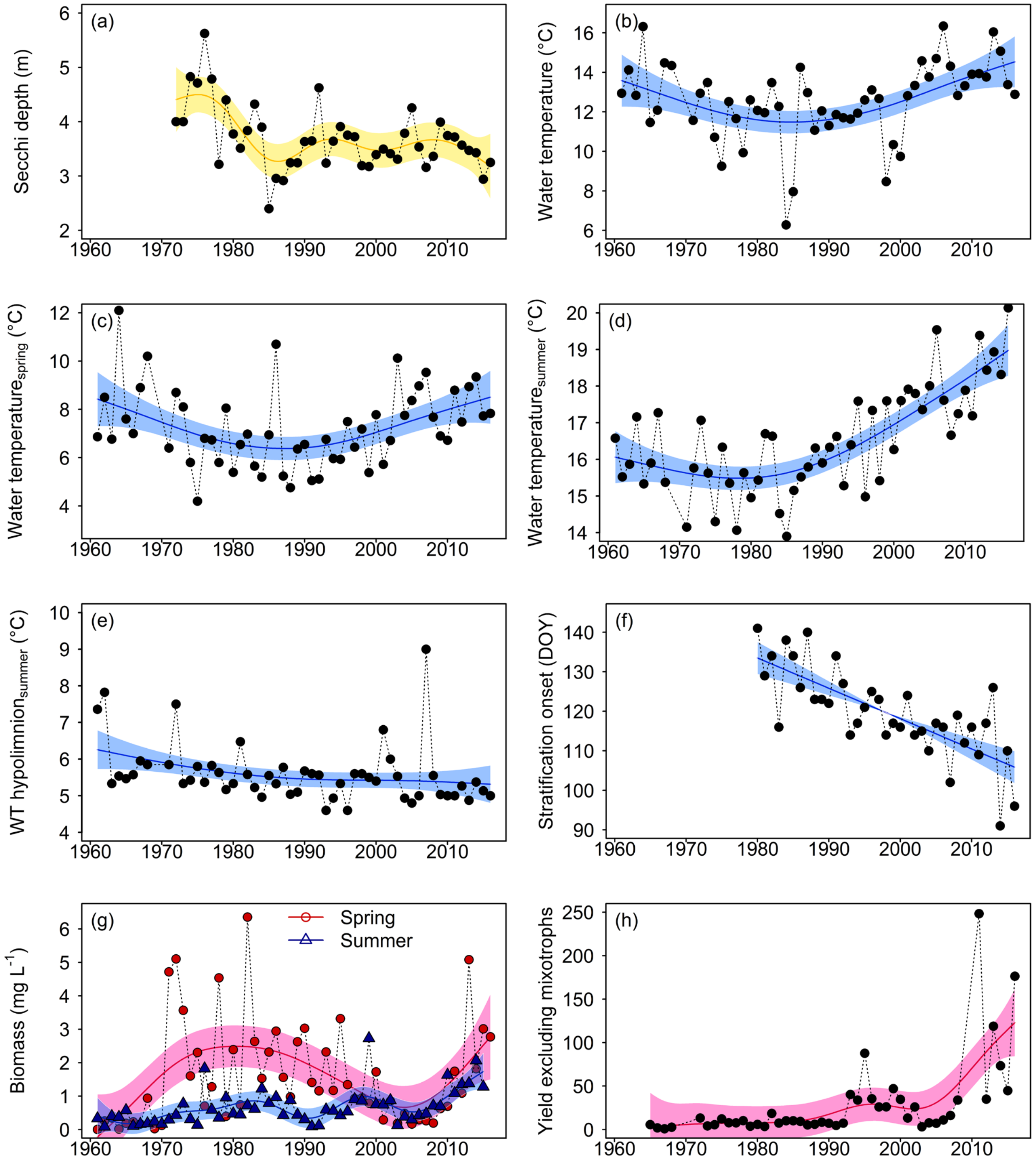
	Aulacoseira sp. (italica/granulata) *Mallomonas sp.* *Fragillaria sp.* *Staurastrum sp.* *Melosira varians*	*Aulacoseira sp. (italica/granulata)* *Cryptomonas sp.* *Fragilaria sp.* *Planktothrix sp.*

Table S9.2.2: Means (± standard deviation) of the variables (environmental parameters, biomass and functional traits) over all dates and results of the generalized additive models (GAM) giving the deviance explained (Dev. exp.) in per cent and the statistical significance judged by F tests (p-value), for the eutrophic and oligotrophic period separately. The p-value was <0.001 for all variables.

	Eutrophic period (1970-1990)		**Oligotrophic period (1996-2016)**	
Variables	**Overall mean (±SD)**	**Dev. exp. (%)**	**Overall mean (±SD)**	**Dev. exp. (%)**
Phytoplankton biovolume (mg L^{-1})	1.280 (2.812)	43.2	0.680 (1.473)	4.58
Water temperature (°C)	10.97 (5.068)	94.6	12.33 (6.151)	90.9
SRP (mg L^{-1})	0.015 (0.009)	42.7	0.004 (0.004)	7.03
NO_3 (mg L^{-1})	6.669 (1.274)	31.3	4.987 (0.829)	57.4
Silica (mg L^{-1})	1.725 (1.046)	76.4	2.120 (0.795)	50.4
Oxygen (mg L^{-1})	10.60 (1.556)	82.2	9.755 (1.327)	79.3
pH	7.612 (0.628)	42.1	7.422 (0.440)	11.9
Secchi depth (m)	3.669 (1.467)	3.6	3.490 (0.838)	14.0
Size of individual cells (μm^3)	3148.7 (6932.5)	15.4	4494.9 (7435.8)	15.4
Phosphate affinity ($L \cdot \mu mol^{-1} \cdot d^{-1}$)	130.89 (119.81)	32.6	241.11 (233.17)	13.9

Light affinity (µmol quanta^{-1} m^2 s day^{-1})	0.031 (0.006)	29.3	0.02 (0.005)	15.7
Maximum growth rate (d^{-1})	0.827 (0.114)	33.4	0.764 (0.152)	12.5
Silica use (fraction)	0.808 (0.297)	46.4	0.626 (0.301)	13.9
Motility (fraction)	0.113 (0.220)	25.2	0.288 (0.268)	15.6
Mixotrophy (fraction)	0.027 (0.108)	5	0.194 (0.209)	9.57
Buoyancy (fraction)	0.051 (0.173)	17.2	0.0711 (0.184)	3.13
Nitrogen fixation (fraction)	0.047 (0.168)	15.4	0.022 (0.087)	12.6
Chain and colony (fraction)	0.744 (0.306)	48.9	0.67 (0.251)	22.8
Edibility for *Daphnia*	0.394 (0.179)	18.9	0.316 (0.078)	11.5

9.3 Supporting information for chapter 4 (S9.3)



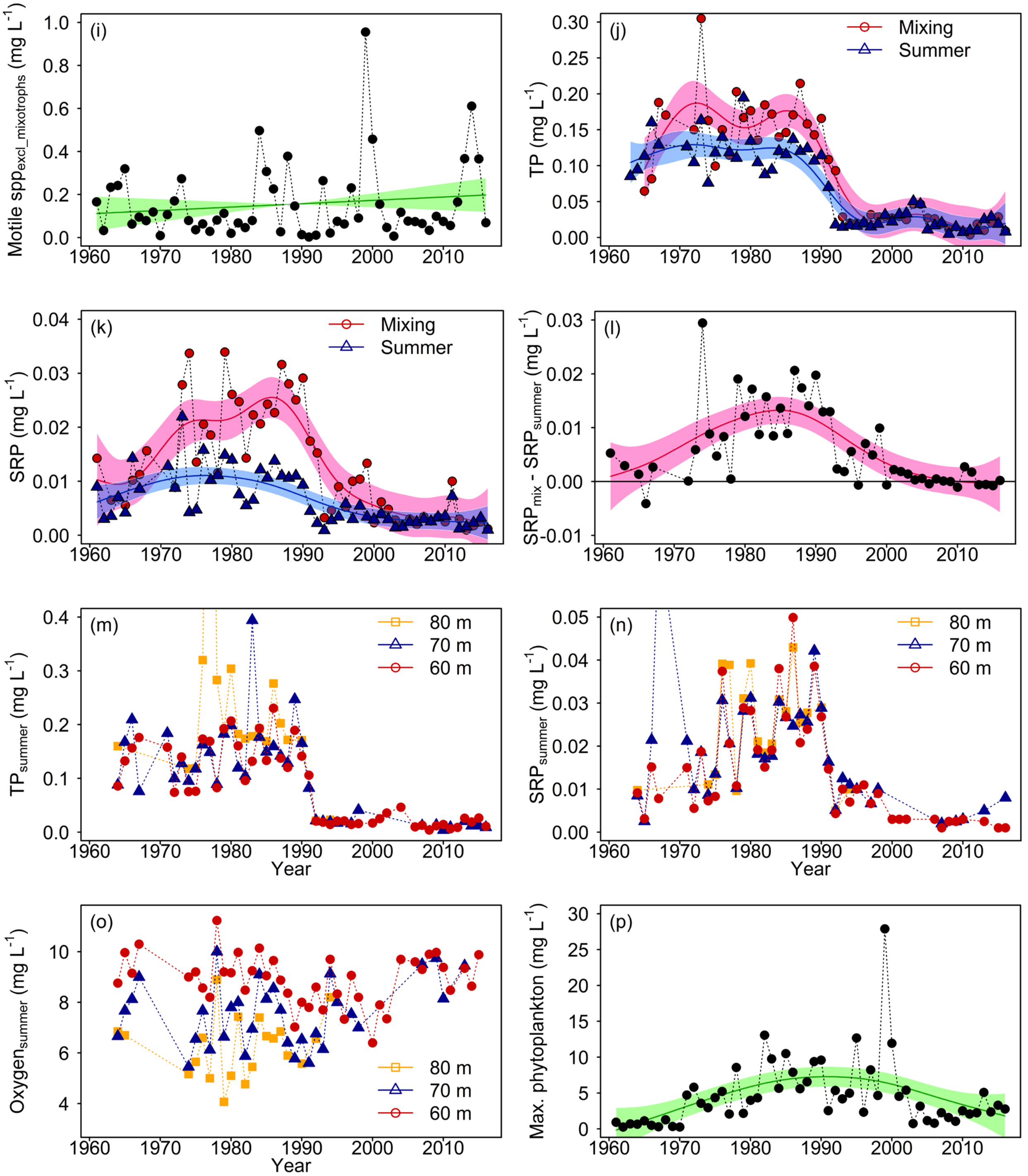


Figure S9.3.1: Long-term development of light conditions, water temperature, stratification onset and phytoplankton in the Rappbode Reservoir. The solid, colored lines are the smoothers from the generalized additive models fitted to the data and the shades are the confidence intervals of these fits. (a) Annual mean Secchi depth. (b) Annual mean surface temperatures. (c) Mean surface temperatures during spring. (d) Mean surface temperatures *during summer. (e) Mean surface temperatures during summer in the hypolimnion. (f) Onset of stratification (Day of Year).(g) Mean phytoplankton biomass in spring (red) and in summer (blue). (h) Annual mean yield of phytoplankton biomass per unit of phosphorus, excluding mixotrophic species. (i) Annual mean biomass of motile species, excluding mixotrophic species. (j) Mean concentrations of total phosphorus during mixing (red) and during summer (blue). (k) Mean concentrations of soluble reactive phosphorus during mixing (red) and during summer (blue). (l) Difference in soluble reactive phosphorus concentrations between the mixing and the summer period. (m) Mean total phosphorus concentrations in summer in the hypolimnion (60 m, 70 m and 80 m depth). (n) Mean soluble reactive phosphorus concentrations in summer in the hypolimnion (60 m, 70 m and 80 m depth). (o) Mean oxygen concentrations in summer in the hypolimnion (60 m, 70 m and 80 m depth). (p) Maximum phytoplankton biomass.*

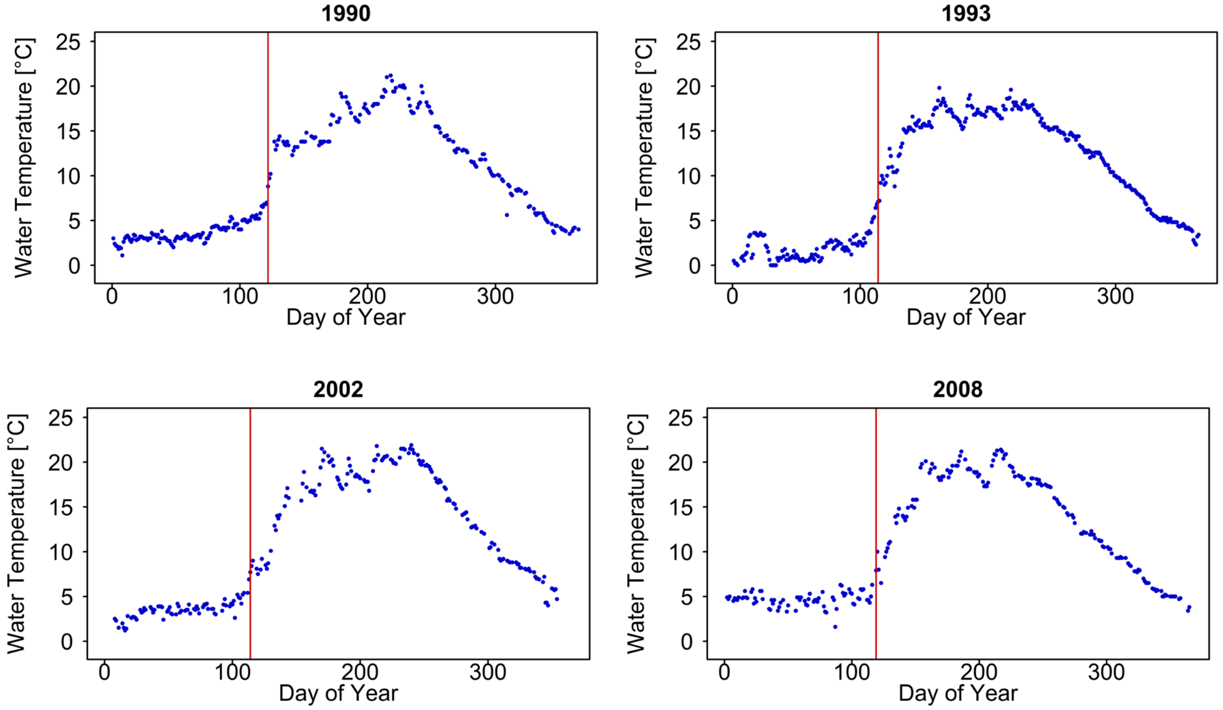


Figure S9.3.2: Examples for the surface temperature development over the season. The red line indicates the calculated day of stratification onset.

Table S9.3.1: Five most abundant genera or species of diatoms in spring, mixotrophic species and motile species excluding mixotrophs during different decades.

Years	Diatoms$_{spring}$	Mixotrophs	Motile species excluding mixotrophs
1961-1970	*Asterionella formosa* *Melosira* sp. *Fragilaria* sp. *Diatoma* sp. *Tabellaria fenestrata*	*Peridinium* sp. *Dinobryon* sp. *Gymnodinium* sp. *Uroglena americana*	*Ceratium hirundinella* *Mallomonas* sp. *Microcystis sp.* *Aphanizomenon fl.a.* *Stephanoon wallichii*
1971-1980	*Asterionella formosa* *Stephanodiscus hantzschii* *Fragilaria* sp. *Cyclotella* sp. *Nitzschia* sp.	*Dinobryon* sp. *Peridinium* sp. *Cryptomonas* sp. *Gymnodinium* sp.	*Stephanoon wallichii* *Ceratium hirundinella* *Mallomonas* sp. *Aphanizomenon.fl.a.* *Microcystis* sp.
1981-1990	*Asterionella formosa* *Stephanodiscus hantzschii* *Diatoma* sp. *Fragilaria* sp. *Melosira* sp.	*Peridinium* sp. *Cryptomonas* sp. *Dinobryon* sp.	*Mallomonas* sp. *Stephanoon wallichii* *Aphanizomenon fl.a.* *Ceratium hirundinella* *Microcystis* sp.
1991-2000	*Asterionella formosa* *Stephanodiscus hantzschii* *Fragilaria* sp. *Diatoma* sp. *Melosira* sp.	*Cryptomonas* sp. *Gymnodinium* sp. *Dinobryon* sp. *Peridinium* sp.	*Planktothrix* sp. *Rhodomonas* sp. *Chloromonas* sp. *Ceratium hirundinella* *Stephanoon wallichii*
2001-2010	*Asterionella formosa* *Tabellaria fenestrata* *Fragilaria* sp. *Diatoma* sp. *Cyclotella* sp.	*Cryptomonas* sp. *Peridinium* sp. *Dinobryon* sp. *Gymnodinium* sp. *Pseudopedinella* sp.	*Synura* sp. *Ceratium hirundinella* *Rhodomonas* sp. *Mallomonas* sp. *Planktothrix* sp.
2011-2016	*Tabellaria fenestrata* *Asterionella formosa* *Urosolenia longiseta* *Diatoma* sp. *Fragilaria* sp.	*Dinobryon* sp. *Gymnodinium* sp. *Peridinium* sp. *Cryptomonas* sp. *Pseudopedinella* sp.	*Synura* sp. *Planktothrix* sp. *Rhodomonas* sp. *Ceratium hirundinella* *Aphanizomenon fl.a.*

9.4 Supporting information for chapter 5 (S9.4)

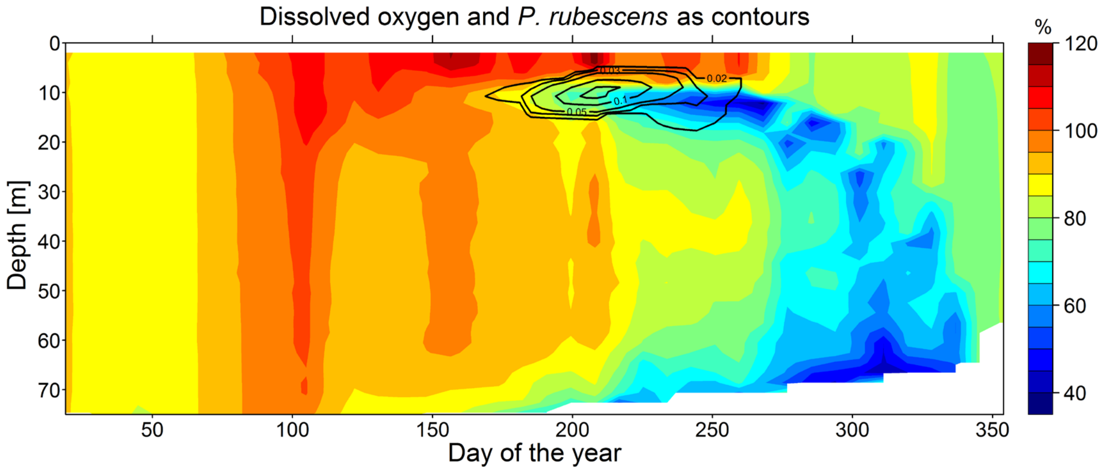


Figure S9.4.1: Seasonal and vertical development (depth vs. time) during the year 2016 of dissolved oxygen (%). Contour lines indicate the biovolume of P. rubescens, obtained by microscopic cell counts (mg L^{-1}).

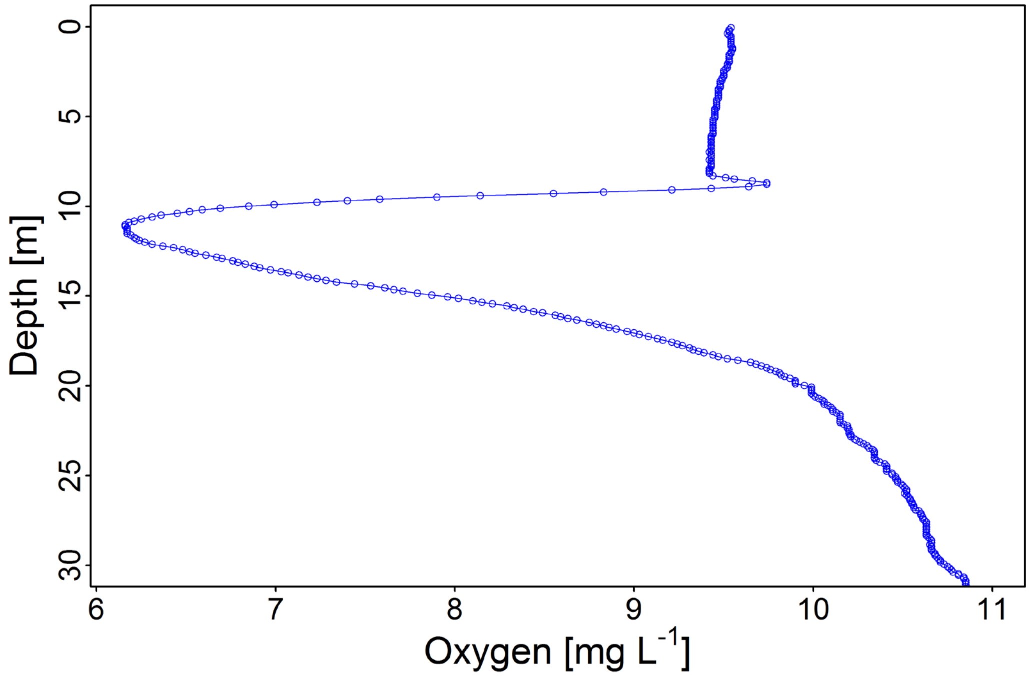


Figure S9.4.2: Example of an oxygen profile (23th August 2016), where a local oxygen maximum was observed, directly above the metalimnetic oxygen minimum.

Table S.9.4.1: Probes and sensors used in this study, including information about their characteristics as given by the manufacturer.

Probe	Sensors for /Discrimination into	Sensor names	Excitation wavelength (for chl-a sensors)	Detected wavelength (for chl-a sensors)	Concentration range	Resolution	Uncertainty/ Accuracy	Detection limit	Can *P. rubescens* be detected?	Data used in	Source
Multiparameter probe: CTD90M, Sea and Sun Technology, Trappenkamp, Germany, serial number: 644	Chlorophyll a fluorescence	Cyclops 7, model number: 2100-000	460 nm	620-715nm	0 – 500 µg/L Chl a			0.03 µg/L Chl a	No	Fig. 2g, Fig. 4a	http://www.turnerdesigns.com/t2/media/cyclopsds_3.pdf
	Temperature	PT100 4 pol			-2 - 36°C	0.0001 °C	± 0.002 °C			Fig. 2a and as contours in Fig. 4a-c	http://www.sea-sun-tech.com/fileadmin/img/pdf_sea/CTD90.pdf
	Electrical conductivity	7-pole platinum cell			0–7 mS/cm	0.0001 mS/cm	± 0.003 mS/cm			Fig. 2b	
	Pressure	piezo-resistive			0-10 bar	0.002 % full scale	± 0.1 % full scale			Fig. 2c	
	pH	single rod electr.			2–10 pH	0.0002 pH	± 0.02 pH			Fig. 2f	
	Dissolved oxygen	Rinko III			0 to 200%	0.01 to 0.04%	±2% of full scale (at 1 atm, 25 °C)			Fig. 2e, Fig. 3, Fig. 4d, Fig. 8b	https://www.jfe-advantech.co.jp/eng/ocean/pdf/RINKO%20Series(E)_201608.pdf
Multi-channel fluorescence probe: FluoroProbe, bbe moldaenke GmbH, Germany, serial number: 2101	Four algal groups: (1) Green algae (rich in chlorophyll a/b), (2) Diatoms/Dinoflagellates (containing xanthophyll and chlorophyll c), (3) phycocyanin-rich Cyanobacteria		370, 470, 525, 570, 590 and 610 nm	680nm	0 - 200 µg chl-a/l	0,01 µg chl-a/l			Yes, it is visible in the signal of the red (3) and the blue group (4)	Fig. 4b, 4c, Fig. 8a and as contours in Fig. 4d	https://www.bbe-moldaenke.de/de/produkte/chlorophyll/details/fluoroprobe.html

	and (4) phycoerythrin-rich Cyanobacteria and Cryptophytes										
Automatic vertical profiler with multiparameter probe: YSI 6820 V2-2-O	Dissolved oxygen	YSI 6150			0 to 500% 0 to 50 mg/L	0.1% 0.01 mg/L	0 to 200%: ±1% of reading or 1% air saturation, whichever is greater 0 to 20 mg/L: ± 0.1 mg/L or 1% of reading, whichever is greater			Fig. 5d	https://www.ysi.com/File%20Library/Documents/Specification%20Sheets/E36-6820-6920-V2.pdf
	Temperature	YSI 6560			-5 to +50°C	0.01°C	±0.15°C			Fig. 5a	
	Chlorophyll a fluorescence	YSI 6025	470 nm	670-700 nm	~0 to 400 µg/L, 0 to 100 RFU	0.1 µg/L Chl 0.1% RFU		~0.1 µg/L Chl a	No	Fig. 5b	
	Chlorophyll a fluorescence excited via phycocyanin for detection of cyanobacteria	YSI 6131	565-605 nm	620-700 nm	~0 to 280,000 cells/mL, 0 to 100 RFU	1 cell/mL 0.1 RFU		~220 cells/mL	Yes	Fig. 5c	
Multiparameter probe DS5, Hydrolab	Oxygen	Hach LDO			0 – 60 mg/L	0.01 mg/L	± 0.1 mg/L at <8 mg/L ± 0.2 mg/L at >8 mg/L ± 10% reading >20 mg/L			Fig. 7	http://www.ott.com/en-uk/products/sensors-108/hach-ldor-dissolved-oxygen-sensor-155/
	Temperature				-5 -50 °C	0.01 °C	± 0.10 °C			Fig. 7	http://www.ott.com/en-uk/products/sensors-108/temperature-sensor-167/

10 Individual contributions to the thesis

In the following, I give an overview of the author's contribution to each chapter.

Chapter 3: A manuscript containing the content and results of chapter 3 has been submitted for publication to Journal of Ecology under the title:

Wentzky, V.C., Tittel, J., Jäger, C.G., Bruggeman, J., Rinke, K.: Seasonal succession of functional traits in phytoplankton communities and their interaction with trophic state. *Under review in Journal of Ecology*.

Valerie C. Wentzky (first author)	Developed the research questions for the paper Analysed the data and made all figures and tables Interpretation of results Composed and wrote the manuscript
Jörg Tittel (Co-author)	Developed the research questions for the paper Interpretation of results Corrected and improved the manuscript
Christoph G. Jäger (Co-author)	Developed the concept for the project Corrected and improved the manuscript
Jorn Bruggeman (Co-author)	Extended and improved the PhyloPars model and provided the resulting trait values

	Wrote Supporting information 1 for chapter 3 (S9.1)
Karsten Rinke (Co-author)	Developed the concept for the project and the research questions for the paper Interpretation of results Corrected and improved the manuscript
Wasserwerk Wienrode (Jan Donner and Wolf-Dieter Skibba, external partners of the project and not author of the paper)	Provided phytoplankton community and environmental data and gave information about the methods of data collection
Talsperren Betrieb Sachsen-Anhalt (Detlef Cöster, external partner of the project and not author of the paper)	Provided daily surface water temperature data

Chapter 4 has already been published with minor alterations under the title:

Wentzky, V.C., Tittel, J., Jäger, C.G., Rinke, K. (2018): Mechanisms preventing a decrease in phytoplankton biomass after phosphorus reductions in a German drinking water reservoir – results from more than 50 years of observation. *Freshwater Biology, 63(9), 1063-1076*.

The chapter differs from the final published article in the numbering of tables and figures, which were adapted to the structure of the thesis.

Valerie C. Wentzky (first author)	Developed the research questions for the paper

	Analysed the data and made figures and tables Interpretation of results Composed and wrote the manuscript
Jörg Tittel (Co-author)	Developed the research questions for the paper Interpretation of results Corrected and improved the manuscript
Christoph G. Jäger (Co-author)	Developed the concept for the project Corrected and improved the manuscript
Karsten Rinke (Co-author)	Developed the concept for the project and the research questions for the paper Interpretation of results Corrected and improved the manuscript
Wasserwerk Wienrode (Jan Donner and Wolf-Dieter Skibba, external partners of the project and not author of the paper)	Provided phytoplankton community and environmental data and gave information about the methods of data collection

Talsperren Betrieb Sachsen-Anhalt (Detlef Cöster, , external partner of the project and not author of the paper)	Provided daily surface water temperature data
Philipp Keller	Prepared the bathymetric map (figure 4.1)

Chapter 5 has already been published with minor alterations under the title:

Wentzky, V.C., Frassl, M.A., Rinke, K., Boehrer, B. (2019): Metalimnetic oxygen minimum and the presence of Planktothrix rubescens in a low-nutrient drinking water reservoir. *Water Research, 148, 208-218*.

The chapter differs from the final published article in the numbering of tables and figures, which were adapted to the structure of the thesis.

Valerie C. Wentzky (first author)	Developed the research questions for the paper Designed, organized and conducted field work for the one-year biweekly monitoring campaign in 2016 Helped with the installation of the automatic profiling station Organized chlorophyll-a analysis and helped in the laboratory Organized and helped with the determination of phytoplankton samples under the microscope Analysed relationship between chlorophyll measured by the multi-parameter probe and HPLC samples Calculation of oxygen budget

	Prepared figures 5.4, 5.6, 5.7 and 5.8 Interpretation of results Composed and wrote the manuscript
Marieke A. Frassl (Co-author)	Developed the research questions for the paper Helped with the installation of automatic profiling station Prepared figure 5.5 Interpretation of results Corrected and improved the manuscript
Karsten Rinke (Co-author)	Interpretation of results Corrected and improved the manuscript
Bertram Boehrer (Co-author)	Initiated the project Developed the research questions for the paper Designed, organized and conducted field work for the transversal and longitudinal transects measured on 10th September 2015 Calculated square buoyancy frequency Prepared figures 5.1 and 5.2

	Interpretation of results Contributed to writing the manuscript
Karsten Rahn and Martin Wieprecht	Conducted field work for the transversal and longitudinal transects measured on 10th September 2015 Conducted field work for the one-year biweekly monitoring campaign in 2016
Burkhard Kühn	Installed high-frequency automatic profiling station
Wasserwerk Wienrode (Jan Donner, external partner of the project and not author of the paper)	Provided phytoplankton count data and oxygen profile data from 2009 to 2016
Kerstin Lerche	Conducted chlorophyll-a analysis in the laboratory
Philipp Keller	Prepared the bathymetric map (figure 5.1)
Erika Ruschak	Analysed phytoplankton samples under the microscope