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Ecosystem stability and regime shifts in interconnected shallow lakes

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“We can’t always see where the road leads. But, GOD promises there is something better up ahead, we just have to trust HIM.”

Contents

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General introduction

1. Lakes and Ponds

Lentic freshwater systems such as shallow lakes and ponds are abundant landscape elements (Beeton, 2002; Downing, 2010; Carpenter, Stanley, & Vander Zanden, 2011) that provide a variety of vital ecosystem services, including water supply, carbon sequestration, nutrient retention, food production and water purification. In addition, they contribute disproportionally to biodiversity at multiple spatial scales (Ormerod et al., 2010; Céréghino et al., 2014; Hermoso et al., 2016; Maes et al., 2016). Yet, they belong to the most threatened ecosystems on earth (Dudgeon et al., 2006; Ormerod et al., 2010; Vörösmarty et al., 2010; Schuler & Relyea, 2018). Many shallow lakes and ponds are located in densely populated areas, which makes them especially vulnerable to human-induced ecosystem alteration and land-use change (Declerck et al., 2006; Céréghino et al., 2008; Schindler, Hecky, & McCullough, 2012; Moss, 2013). Habitat destruction and eutrophication have worldwide been identified as major drivers for the observed severe ecological degradation of many freshwater ecosystems. Ponds and shallow lakes are ideal model systems for ecological research (De Meester et al., 2005). Lakes and ponds are generally abundant throughout the world (Verpoorter et al., 2014), which allows carrying out field surveys and experiments covering large spatial and ecological gradients. They also have well-delineated habitat boundaries in a terrestrial habitat matrix, which makes lentic waterbodies excellent model systems for quantitative research on metacommunity ecology. In addition, their relative small size facilitates repeatable representative quantitative sampling, allowing a reliable quantification of biodiversity and ecosystem functioning (Jeppesen et al., 1997). Despite having relatively simple biotic communities, pond and shallow lakes communities show almost all characteristics of complex ecosystems, making them highly interesting systems to use in mesocosm experiments (De Meester et al., 2005).

A simplified representation of the food web in shallow lakes and ponds typically consists of four trophic levels: top predators (piscivorous fish), intermediate predators (planktivorous fish and planktivorous invertebrates), herbivores (zooplankton) and primary producers (phytoplankton) (Carpenter & Kitchell, 1996, Figure 1). Different trophic levels are directly and indirectly linked by multiple food web interactions.

Zooplankton plays a central role in the structure and the functioning of lake and pond food webs as they are major grazers on phytoplankton, contribute to nutrient recycling, and represent an important food source for many predators. Multiple studies clearly demonstrate that zooplankton can suppress algae growth and phytoplankton blooms (Gerasimova, Pogozhev, & Sadchikov, 2018; Iacarella et al., 2018), and this is especially the case for large bodied taxa such as *Daphnia* (Lampert & Sommer, 2007; Verreydt et al., 2012; Gianuca, Pantel, & De Meester, 2016). Predation by fish is an important determinant of variation in zooplankton and phytoplankton community characteristics in lakes and ponds (Carpenter et al., 2001; Jeppesen et al., 2003). Fishes are positive size selective predators that do not only affect the biomass of their prey communities, but also determine qualitative characteristics of prey communities such as their size distribution, species composition and taxon richness (Cottenie & De Meester, 2003; Declerck & De Meester, 2003; Lampert et al., 2007; Lemmens et al., 2018). Selective predation by fish also affects prey populations, influencing age distributions, life history characteristics, habitat use, sex ratios and behavior (Lampert et al., 2007; Latta et al., 2007), as well as micro-evolutionary trajectories (Cousyn et al., 2001; Stoks et al., 2016). Predation by fish might indirectly also affect the abundance and biomass of primary producers through tropic cascades (Carpenter, Kitchell, & Hodgson, 1985; Carpenter & Kitchell, 1996).

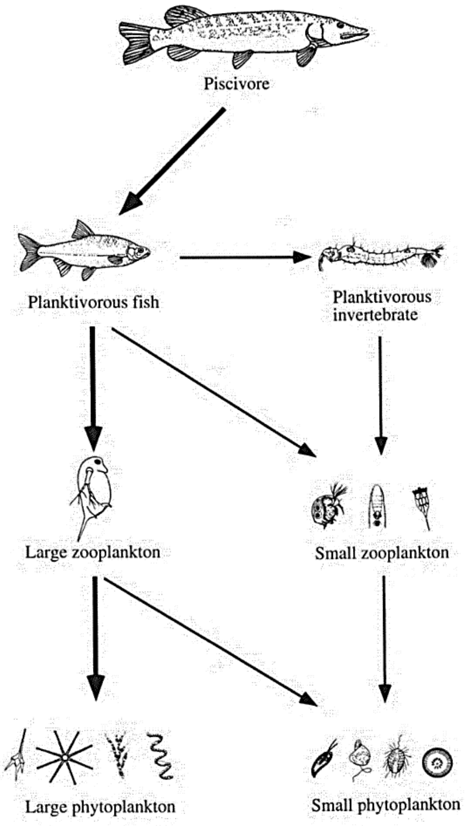

Figure1. A simple representation of a typical shallow lake food web. The size of the arrows indicates the strength of the predatory pressure (from Carpenter and Kitchell, 1996).

2. Ecosystem stability

Ecosystem stability has become a central research topic in ecology and environmental management during the last decades (Donohue et al., 2013; Donohue et al., 2016). Ecosystem stability can be defined as the ability of an ecosystem to resist changes in the presence of disturbances (Rockström et al., 2009; Pereira, Navarro, & Martins, 2012; Donohue et al., 2013), and clearly is a multidimensional concept that captures different aspects of ecosystem dynamics and its responses to perturbations (Pimm, 1984;

Ives & Carpenter, 2007; Donohue et al., 2013). Most ecologists describe ecosystem stability as the ability of an ecosystem to maintain its structure and functioning over longer time periods despite the occurrence of disturbances.

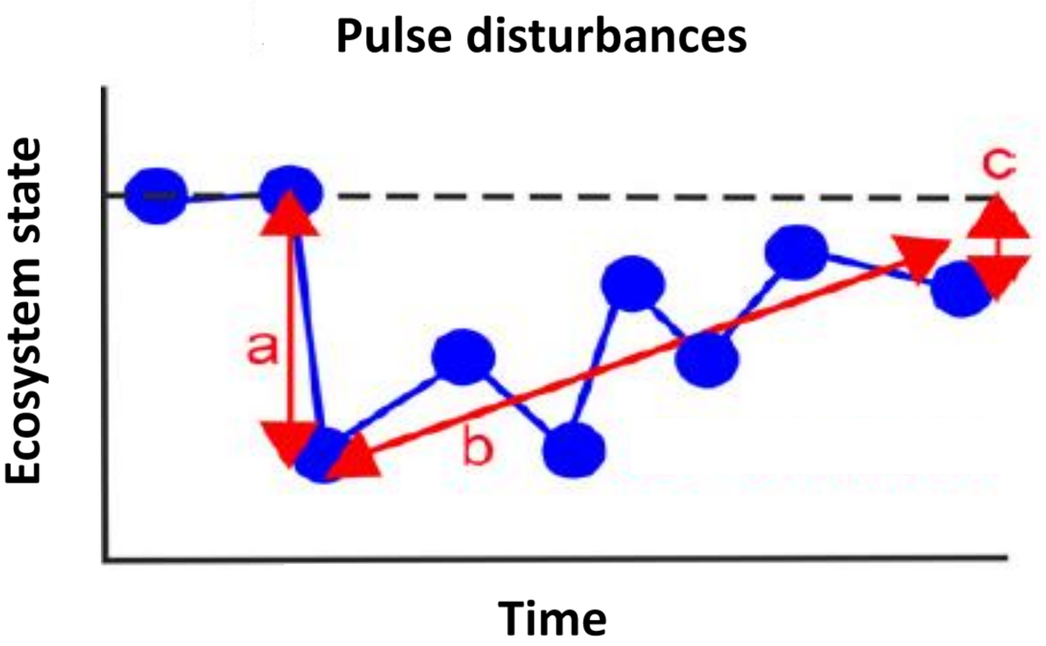

Figure 2. Schematic representation of key components of stability. Arrow a captures resistance, arrow b captures resilience, and arrow c captures recovery. For resistance and recovery, the length of the arrow is inversely related to the measure of stability. The slope of arrow b quantifies resilience (from Hillebrand et al., 2018).

The key concepts specifying ecological stability include resilience, recovery rate and resistance (Pimm, 1984; Donohue et al., 2013; Hillebrand et al., 2018; van de Leemput et al., 2018; Figure 2). Resistance describes the degree to which an ecosystem state or ecosystem characteristic is immune to change upon perturbation (Pimm, 1984; Vogel, Scherer-Lorenzen, & Weigelt, 2012; Donohue et al., 2013). Resilience can be defined as the capacity and speed of the system to return towards its equilibrium following a perturbation (Pimm, 1984). Recovery refers to the degree to which the system returns to its pre-disturbance state following a perturbation (Pimm, 1984; van de Leemput et al., 2018). A higher resistance, resilience and recovery all increase ecosystem stability. Most theoretical, experimental and empirical studies quantify stability as the inverse of temporal variability, directly estimated on time series data (Tilman et al., 2006; Jiang & Pu, 2009; Campbell et al., 2011; Donohue et al., 2013). More specifically, a higher stability translates into a lower coefficient of variation of an ecosystem property over time (Donohue et al., 2013).

Ecological stability can be measured at multiple levels of ecosystem organization (ecosystem, community, population, and individual) and spatial scales (local community and ecosystem *versus* metacommunity and meta-ecosystem). Importantly, stability at one level of organization can imply or even be achieved through instability at other organization levels. For example, an ecosystem characteristic such as total biomass might be stable over time because community composition is dynamic as the environment changes. Similarly, community biomass can be stable due to differential dynamics of individual populations (Donohue et al., 2013), while evolutionary dynamics and temporal variation in genotype abundances can foster stability at the population level (Maebe et al., 2016) (see

Box 1. Eco-evolutionary dynamics

Ecological and evolutionary dynamics have long been considered as largely uncoupled and independent processes. More recently, it has become increasingly clear that both processes are strongly intertwined and can occur on the same time scales (Hairston et al., 2005; Whitham et al., 2006; Ellner, Geber, & Hairston, 2011; Schoener, 2011; Hendry, 2016). An increasing number of studies unequivocally demonstrate the existence of important feedbacks between evolutionary change and ecological dynamics (Bassar et al., 2010; Matthews et al., 2011; Pantel, Duvivier, & Meester, 2015). For example, genetic diversity can profoundly alter population, community, and ecosystem characteristics (Crutsinger et al., 2006; Whitham et al., 2006; Johnson, Vellend, & Stinchcombe, 2009). Evolutionary trait change can mediate changes in population dynamics, community composition (Urban et al., 2008; Bassar et al., 2010; Matthews et al., 2011; Terhorst, Lennon, & Lau, 2014; Pantel et al., 2015), and ecosystem functions (Fussmann, Loreau, & Abrams, 2007). Evolutionary dynamics can also mediate ecosystem stability (van Moorsel et al., 2018). This insight led to an upsurge in research interest on how ecological and evolutionary dynamics interact.

also Box 1). The latter is an example of how evolutionary dynamics can contribute to ecosystem stability.

The relationship between biodiversity and ecosystem stability has stimulated much scientific debate (McCann, 2000; Ives et al., 2007; Loreau & Mazancourt, 2013). A rapidly growing number of studies indicate that biodiversity can promote ecosystem stability through multiple mechanisms (Tilman, Reich, & Knops, 2006; Downing et al., 2012; Lefcheck et al., 2015; Wang & Brose, 2018). Functional redundancy and asynchrony of species' responses to environmental fluctuations have long been considered the major factors that drive the positive effect of biodiversity on ecosystem stability (e.g. Insurance Hypothesis; Yachi & Loreau, 1999). Recent investigations suggest that differences in speed at which species respond to perturbations and reduction in the strength of competition with increasing diversity can also be important drivers for ecosystem stability (Loreau et al., 2013).

3. Regime Shifts, Alternative Stable States and Early Warning Indicators

The occurrence of alternative stable states and regime shifts in ecosystems is increasingly reported and receives growing attention in ecology (Scheffer et al., 2009). A vast body of studies clearly demonstrate the occurrence of alternative stable states in multiple types of ecosystems, including oceans (Knowlton 1992; Hare & Mantua, 2000), freshwaters (Scheffer & van Nes, 2007; Ramstack et al., 2016), forests (Liu et al., 2018), coral reefs (Hempson et al., 2018), rangelands (Reeves et al., 2018), and agro-ecosystems (Gordon, Peterson, & Bennett, 2008). Although the precise mechanisms that result in the occurrence of alternative stable states may vary between different types of ecosystems, a general characteristic is that stable states largely depend on multiple positive and negative feedback mechanisms that structure the dynamics and functioning of the food web (Scheffer et al., 2009). Regime shifts can occur when the organization of ecosystems changes profoundly in response to perturbations that lead to the breakdown of internal feedback mechanisms.

Alternative stable states and regime shifts are intrinsically related to ecological resilience theory (Holling, 1973; May, 1977; Scheffer et al., 2001). It is the fact that ecosystems show strong resilience in the face of environmental perturbation combined with a sudden breakdown of the mechanisms that underpin this resilience when a threshold in environmental perturbation is surpassed that leads to a sudden regime shift from one stable state to another. Ecological resilience theory suggests that regime shifts are preceded by subtle changes in ecosystem behavior that can be detected using quantifiable indicators or early warning signals. Since regime shift are common and frequently lead to severe ecological and economic losses, an increasing number of studies has suggested the use of generic early warning signals or leading indicators that can detect the proximity of a system to a critical transition. Identifying early warning indicators that signal a system may be in danger of shifting to an alternative state has thus been a primary focus of research into regime shifts (e.g., Scheffer et al., 2009; Carpenter, Cole, et al., 2011; Graham et al., 2015; Hicks et al., 2016). Critical transitions are defined as the abrupt qualitative changes in the state of an ecosystem that occur close to bifurcation points (Kuehn, 2011). Critical transitions are characterized by the occurrence of alternative regimes under the same environmental conditions and by abrupt, discontinuous transitions between regimes when a critical threshold is exceeded. As each regime is stabilized by feedback loops, the thresholds for the forward and backward shifts may differ, resulting in hysteresis (Scheffer et al., 2001). Early warning indicators (EWIs) have been shown to precede critical transitions in modeled time series (Carpenter et al., 2008; Dakos et al., 2012), experimental time series (Dai et al., 2012; Veraart et al., 2012), reconstructed paleo-climate record (Dakos et al., 2008; Lenton et al., 2012) and whole-lake experiments (Carpenter et al., 2001).

EWIs are statistical metrics that quantify the loss of temporal or spatial resilience and thereby provide advance warning of the potential proximity to a critical threshold (Scheffer et al., 2009). Several EWIs are related to critical slowing down, a characteristics property of dynamic systems close to catastrophic local bifurcations (Van Nes & Scheffer, 2007). A bifurcation marks a threshold at which the stabilizing properties of the system change. As the system approaches such a threshold, the return rate to equilibrium after a small perturbation slows, so that the system tends to become more similar to its own past, resulting an increase in autocorrelation at lag-1 (AR-1) (Ives, 1995).

Abrupt changes in the state of an ecosystem can develop from several mechanisms, including (i) linear tracking of large and abrupt changes in environmental conditions, (ii) nonlinear but continuous (reversible) responses to gradual changes in environmental conditions, and(iii) nonlinear discontinuous (irreversible) responses to gradual changes in environmental conditions (Andersen et al., 2009). Whereas the first mechanism is distinguished by concurrent large changes in environmental drivers (e.g. sudden increase in nutrients), the difference between the other two mechanisms becomes apparent only when the driver is reversed.

Early warning signals are temporal and spatial statistical signatures of the phenomenon of critical slowing down (CSD) that arises in the vicinity of bifurcations (Carpenter & Brock, 2006; Van Nes et al., 2007; Drake & Griffen, 2010). Critical slowing down can be interpreted as an indication of low resilience and is characterized by a reduction in the speed of ecological recovery after disturbance as an ecosystem approaches a critical threshold (Scheffer et al., 2012; Dakos et al., 2015). Increased variance and autocorrelation are critical slowing down indicators that can be used to estimate the loss of ecosystem resilience.

The most straightforward implication of critical slowing down is that the recovery rate after a small perturbation can be used as an indicator of how close a system is to a bifurcation point. For most natural systems, it would be impractical or impossible to monitor them by systematically testing recovery rates. However, almost all real systems are permanently subject to natural perturbations. One important prediction is that the slowing down should lead to an increase in autocorrelation in the resulting pattern of fluctuations (Figure 3H). Because slowing down causes the intrinsic rate of change in the system to decrease, the state of the system at any given moment becomes more and more like its past state. The resulting increase in memory of the system can be measured using indicators. The simplest approach is to look at lag-1 autocorrelation (Carpenter et al., 2006; Kuehn, 2011), which can be directly interpreted as slowness of recovery upon natural perturbation.

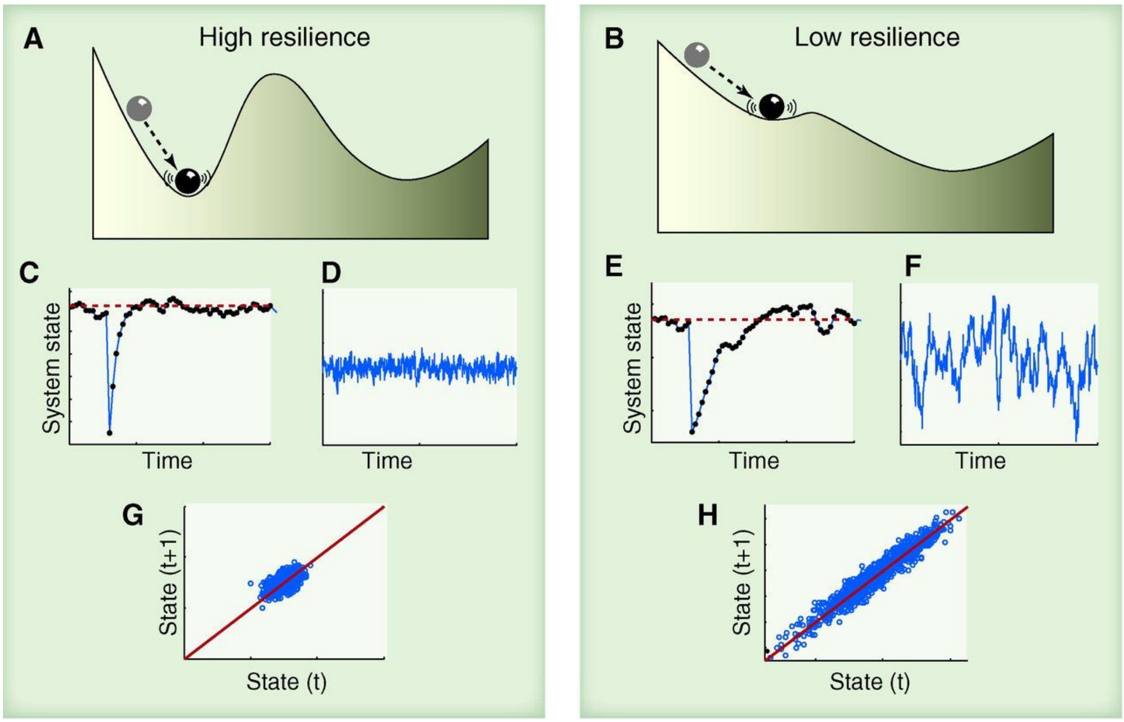

Figure 3. Critical slowing down as an indicator that the system has lost resilience and may therefore be tipped more easily into an alternative state. Recovery rates upon small perturbations (C and E) are slower if the basin of attraction is small (B) than when the attraction basin is larger (A). The effect of this slowing down may be measured in stochastically induced fluctuations in the state of the system (D and F) as increased variance and “memory” as reflected by Lag-1 autocorrelation (G and H). In a system far from the bifurcation point (A), resilience is high in two respects: the basin of attraction is large and the rate of recovery from perturbation is relatively high. If such system is stochastically forced, the resulting dynamics are characterized by low correlation between the states at subsequent time intervals (D, G). When a system is closer to the transition point (B), resilience decreases in two senses: the basin of attraction shrinks and the rate of recovery from small perturbation is lower. As a consequence of this slowing down, the system has a longer memory for perturbations, and its

dynamics in a stochastic environment are characterized by a larger variance and stronger correlation between subsequent states (F, H), (from Scheffer et al., 2012).

4. Regime Shifts and Alternative Stable States in shallow lakes

Ponds and shallow lakes have been central to the development of the concept of alternative stable states and regime shifts in ecosystems (Scheffer et al., 1993; Søndergaard et al., 2007; Kéfi et al., 2013) and they are excellent model systems for studying regime changes (Scheffer et al., 2009) and to test for EWIs associated with critical transitions (Carpenter & Kinne, 2003). Ponds and shallow lakes are characterized by the occurrence of two distinct alternative stable states: a clear-water state and a turbid-water state. The clear-water state is characterized by a high coverage with submerged vegetation, low phytoplankton density and relative high zooplankton grazing rates, whereas the turbid state is characterized by dominance of phytoplankton, the lack of submerged vegetation, and relative low zooplankton grazing rates (Scheffer et al., 1993). The interaction between submerged plants and water turbidity is the major stabilizing mechanism of both alternative stable states (Figure 4). Submerged aquatic plants stabilize the clear-water state by promoting high water transparency via stabilizing the sediments, increasing sedimentation rates of particles, suppression of phytoplankton through competition for resources and allelopathy, and enhancing grazing rates on phytoplankton by providing shelter to zooplankton against predation by fish (Timms & Moss, 1984; Van Donk et al., 1990; Jeppesen et al., 2012; Vanderstukken et al., 2014; Figure 4; Kéfi, Holmgren, & Scheffer, 2016). The extent to which lakes and ponds occur in a clear-water or turbid state strongly depends on the nutrient status. A clear-water state generally dominates under oligotrophic to mesotrophic conditions, while the likelihood of a turbid state increases as nutrient loads increase. An increase in nutrient concentrations can enhance phytoplankton production through bottom-up effects. This reduces light availability in the water column, which subsequently results in the disappearance of submerged macrophytes, because macrophytes need light to grow. Disappearance of submerged vegetation leads to a breakdown of the above mentioned feedback mechanisms stabilizing the clear-water state (Figure 4) and thus leads to a shift from a clear-water to a turbid state. Once in the turbid state, nutrient loading needs to reduce to much lower values compared to the threshold at which a regime shift to the turbid state occurred before the system will spontaneously revert to a clear-water state. This is because the turbid state is stabilized by the fact that an algal bloom induces light limitation reducing establishment of macrophytes. As also the turbid state is stabilized (phytoplankton dominance leading to suppression of macrophytes, Figure 4), the system shows hysteresis.

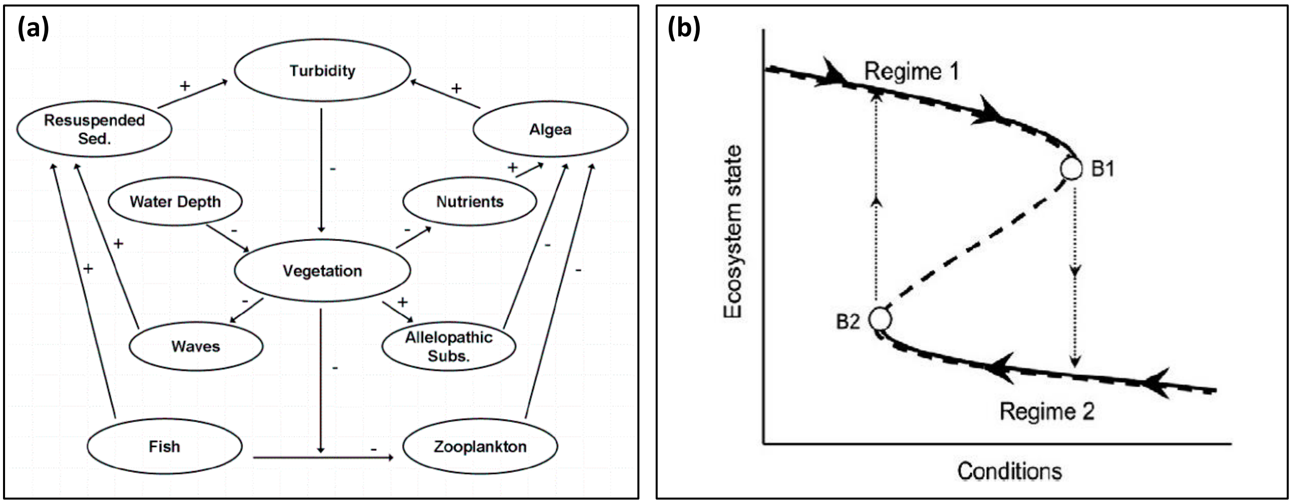

Figure 4. (a) A scheme providing an overview of the multiple positive and negative feedback mechanisms that maintain the two alternative stable states in shallow lakes and ponds (from Scheffer *et al.*, 1993). A minus-sign next to an arrow denotes a negative effect of one variable on another, whereas a plus-sign denotes a positive effect. Feedback loops are negative when the product of all signs along a loop is negative. (b) Graphical illustration of hysteresis depicting path dependence along a trajectory of change in ecosystem state. If the system is in regime 1 close to bifurcation point B1, an incremental change in conditions may induce a discontinuous shift to the alternative state, or regime 2 (down arrow). If one then tries to restore regime 1 by reversing the conditions, the system shows hysteresis. A return shift to regime 1 only occurs if conditions are reversed far enough to reach the other bifurcation point, B2 (up arrow) (from Scheffer et al., 2001).

A tipping point in the context of clear-water and turbid states in shallow lakes corresponds to a critical level an external condition (for example nutrient inflow) where the lake shifts to an alternative state (Lenton et al., 2008). Multiple statistical methods for detecting changes in ecological stability of shallow lakes have been developed and tested. For example, an increase in autocorrelation or variance in phytoplankton biomass due to critical slowing down or flickering seems to precede the transition from a clear-water state to turbid-water state in shallow lakes and ponds (Figure 5). Laboratory and field experiments indeed suggested that generic early warning indicators (i.e. variance, skewness and autocorrelation at lag-1) may indeed be detected in time series of real systems prior to transitions (Drake et al., 2010; Figure 5; Carpenter, Cole, et al., 2011; Dakos et al., 2012). An important application of these methods is their potential real-time use as warnings of increased risk of upcoming transitions to the turbid state (Carpenter et al., 2006; Scheffer et al., 2009). To date, the performance of these methods has largely been tested using experimental data and simulations (Carpenter et al., 2014).

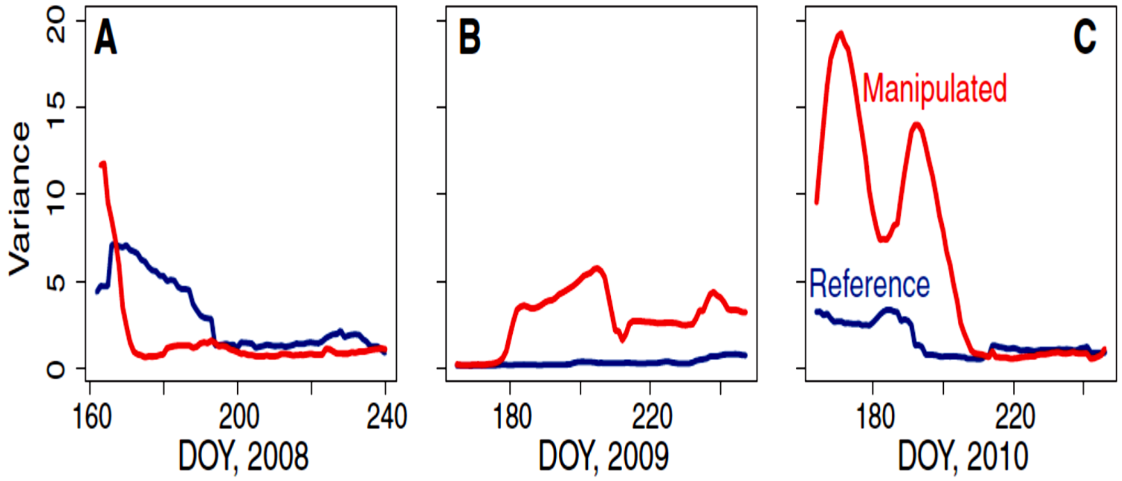

Figure 5. Potential early warning indicator computed from daily chlorophyll *a* time series in the manipulated (red) and control (blue) lakes in 2008, 2009, and 2010. High values of variance in chlorophyll *a* are associated with nonlinear transitions (from Carpenter et al., 2011).

5. Debate and open questions on Alternative Stable States in Shallow Lakes

While shallow lakes have been central to the development of the research on alternative stable states and regime shifts in ecosystems, in the recent decade an increasing number of papers have questioned the ubiquity of alternative stable states in shallow lakes and have suggested that not all variation in ecosystem characteristics in shallow lakes should be pushed in the framework of alternative stable states (Schröder, Persson, & De Roos, 2005). Several studies emphasized that gradients in ecosystem state in surveys of shallow lakes often seem rather smooth and gradual, and that strong shifts in ecosystem state are often linked to strong shifts in environmental condition (e.g. massive change in fish predation pressure or eutrophication event, etc.) (Jeppesen et al., 2000; McGowan et al., 2005; Bayley et al., 2007; Zimmer et al., 2009; Hanson et al., 2012). While the debate has been at times quite vivid, the many studies that link observations to alternative stable states and those that question the ubiquity of alternative stable states are likely not in conflict with each other, but rather emphasize different aspects of the whole spectrum of possibilities. While there is no doubt that some of the key mechanisms (linked to food web structure and the presence of macrophytes) studied in the context of alternative stable state, regime shifts and biomanipulation, are widespread and important in many shallow lakes and ponds, this does not imply that all lakes will regularly shift from one state to another or that all profound changes in ecosystem state reflect regime shifts.

Insight in how common regime shifts are in nature is important not only for ecology but also for developing ecosystem management that assures the provision of ecosystem services to humanity. We here identify three knowledge gaps that are linked to the detection and interpretation of sudden shifts in the state of shallow lakes and ponds (knowledge gap 1 and 2) and to the mechanisms underlying regime shifts in lakes:

- *Knowledge Gap 1*: Interpretation of variation in lake state in space. We often do not know how **stable** shallow lakes and ponds are in time. To what extent do the patterns that we find in nature reflect the co-occurrence in the landscape of systems that behave differently from each other because they are exposed to different environmental conditions rather than that they reflect different states of highly dynamic systems that show regular regime shifts?

- *Knowledge Gap 2*: Interpretation of **sudden changes** in regime state of lakes through time. We often do not know to what extent changes between the clear-water and turbid state in shallow lakes and ponds in time reflect regime shifts or rather responses to strong shifts in environmental conditions.
- *Knowledge Gap 3:* The role of *evolutionary trait change* in determining turbidity in lakes. The processes stabilizing the turbid and clear-water states of lakes (Figure 4) are mediated by both the abundances and the phenotype of organisms (McPeek, 2017). As a result, genetic changes in phenotype have the potential to impact shifts in lake state, but this aspect is understudied.

The first knowledge gap refers to a problem in literature, where the existence of both clear-water and turbid lakes in a survey of shallow lakes and ponds in a landscape is often interpreted as reflecting the occurrence of alternative stable states and regime shifts. While this interpretation might be correct, it is not necessarily so, as it may also reflect spatial differences in environmental conditions. It is necessary to sample the same lakes multiple times to assess to what degree the spatial patterns are informative on the dynamics in time. If the spatial pattern reflects true alternative states independent of environmental gradients, then variation in time within one system should reflect differences among systems in space. In reality, landscapes are often heterogeneous, so in nature part of the differences might reflect straightforward responses to environmental gradients, while some variation might reflect the occurrence of two alternative stable states under similar environmental conditions.

The second knowledge gap also refers to a problem in literature, where sudden shifts from a turbid to a clear-water state or *vice versa* are often interpreted as reflecting regime shifts. Again, while this interpretation can be correct, it might also be wrong, as sudden shifts in turbidity may simply reflect a response to a sudden, major environmental change. Regime shifts reflect profound changes in ecosystem state in the absence of a strong change in environmental conditions. One way to test whether a regime shift occurred might be to explore to what extent there were early warning signals (EWS) prior to the state shift. Yet most studies testing for the occurrence of EWS used laboratory experiments or simulation data rather than field data, and as a result this field is still in its infancy. For a proper assessment of the generality and the detection power of early warning indicators, detailed and long-term monitoring data in aquatic systems are necessary (Seekell et al., 2012).

Based on the mechanistic scheme outlined by Scheffer et al. (1993; Figure 4), we here identify four different scenarios for changes in lake state in time and space (Figure 6). In Scenario 1, different ponds differ in their state (clear-water state characterized by an abundant underwater vegetation or turbid state dominated by algae) and these differences are associated with strong differences in environmental conditions. While such a pattern is not in disagreement with the theory of alternative stable states and regime shifts, the two different states as observed in the landscape might simply reflect ecological responses along an environmental gradient and do not necessarily reflect regime shifts. The lakes might differ in state because there are strong differences in environmental conditions. In Scenario 2, there is no change in state of a lake while there is also no change in environmental conditions. Again, while this pattern is not in contradiction with the idea of alternative stable states and regime shifts, it also does not provide any proof of regime shift or alternative stable states. In Scenario 3, there is change in state of a lake that is linked to a strong change in environmental conditions. Again, while this is not in contradiction with the theory of alternative stable states, it can also not be considered a typical regime shift. The change in state we observe might reflect a response to the pronounced change in environmental conditions, and thus does not reflect a typical regime shift, which is characterized by a strong non-linear response of the system to a small change in environmental stress. Scenario 4 depicts a change in state of a lake without a strong environmental change (either no detectable or a weak change in environmental conditions). The pattern observed in Scenario 4 is the only pattern that provides strong proof of the occurrence of alternative stable states and regime shifts. From these four scenarios, one can derive that one needs to have data on multiple systems through time to assess the occurrence and frequency of regime shifts with some certainty. In a survey of multiple lake systems repeated through time, direct evidence of regime shifts only comes from spatial or temporal variation in lake state in the absence of pronounced differences in environmental conditions. Differences among lakes, among years or among seasons that can be linked to strong external forcing should not be seen as evidence of regime shifts. Importantly, however, they also do not refute the theory.

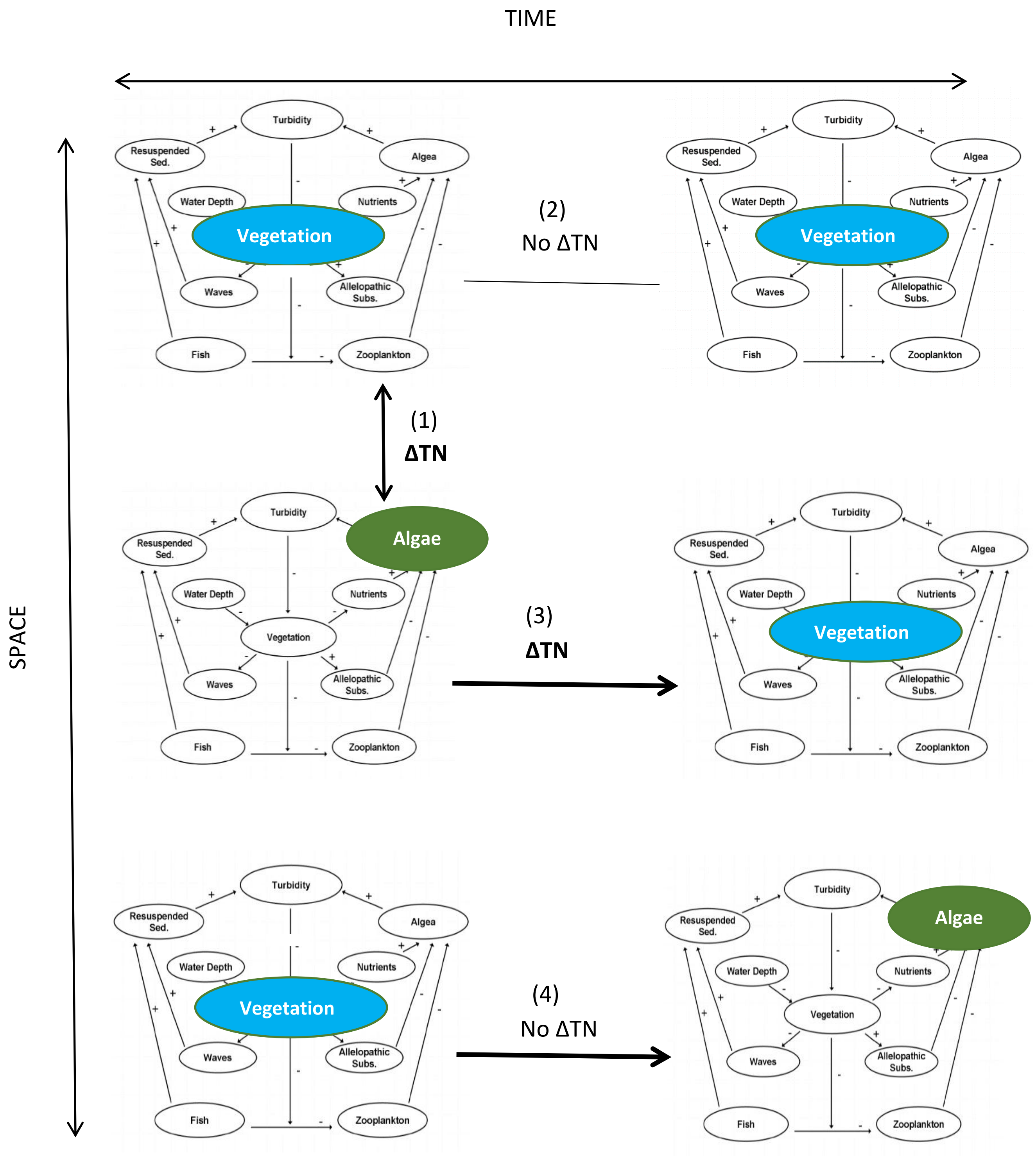

Figure 6. Scheme illustrating different scenarios for variation in lake state in time (columns) and space (rows) linked to the mechanistic scheme outlined by Scheffer et al. (1993). A highlight of “algae” (green) refers to a lake in turbid state, a highlight of “vegetation” (blue) refers to a lake in the clear-water state. (No) ΔTN refers to the (absence of) a strong change in environmental conditions in time. Scenario 1

refers to variation in lake state in space. Scenario 2 refers to a lake that is stable in one state in the absence of a strong environmental change. Scenario 3 refers to a lake that shows a shift in state linked to a strong shift in environmental conditions, and Scenario 4 refers to a lake that shows a shift in state in the absence of a strong environmental change.

The third knowledge gap refers to the fact that the mechanisms underlying regime shifts in part are linked to phenotypes of organisms. For example, grazing pressure of zooplankton on algae is an important component stabilizing the clear-water state and is directly linked to body size of the zooplankton (Verreydt et al., 2012; Gianuca et al., 2016). The study of Walsh et al. (2012) showed that genetic differentiation in populations of the water flea *Daphnia* inhabiting different lakes can impact top-down control of phytoplankton. Yet, so far, no studies demonstrated that evolution in a single zooplankton population through time has an impact on top-down control of phytoplankton and thus on lake turbidity. Such studies should involve either experimental evolution (e.g. Pantel, Duvivier, & De Meester, 2015), resurrection ecology (e.g. Stoks et al., 2016), or repeated sampling of active populations in the field across years.

6. Objectives

The general objectives of my PhD thesis are:

- to provide a better understanding of ecosystem stability in shallow lake systems at different temporal scales, from among-year to seasonal and weekly variation, and both within as well as among lakes.

- to explore to what extent evolutionary trait change can influence top-down control of algae and thus ecosystem stability.

7. Study area

The study was carried out in “Vijvergebied Midden-Limburg” (Figure 7), located in the north-eastern part of Belgium (50° 59’ 00.92” N; 5° 19’ 55.85” O). Vijvergebied Midden-Limburg comprises a total area of 4000 ha and consists more than 1000 interconnected shallow ponds, dry and wet heats, forest and reed vegetation (Lemmens et al., 2013). All ponds share a common water source (Roosterbeek), which brings well-buffered water to the system. Although most ponds are of anthropogenic origin and have historically been managed for purposes of fish farming, the region now is a biodiversity hotspot of European importance. Fish farming is still an important local practice, but the majority of ponds is currently property of the Flemish Government (Agency for Nature and Forest) and managed for purposes of biodiversity conservation (see Lemmens et al., 2013 for more detail). Vijvergebied Midden-Limburg is designated a Natura 2000 site and also protected by the EU Birds directive and the Habitats directive. The ponds differ widely in management. A number of ponds are managed for the purpose of nature conservation, others are used for extensive fish farming , still others are used for intensive fish farming, and a substantial number of ponds are not managed at all (Lemmens 2014). In all management types, fish is harvested during pond drainage. Most of the ponds that are managed for nature conservation are drained every few years and are not subject to fish stocking. Some of them are fishless; others get colonized spontaneously through overflows. Extensive fish stocking and farming juveniles are also applied on ponds that are used for biodiversity conservation (Lemmens et al., 2013). Ponds under extensive fish farming management are occasional drained (2-3 years) and are initially stocked with moderate density of planktivorous, benthivorous and piscivorous fish (30-80 kg/ha/year). In these extensive fish farming management, no additional fish feeds are used. Ponds in use for intensive fish farming are drained annually in autumn, and are stocked in spring with high densities of planktivorous and benthivorous fish (100 kg/ha/year). Commercial feeds are used to increase fish production in these systems, resulting in very high biomasses of fish (Lemmens et al., 2013, 2015). Partially linked to these different management systems, the ponds in the study system widely differ in their phytoplankton biomass (3.92-332.67µg/L), nutrients (TN = 0.7-10mg/L; TP = 0.05-5mg/L), and macrophyte coverage (0-95 %) (Lemmens et al. 2013, 2015).

Figure 7. Areal picture of part of “Vijvergebied Midden-Limburg” (from Lemmens, 2014)

Outline of the thesis

Given the two major aims of my thesis, it consists of two major parts. The first part (Chapter 1-3) focuses on the change in phytoplankton biomass and zooplankton community composition in relation to environmental changes within and among shallow lakes that are part of the interconnected fish pond system of Vijvergebied Midden-Limburg, at different temporal scales. The second part comprises one chapter (Chapter 4) and focuses on eco-evolutionary dynamics. In this chapter, the extent to which and how evolutionary trait change as it occurred in nature can influence population dynamics, ecosystem state and functioning is studied in a mesocosm experiment.

In Chapter 1, I study the differences in phytoplankton biomass (chlorophyll *a* concentration) within and among years in a set of 25 fish ponds, and relate these differences to variation in zooplankton body size and macrophyte cover. To this end I sampled the set of 25 ponds at weekly intervals during two years. My aim was to differentiate among ponds that are consistently different from each other (being in the turbid or clear-water state), ponds that show repeatable seasonal changes in state, and ponds that behave more erratic. Of these different scenarios, only the latter one provides direct evidence for the occurrence of regime shifts. In chapter 2, I studied year-to-year turnover in environmental conditions and zooplankton community composition in largely the same set of ponds during three years, and relate zooplankton community composition and system state to environmental variables across years to analyze the repeatability of the observed patterns. In Chapter 3, I zoom in to a much higher temporal resolution by analyzing data from chlorophyll *a* loggers that were positioned in four of the systems during two consecutive growing season and collected data on chlorophyll *a* and phycocyanin at fifteen minute intervals. We quantify differences among years as well as among systems, and explore to what extent early-warning indicators provide evidence for the occurrence of regime shifts.

Chapters 1-3 thus focus on the dynamics of phytoplankton biomass in time and space as well as its association with environmental change and zooplankton community composition, reflecting both bottom-up and top-down ecological impact. Grazing efficiency might, however, not only be determined by changes in species composition and its associated changes in body size distribution, but may also be impacted by evolution. In Chapter 4, I capitalize on a resurrection ecology study to quantify to what extent evolutionary change can impact top-down control of zooplankton on phytoplankton biomass. To that end, I compare phytoplankton dynamics, *Daphnia* population densities and population composition in mesocosms that were inoculated with clone sets that represent random samples of three subpopulations separated in time, hatched from the layered dormant egg bank of single pond that was exposed to strongly varying levels of fish predation pressure. As we purged for maternal effects, the observed differences in population dynamics and top-down control of algae in the experimental mesocosms could be ascribed to genetic differences, reflecting an impact of evolution on ecosystem functioning.

References

Andersen, T., Carstensen, J, Hernandez-Garcia, E., & Duarte, C. M. (2009). Ecological thresholds and regime shifts: Approaches to identification. *Trends in Ecology & Evolution, 24*(1), 49-57.

Bassar, R. D., Marshall, M. C., Lopez-Sepulcre, A., Zandona, E., Auer, S. K., Travis, J., . . . Reznick, D. N. (2010). Local adaptation in Trinidadian guppies alters ecosystem processes. *Proceedings of the National Academy of Sciences of the United States of America, 107*(8), 3616-3621.

Bayley, S. E., Creed, I. F., Sass, G. Z., & Wong, A. S. (2007). Frequent regime shifts in trophic states in shallow lakes on the boreal plain: Alternative" unstable" states? *Limnology and Oceanography, 52*(5), 2002-2012.

Beeton, A. M. (2002). Large freshwater lakes: Present state, trends, and future. *Environmental Conservation, 29*(1), 21-38.

Carpenter, S. R, Brock, W. A., Cole, J. J., Kitchell, J. F., & Pace, M. L. (2008). Leading indicators of trophic cascades. *Ecology Letters, 11*(2), 128-138.

Carpenter, S. R, Stanley, E. H., & Vander Zanden, M. J. (2011). State of the world's freshwater ecosystems: Physical, chemical, and biological changes. *Annual Review of Environment and Resources, 36*(1), 75-99. doi: 10.1146/annurev-environ-021810-094524

Carpenter, S. R., & Brock, W. A. (2006). Rising variance: A leading indicator of ecological transition. *Ecology Letters, 9*(3), 311-318.

Carpenter, S. R., Brock, W. A., Cole, J. J., & Pace, M. L. (2014). A new approach for rapid detection of nearby thresholds in ecosystem time series. *Oikos, 123*(3), 290-297.

Carpenter, S. R., Cole, J. J., Hodgson, J. R., Kitchell, J. F., Pace, M. L., Bade, D., . . . Schindler, D. E. (2001). Trophic cascades, nutrients, and lake productivity: Whole-lake experiments. *Ecological Monographs, 71*(2), 163-186.

Carpenter, S. R., Cole, J. J., Pace, M. L., Batt, R., Brock, W. A., Cline, T., . . . Seekell, D. A. (2011). Early warnings of regime shifts: A whole-ecosystem experiment. *Science, 332*(6033), 1079-1082.

Carpenter, S. R., & Kinne, O. (2003). *Regime shifts in lake ecosystems: Pattern and variation* (Vol. 15): International Ecology Institute Oldendorf/Luhe.

Carpenter, S. R., & Kitchell, J. F. (1996). *The trophic cascade in lakes*: Cambridge University Press.

Carpenter, S. R., Kitchell, J. F., & Hodgson, J. R. (1985). Cascading trophic interactions and lake productivity. *Bioscience, 35*(10), 634-639.

Céréghino, R., Biggs, J., Oertli, B., & Declerck, S. (2008). The ecology of European ponds: Defining the characteristics of a neglected freshwater habitat. *Hydrobiologia, 597*(1), 1-6.

Céréghino, R., Boix, D., Cauchie, Henry-Michel, Martens, K., & Oertli, B. (2014). The ecological role of ponds in a changing world. *Hydrobiologia, 723*(1), 1-6. doi: 10.1007/s10750-013-1719-y

Cottenie, K., & De Meester, L. (2003). Connectivity and cladoceran species richness in a metacommunity of shallow lakes. *Freshwater Biology, 48*(5), 823-832.

Cousyn, C., De Meester, L., Colbourne, J. K., Brendonck, L., Verschuren, D., & Volckaert, F. (2001). Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. *Proc Natl Acad Sci U S A, 98*(11), 6256-6260. doi: 10.1073/pnas.111606798

Crutsinger, G. M., Collins, M. D., Fordyce, J. A., Gompert, Z., Nice, C. C., & Sanders, N. J. (2006). Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science, 313*(5789), 966-968. doi: 10.1126/science.1128326

Dai, L., Vorselen, D., Korolev, K. S., & Gore, J. (2012). Generic indicators for loss of resilience before a tipping point leading to population collapse. *Science, 336*(6085), 1175-1177.

Dakos, V., Carpenter, S. R., Brock, W. A., Ellison, A. M., Guttal, V., Ives, A. R., . . . van Nes, E. H. (2012). Methods for detecting early warnings of critical transitions in time series illustrated using simulated ecological data. *PloS one, 7*(7), e41010.

Dakos, V., Carpenter, S. R., van Nes, E. H., & Scheffer, M. (2015). Resilience indicators: Prospects and limitations for early warnings of regime shifts. *Philosophical Transactions of the Royal Society B: Biological Sciences, 370*(1659), 20130263.

Dakos, V., Scheffer, M., van Nes, E. H., Brovkin, V., Petoukhov, V., & Held, H. (2008). Slowing down as an early warning signal for abrupt climate change. *Proceedings of the national Academy of Sciences, 105*(38), 14308-14312.

De Meester, L., Declerck, S., Stoks, R., Louette, G., Van De Meutter, F., De Bie, T., . . . Brendonck, L. (2005). Ponds and pools as model systems in conservation biology, ecology and evolutionary biology. *Aquatic Conservation: Marine and Freshwater Ecosystems, 15*(6), 715-725.

Declerck, S., De Bie, T., Ercken, D., Hampel, H., Schrijvers, S., Van Wichelen, J., . . . Bauwens, D. (2006). Ecological characteristics of small farmland ponds: Associations with land use practices at multiple spatial scales. *Biological Conservation, 131*(4), 523-532.

Declerck, S., & De Meester, L. (2003). Impact of fish predation on coexisting daphnia taxa: A partial test of the temporal hybrid superiority hypothesis. *Hydrobiologia, 500*(1-3), 83-94.

Donohue, I., Hillebrand, H., Montoya, J. M., Petchey, O. L., Pimm, S. L., Fowler, M. S., . . . McClean, D. (2016). Navigating the complexity of ecological stability. *Ecology Letters, 19*(9), 1172-1185.

Donohue, I., Petchey, O. L., Montoya, J. M., Jackson, A. L., McNally, L., Viana, M., . . . Emmerson, M. C. (2013). On the dimensionality of ecological stability. *Ecology Letters, 16*(4), 421-429.

Downing, A. S., van Nes, E. H., Mooij, W. M., & Scheffer, M. (2012). The resilience and resistance of an ecosystem to a collapse of diversity. *PloS one, 7*(9), e46135.

Downing, J. A. (2010). Emerging global role of small lakes and ponds. *Limnetica, 29*(1), 0009-0024.

Drake, J. M., & Griffen, B. D. (2010). Early warning signals of extinction in deteriorating environments. *Nature, 467*(7314), 456.

Dudgeon, David, Arthington, Angela H, Gessner, Mark O, Kawabata, Zen-Ichiro, Knowler, Duncan J, Lévêque, Christian, . . . Stiassny, Melanie LJ. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological reviews, 81*(2), 163-182.

Ellner, S. P., Geber, M. A., & Hairston, N. G. (2011). Does rapid evolution matter? Measuring the rate of contemporary evolution and its impacts on ecological dynamics. *Ecology Letters, 14*(6), 603-614. doi: 10.1111/j.1461-0248.2011.01616.

Fussmann, GF, Loreau, M, & Abrams, PA. (2007). Eco-evolutionary dynamics of communities and ecosystems. *Functional Ecology, 21*(3), 465-477.

Gerasimova, T. N., Pogozhev, P. I., & Sadchikov, A. P. (2018). Suppression of alga blooming by zooplankton filter feeders in small water bodies. *Water Resources, 45*(2), 199-204.

Gianuca, A. T., Pantel, J. H., & De Meester, L. (2016). Disentangling the effect of body size and phylogenetic distances on zooplankton top-down control of algae. *Proceedings. Biological sciences / The Royal Society, 283*(1828). doi: 10.1098/rspb.2016.0487

Gordon, Line J, Peterson, Garry D, & Bennett, Elena M. (2008). Agricultural modifications of hydrological flows create ecological surprises. *Trends in Ecology & Evolution, 23*(4), 211-219.

Graham, N. A. J., Jennings, S., MacNeil, M. A., Mouillot, D., & Wilson, S. K. (2015). Predicting climate-driven regime shifts versus rebound potential in coral reefs. *Nature, 518*(7537), 94-97.

Hairston, N. G., Ellner, S. P., Geber, M. A., Yoshida, T., & Fox, J. A. (2005). Rapid evolution and the convergence of ecological and evolutionary time. *Ecology Letters, 8*(10), 1114-1127.

Hanson, M. A., Herwig, B. R., Zimmer, K. D., Fieberg, J., Vaughn, S. R., Wright, R. G., & Younk, J. A. (2012). Comparing effects of lake-and watershed-scale influences on communities of aquatic invertebrates in shallow lakes. *PloS one, 7*(9), e44644.

Hempson, T. N., Graham, N. A. J., MacNeil, M. A., Hoey, A. S., & Wilson, S. K. (2018). Ecosystem regime shifts disrupt trophic structure. *Ecological Applications, 28*(1), 191-200.

Hendry, A. P. (2016). *Eco-evolutionary dynamics*: Princeton University Press.

Hermoso, V., Abell, R., Linke, S., & Boon, P. (2016). The role of protected areas for freshwater biodiversity conservation: Challenges and opportunities in a rapidly changing world. *Aquatic Conservation: Marine and Freshwater Ecosystems, 26*(S1), 3-11.

Hicks, C. C., Crowder, L. B., Graham, N. A. J., Kittinger, J. N., & Cornu, E. L. (2016). Social drivers forewarn of marine regime shifts. *Frontiers in Ecology and the Environment, 14*(5), 252-260.

Hillebrand, H., Langenheder, S., Lebret, K, Lindström, E., Östman, Ö., & Striebel, M. (2018). Decomposing multiple dimensions of stability in global change experiments. *Ecology Letters, 21*(1), 21-30.

Holling, C. S. (1973). Resilience and stability of ecological systems. *Annual Review of Ecology and Systematics, 4*(1), 1-23.

Iacarella, J. C., Barrow, J. L., Giani, A., Beisner, B. E., & Gregory-Eaves, Irene. (2018). Shifts in algal dominance in freshwater experimental ponds across differing levels of macrophytes and nutrients. *Ecosphere, 9*(1), e02086. doi: doi:10.1002/ecs2.2086

Ives, A. R. (1995). Measuring resilience in stochastic systems. *Ecological Monographs, 65*(2), 217-233.

Ives, A. R., & Carpenter, S. R. (2007). Stability and diversity of ecosystems. *Science, 317*(5834), 58-62.

Jeppesen, E., Jensen, J. P., Jensen, C., Faafeng, B., Hessen, D. O., Søndergaard, M., . . . Christoffersen, K. (2003). The impact of nutrient state and lake depth on top-down control in the pelagic zone of lakes: A study of 466 lakes from the temperate zone to the arctic. *Ecosystems, 6*(4), 313-325.

Jeppesen, E., Peder, J. J., SØndergaard, M., Lauridsen, T., & Landkildehus, F. (2000). Trophic structure, species richness and biodiversity in danish lakes: Changes along a phosphorus gradient. *Freshwater Biology, 45*(2), 201-218.

Jeppesen, E., Sondergaard, M., Sondergaard, M., & Christofferson, K. (2012). *The structuring role of submerged macrophytes in lakes* (Vol. 131): Springer Science & Business Media.

Johnson, M. T., Vellend, M., & Stinchcombe, J. R. (2009). Evolution in plant populations as a driver of ecological changes in arthropod communities. *Philos Trans R Soc Lond B Biol Sci, 364*(1523), 1593-1605. doi: 10.1098/rstb.2008.0334

Kéfi, S., Holmgren, M, & Scheffer, M. (2016). When can positive interactions cause alternative stable states in ecosystems? *Functional Ecology, 30*(1), 88-97.

Kuehn, C. (2011). A mathematical framework for critical transitions: Bifurcations, fast–slow systems and stochastic dynamics. *Physica D: Nonlinear Phenomena, 240*(12), 1020-1035.

Lampert, W., & Sommer, U. (2007). *Limnoecology: The ecology of lakes and streams*: Oxford university press.

Latta, L. C., Bakelar, J. W., Knapp, R. A., & Pfrender, M. E. (2007). Rapid evolution in response to introduced predators ii: The contribution of adaptive plasticity. *BMC Evolutionary Biology, 7*, 21. doi: 10.1186/1471-2148-7-21

Lefcheck, J. S., Byrnes, J. E. K., Isbell, F., Gamfeldt, L., Griffin, J. N., Eisenhauer, N., . . . Duffy, J. (2015). Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. *Nature communications, 6*, 6936.

Lemmens, P., Declerck, S. A. J., Tuytens, K., Vanderstukken, M., & De Meester, L. (2018). Bottom-up effects on biomass versus top-down effects on identity: A multiple-lake fish community manipulation experiment. *Ecosystems, 21*(1), 166-177. doi: 10.1007/s10021-017-0144-x

Lenton, T. M., Held, H., Kriegler, E., Hall, J. W., Lucht, W., Rahmstorf, S., & Schellnhuber, H. J. (2008). Tipping elements in the earth's climate system. *Proceedings of the national Academy of Sciences, 105*(6), 1786-1793.

Lenton, T. M., Livina, V. N., Dakos, V., & Scheffer, M. (2012). Climate bifurcation during the last deglaciation? *Climate of the Past, 8*(4), 1127-1139.

Liu, D., Ogaya, R., Barbeta, A, Yang, X., & Peñuelas, J. (2018). Long-term experimental drought combined with natural extremes accelerate vegetation shift in a mediterranean holm oak forest. *Environmental and Experimental Botany*.

Loreau, M., & Mazancourt, C. (2013). Biodiversity and ecosystem stability: A synthesis of underlying mechanisms. *Ecology Letters, 16*(s1), 106-115.

Maes, J., Liquete, C., Teller, A., Erhard, M., Paracchini, M. L., Barredo, J. I., . . . Petersen, Jan-Erik. (2016). An indicator framework for assessing ecosystem services in support of the eu biodiversity strategy to 2020. *Ecosystem services, 17*, 14-23.

Matthews, B., Narwani, A., Hausch, S., Nonaka, E., Peter, H., Yamamichi, M., . . . Hanley, T.. C. (2011). Toward an integration of evolutionary biology and ecosystem science. *Ecol Lett, 14*(7), 690-701.

May, R. M. (1977). Thresholds and breakpoints in ecosystems with a multiplicity of stable states. *Nature, 269*(5628), 471-477.

McCann, K. S. (2000). The diversity–stability debate. *Nature, 405*(6783), 228.

McGowan, S., Leavitt, P. R., Hall, R. I., Anderson, N. J., Jeppesen, E., & Odgaard, B. V. (2005). Controls of algal abundance and community composition during ecosystem state change. *Ecology, 86*(8), 2200-2211.

McPeek, M. A. (2017). The ecological dynamics of natural selection: Traits and the coevolution of community structure. *The American Naturalist, 189*(5), E91-E117.

Moss, B. R. (2013). *Ecology of fresh waters: A view for the twenty-first century*: John Wiley & Sons.

Ormerod, SJ, Dobson, M, Hildrew, AG, & Townsend, CR. (2010). Multiple stressors in freshwater ecosystems. *Freshwater Biology, 55*(s1), 1-4.

Pantel, J. H., Duvivier, C., & De Meester, L. (2015). Rapid local adaptation mediates zooplankton community assembly in experimental mesocosms. *Ecol Lett, 18*(10), 992-1000. doi: 10.1111/ele.12480

Pantel, J. H., Duvivier, C., & Meester, L. D. (2015). Rapid local adaptation mediates zooplankton community assembly in experimental mesocosms. *Ecol Lett, 18*(10), 992-1000. doi: 10.1111/ele.12480

Pereira, H. M., Navarro, L. M., & Martins, I. S. (2012). Global biodiversity change: The bad, the good, and the unknown. *Annual Review of Environment and Resources, 37*.

Pimm, S. L. (1984). The complexity and stability of ecosystems. *Nature, 307*(5949), 321.

Ramstack H., Joy M, H., William O, Edlund, M. B., Zimmer, K. D., Theissen, K. M., Hoidal, N., . . . Cotner, J. B. (2016). The legacy of large regime shifts in shallow lakes. *Ecological Applications, 26*(8), 2662-2676.

Reeves, M. C., Manning, M. E., DiBenedetto, J. P., Palmquist, K. A., Lauenroth, W. K., Bradford, J. B., & Schlaepfer, Daniel R. (2018). Effects of climate change on rangeland vegetation in the northern Rockies. *Climate change and rocky mountain ecosystems* (pp. 97-114): Springer.

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin III, F. S., Lambin, E. F., . . . Schellnhuber, H. J. (2009). A safe operating space for humanity. *Nature, 461*(7263), 472.

Scheffer, M., Bascompte, J., Brock, W. A., Brovkin, V., Carpenter, S. R., Dakos, V., . . . Sugihara, G. (2009). Early-warning signals for critical transitions. *Nature, 461*(7260), 53-59.

Scheffer, M., Carpenter, S. R., Foley, J. A., Folke, C., & Walker, B. (2001). Catastrophic shifts in ecosystems. *Nature, 413*(6856), 591.

Scheffer, M., Carpenter, S. R., Lenton, T. M., Bascompte, J., Brock, W., Dakos, V., . . . Van Nes, E. H. (2012). Anticipating critical transitions. *science, 338*(6105), 344-348.

Scheffer, M., Hosper, S. H, Meijer, M. L, Moss, B., & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution, 8*(8), 275-279.

Scheffer, M., & van Nes, E. H. (2007). Shallow lakes theory revisited: Various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia, 584*(1), 455-466.

Schindler, D. W., Hecky, R. E., & McCullough, G. K. (2012). The rapid eutrophication of lake winnipeg: Greening under global change. *Journal of Great Lakes Research, 38*, 6-13.

Schoener, T. W. (2011). The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. *Science, 331*(6016), 426-429. doi: 10.1126/science.1193954

Schröder, A., Persson, L., & De Roos, A. M. (2005). Direct experimental evidence for alternative stable states: A review. *Oikos, 110*(1), 3-19.

Schuler, M. S., & Relyea, R. A. (2018). A review of the combined threats of road salts and heavy metals to freshwater systems. *Bioscience*.

Seekell, D. A., Carpenter, S. R., Cline, T. J., & Pace, M. L. (2012). Conditional heteroskedasticity forecasts regime shift in a whole-ecosystem experiment. *Ecosystems, 15*(5), 741-747.

Stoks, R., Govaert, L., Pauwels, K, Jansen, B., & De Meester, L. (2016). Resurrecting complexity: The interplay of plasticity and rapid evolution in the multiple trait response to strong changes in predation pressure in the water flea daphnia magna. *Ecology Letters, 19*(2), 180-190. doi: 10.1111/ele.12551

Terhorst, C. P., Lennon, J. T., & Lau, J. A. (2014). The relative importance of rapid evolution for plant-microbe interactions depends on ecological context. *Proc Biol Sci, 281*(1785), 20140028. doi: 10.1098/rspb.2014.0028

Tilman, D., Reich, P. B., & Knops, J. M. H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature, 441*(7093), 629.

Timms, R. M., & Moss, B. (1984). Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. *Limnology and Oceanography, 29*(3), 472-486.

Urban, M. C., Leibold, M. A., Amarasekare, P., De Meester, L., Gomulkiewicz, R., Hochberg, M. E., . . . Wade, M. J. (2008). The evolutionary ecology of metacommunities. *Trends Ecol Evol, 23*(6), 311-317. doi: 10.1016/j.tree.2008.02.007

van de Leemput, I. A., Dakos, V., Scheffer, M., & van Nes, E. H. (2018). Slow recovery from local disturbances as an indicator for loss of ecosystem resilience. *Ecosystems, 21*(1), 141-152.

Van Donk, E., Grimm, M. P., Gulati, R. D., & Breteler, J.P.G.K. (1990). Whole-lake food-web manipulation as a means to study community interactions in a small ecosystem. *Hydrobiologia, 200*(1), 275-289.

Van Moorsel, S., Hahl, T., Petchey, O. L., Ebeling, A., Eisenhauer, N., Schmid, B., & Wagg, C. (2018). Evolution increases ecosystem temporal stability and recovery from a flood in grassland communities. *bioRxiv*, 262337.

Van Nes, E. H., & Scheffer, M. (2007). Slow recovery from perturbations as a generic indicator of a nearby catastrophic shift. *The American Naturalist, 169*(6), 738-747.

Vanderstukken, M., Declerck, S. A. J., Decaestecker, E, & Muylaert, K. (2014). Long-term allelopathic control of phytoplankton by the submerged macrophyte elodea nuttallii. *Freshwater Biology, 59*(5), 930-941.

Veraart, A. J., Faassen, E. J., Dakos, V., van Nes, E. H., Lürling, M., & Scheffer, M. (2012). Recovery rates reflect distance to a tipping point in a living system. *Nature, 481*(7381), 357.

Verpoorter, C., Kutser, T., Seekell, D. A., & Tranvik, L. J. (2014). A global inventory of lakes based on high-resolution satellite imagery. *Geophysical Research Letters, 41*(18), 6396-6402. doi: doi:10.1002/2014GL060641

Verreydt, D., De Meester, L., Decaestecker, E., Villena, M. J., Van Der Gucht, K., Vannormelingen, P., . . . Declerck, S. (2012). Dispersal-mediated trophic interactions can generate apparent patterns of dispersal limitation in aquatic metacommunities. *Ecology Letters, 15*(3), 218-226.

Vogel, A., Scherer-Lorenzen, M., & Weigelt, A. (2012). Grassland resistance and resilience after drought depends on management intensity and species richness. *PloS one, 7*(5), e36992.

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., . . . Liermann, C. R. (2010). Global threats to human water security and river biodiversity. *Nature, 467*(7315), 555.

Walsh, M. R., DeLong, J. P., Hanley, T. C., & Post, D. M. (2012). A cascade of evolutionary change alters consumer-resource dynamics and ecosystem function. *Proceedings. Biological sciences / The Royal Society, 279*(1741), 3184-3192. doi: 10.1098/rspb.2012.0496

Wang, S., & Brose, U. (2018). Biodiversity and ecosystem functioning in food webs: The vertical diversity hypothesis. *Ecology Letters, 21*(1), 9-20.

Whitham, T. G., Bailey, J. K., Schweitzer, J. A., Shuster, S. M., Bangert, R. K., LeRoy, C. J., . . . Potts, B. M. (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nature Reviews Genetics, 7*(7), 510-523.

Yachi, S., & Loreau, M. (1999). Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings of the national Academy of Sciences, 96*(4), 1463-1468.

Zimmer, K. D., Hanson, M. A., Herwig, B. R., & Konsti, M. L. (2009). Thresholds and stability of alternative regimes in shallow prairie–parkland lakes of central North America. *Ecosystems, 12*(5), 843-852.

Chapter 1: Seasonality and stability in ecosystem state in a set of interconnected shallow ponds

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[Unpublished manuscript]

Abstract

Ecosystem stability and regime shifts gain increasing attention in ecology. The turbid and clear-water states of shallow lakes are amongst the best studied examples of such regime shifts, and much is already known about the mechanisms that stabilize the two distinct states. However, most studies on ecosystem stability either focus on a high temporal resolution in a single system or engage in large scale surveys that are not replicated in time. Here we present results from a weekly monitoring, across two years, of 25 shallow lakes that are interconnected and part of a large fish pond complex. The study ponds share a similar water source but are different in ecology, ranging from clear-water to turbid systems. In 2014 and 2015, we weekly monitored chlorophyll *a* concentration and macrophyte cover during the growth season (April-November). Zooplankton biomass and community composition was assessed three times during each growth season. Our results allow a differentiation among lakes that are always clear, always turbid, lakes that shift from the clear-water to the turbid state as the season progresses, and lakes that shift back and forth between the two states. We find significant associations between chlorophyll *a* concentration across the season and both zooplankton body size during spring as well as macrophyte cover during summer, pointing to a key role for mechanisms that are known to be important role in stabilizing the two alternative stable states in shallow lakes. While fixed and pronounced differences in turbidity among lakes might reflect important differences in environmental conditions (e.g. in pond management and fish predation), and repeatability of a seasonal state shift across years within ponds can similarly be interpreted as a transition between states that is linked to a seasonal change in environmental conditions (e.g. juvenile fish entering the pelagic), differences in the dynamics of state shifts among years within ponds more likely reflect true regime shifts that occurred in the absence of strong environmental change. Our data support the existence of stable alternative states in shallow lakes, the proposed mechanisms stabilizing them, and show that a substantial portion of lakes shows dynamic changes in turbidity. They also suggest, however, that approximately half of the studied lakes did not show regime shifts or did so in a seasonally predictable way. We suggest that it is important to distinguish between the existence of alternative stable states and the mechanisms

stabilizing them on the one hand and the frequency of occurrence of true regime shifts at the other hand.

Introduction

Ecosystem stability and the occurrence of regime shifts in ecosystems have received rapidly growing attention in science and policy during the last decades (Carpenter & Brock, 2006; Scheffer & van Nes, 2007; Donohue et al., 2013; Loreau & Mazancourt, 2013; Donohue et al., 2016). Many ecosystems are characterized by the occurrence of multiple alternative stable states that are stabilized by positive feedback loops (Scheffer et al., 1993; Kéfi, Holmgren, & Scheffer, 2016). Such systems typically show resilience, but once a threshold is surpassed, it may show a dramatic shift in state (Holling, 1973; May, 1977; Scheffer et al., 2001). Regime shifts are important as they can lead to sudden and profound alterations of ecosystem properties, and can also undermine the provisioning of vital ecosystem services (Worm et al., 2006; Jiao, 2009) in a setting where environmental stress is just marginally increased. In addition, once a regime has occurred, hysteresis intrinsically linked to regime shifts implies that it often takes major efforts to reverse the state shift (Scheffer et al., 2001; Carpenter, Kinne, & Wieser, 2003).

A well-known example of alternative stable states is the occurrence of the clear-water and turbid state in shallow lakes and ponds (Van Donk et al., 1990; Scheffer et al., 1993; Jeppesen et al., 1997; Kéfi et al., 2016). The clear-water state is characterized by low phytoplankton densities, the occurrence of a well-developed submerged vegetation, and relative high abundances of large bodied zooplankton. In contrast, the turbid state is characterized by high phytoplankton densities and the lack of both submerged aquatic vegetation and large bodied zooplankton (Scheffer et al., 1993; Scheffer, 1998). Submerged macrophytes play a central role in stabilizing the clear-water state as they promote high water transparency by suppressing phytoplankton via competition for nutrients and by allelopathy (Gross, 2003; Vanderstukken et al., 2014). Macrophytes also reduce resuspension of sediments (Carpenter & Lodge, 1986) and can enhance grazing pressure of zooplankton on phytoplankton by providing shelter to zooplankton against predation by fish (Irvine, Moss, & Stansfield, 1990). Large-bodied zooplankton are preferred prey of fish but also the most efficient grazers of phytoplankton, and play a crucial role in the top-down control of algae (Tessier, Leibold, & Tsao, 2000; Lampert & Sommer, 2007; Gianuca, Pantel, & De Meester, 2016).

Shallow lakes and ponds have been pivotal to the development of the theory of the occurrence of alternative stable states and regime shifts in ecosystems (Scheffer et al., 1993; Scheffer et al., 2001; Scheffer et al., 2009). The theory was largely inspired by the observation that lakes seem to shift rather abruptly from a clear, macrophyte dominated state to a turbid, phytoplankton dominated state under conditions of gradually increasing nutrient concentrations (Scheffer et al., 1993). There is a large number of studies documenting the occurrence of alternative stable states, the mechanisms underpinning their stability, and the occurrence of regime shifts (Scheffer et al., 1993; Scheffer et al., 2001; Carpenter et al., 2003; Foley et al., 2003; Søndergaard et al., 2007; Scheffer et al., 2009; Carpenter et al., 2011; Kéfi et al., 2013). Most field studies, however, either involve sampling of single lakes at a high temporal resolution or a large scale spatial survey that is, however, not replicated in time. Monitoring a single system that changes through time does not provide insight into the extent to which regime shifts are common, and spatial surveys only show the co-occurrence of systems in different states in the landscape, but do not show whether this corresponds to alternative stable states that can occur under similar environmental conditions or just reflects linear responses of the systems to environmental gradients. Such surveys also do not show whether the lakes are fixed in a state or show regime shifts through time. There is also growing controversy with respect to the importance of alternative stable states and regime shifts in shallow lakes (Schröder, Persson, & De Roos, 2005; Petraitis, 2013). This is in part related to the fact that not all sudden shifts between a clear-water and turbid state reflect a true regime shift. Indeed, the theory of regime shifts implies that they occur under gradual environmental change, reflecting a non-linear response to a change in driver (Schmitz, 2004; Bestelmeyer et al., 2011). A change from one state to another one can also be caused by sudden and profound changes in environmental conditions or food web dynamics (Jeppesen et al., 1997). For example, a shift from the clear-water to the turbid state in late spring might reflect a massive migration of juvenile fish into the pelagic zone, exerting strong predation pressure on zooplankton; as this prevents top-down control of algae, it can lead to a sudden and severe algal bloom. Such a change would be in line with what we know on the mechanisms that stabilize the clear-water and turbid states (Scheffer et al. 1993), but it would not be a typical regime shift; rather it would be a strong shift in system state as a consequence of a pronounced change in environmental conditions. Thus, it is necessary to carry out repeated spatial surveys over time, including seasonally, to get a better grasp on to what extent the two major states occur in shallow lakes and to what extent they show regime shift in the absence of strong environmental change.

The aim of the present study was to obtain more insight into the occurrence of alternative states and regime shifts in a set of shallow lakes that are very similar in water source and morphometry, and into the repeatability of system characteristics across lakes, years and seasons. Fixed differences in state among lakes within and across years might reflect pronounced environmental differences between the systems (e.g. in fish predation pressure or nutrient loading). Similarly, repeatability of a seasonal state shift across years within ponds might reflect a transition between states that is linked to a seasonal change in environmental conditions, such as the migration of juvenile fish to the open water. More dynamic ponds that differ in the dynamics of state shifts among years more likely show true regime shifts. To document these dynamics we engaged in a weekly monitoring during the growing season (April – November) of two consecutive years, of chlorophyll *a* concentration and macrophyte cover in 25 shallow lakes that are interconnected and part of a large fish pond complex (Vijvergebied Midden-Limburg; Lemmens et al., 2013). The study ponds share similar water input and thus show similar chemical characteristics such as nutrient levels. Yet they show pronounced differences in their ecology, and range from clear-water to turbid systems (Lemmens et al., 2013). We tested (1) to what extent ponds systematically differ in their state, show repeatable seasonal shifts in state in different years, or show a more irregular behavior of state shifts pointing to regime shifts; and (2) tested the hypothesis that in this set of interconnected ponds that share a similar water source, the mechanisms that have been put forward as stabilizing the turbid and clear-water state, being zooplankton grazing and macrophyte cover can explain variation in state of shallow lakes in the study system. Our results shed light on the mechanisms that stabilize alternative states in shallow lakes and on the nature of the dynamics that underlie patterns that are found in snapshot surveys.

Material and Methods

Study area and sampling

This study was conducted in Vijvergebied Midden-Limburg, located in the North-Eastern part of Belgium (50°59’00.92” N, 5°19’55.85” and surroundings). The region consists of a large number of interconnected shallow ponds that are surrounded by marshes, heather and forests. Most of the ponds in this region are man-made and have historically been managed for purposes of fish farming. More recently, many ponds have become property of the governmental Agency of Nature and Forest and are now managed for purposes of biodiversity conservation (Lemmens et al., 2013). The ponds share a common water source, and are connected by rivulets. As a result, chemical characteristics of the ponds are similar, except for changes that are caused by differences in food web characteristics.

We selected 25 ponds in the central part of the Vijvergebied (Figure 1) to be monitored weekly for phytoplankton biomass and macrophyte cover during the growth season (April – November) of two subsequent years (2014 and 2015). In addition, all 25 ponds were sampled for their zooplankton community characteristics at three time points each year, and nutrient concentrations in each pond were quantified once yearly, in summer. Phytoplankton biomass was quantified by measuring the concentration of in *vivo* chlorophyll *a* in a water sample collected in the upper 20 cm of the water column using a vial on a long handle. *In vivo* chlorophyll *a* was used as a proxy of phytoplankton biomass and measured directly in the field using a handheld fluorometer (AquaFluor, Turner Designs, Sunnyvale, CA).

Samples (50ml) were taken once in July of each year for the quantification of total nitrogen and total phosphorus in the laboratory. These samples were kept cool in the dark in the field and stored at -20°C in the laboratory until further processing. TN and TP concentrations were quantified using a Technicon Autoanalyzer II (Technicon, Tarrytown, New York, USA) after alkaline persulphate digestion (Koroleff, 1970). Direct spectrophotometric determination of ammonia in natural waters as indophenol blue was done using a Technicon Autoanalyzer II (Technicon, Tarrytown, New York, USA). The percentage of submerged macrophytes was visually estimated and scored following the Braun-Blanquet classification (Braun-Blanquet et al., 1932; 1: < 5%, 2: 5-25%, 3: 25-50%, 4: 50-75%, 5: >75%).

Figure 1. Overview of a part of “Vijvergebied Midden –Limburg” with the selected ponds and their code.

Zooplankton communities were sampled by collecting a depth-integrated water sample from the open and vegetated meso-habitats using a tube sampler (De Bie et al., 2012). In each pond eight depth-integrated samples were taken for a total volume of approximately 48 liter. The relative amount of water collected in each meso-habitat was adjusted to our estimates of their relative importance around the sampling location (20 m perimeter) using the Braun-Blanquet score (score 1: 0L from littoral, 8L from pelagic; 2: 2L from littoral, 6L from pelagic; 3: 4L from littoral, 4L from pelagic; 4: 6L from littoral, 2L from pelagic; 5: 8L from littoral, 0L from pelagic). The water was pooled and gently mixed to then filter a 30 L subsample through a conical plankton net with mesh size 64 µm. Samples were preserved in 50 ml jars and fixed with 4% formaldehyde concentration. In the laboratory, subsamples were taken with a wide-mouthed pipet and counted under a stereomicroscope (Olympus ZS X 12) until a lower limit of 300 individuals of cladocerans was reached. The counts were extrapolated to the total volume of the sample and transformed to abundances per liter (number of individuals/liter). Cladocerans were identified to species level following Flössner (2000), except for the genera *Chydorus* and *Ceriodaphnia* that were identified to genus level. Copepods were classified as cyclopoids and calanoids. We focused on cladocerans, as they are the most important grazers during the summer season in the study ponds. Especially larger species of the genus *Daphnia* are well known for their high capacity for top-down control of phytoplankton (Carpenter, Cottingham, & Schindler, 1992; Lampert et al., 2007; Verreydt et al., 2012; Gianuca et al., 2016).

The body size of 10 random individuals of each cladoceran taxon in each sample was measured to quantify the abundance-weighted mean zooplankton body size in each pond, calculated using taxon abundances and pond- specific mean species body sizes. Zooplankton species composition and body size were quantified during spring (last week of May), early summer (first week of July) and late summer (third week of August) in both years.

We used phytoplankton biomass to assess whether a given lake at a given time was in the turbid or clear-water state because the turbid state typically is characterized by high phytoplankton biomass and the clear-water state by low phytoplankton biomass.

Data analysis

We first categorized ponds into three different groups: ponds that remain clear throughout the growing season (“stable clear”), ponds that remain turbid during the growing season (“stable turbid”), and ponds that show shifts from a clear-water state to a turbid state (“dynamic”). Our criterion to categorize a pond as turbid was set at a chlorophyll *a* concentration of >20 µg/L. This criterion was set based on literature (Free et al., 2006; Søndergaard et al., 2010; Poikane et al., 2014) and visual observations during sampling. Ponds that had chlorophyll *a* concentrations between 0-20 µg/L were also visually clearly in a clear-water state during sampling. Ponds that showed one or multiple changes across this boundary were considered dynamic.

To test for consistency in the among-pond differences in mean phytoplankton biomass as well as in variation in phytoplankton biomass across years, we calculated Pearson’s correlations between values for 2014 and 2015 of mean phytoplankton biomass and its coefficient of variation (i.e. the ratio of the standard deviation of chlorophyll *a* concentration to the mean chlorophyll *a* concentration for each pond), pairing data according to lake. To test for differences in both mean phytoplankton biomass and its variation among years, we used a paired t-test and Wilcoxon signed rank tests, respectively. We also tested for differences among seasons, using a linear mixed-effect model for repeated measures for each year separately, grouping the data according to season: spring (last week of April - third week of June), summer (last week of June - third week of September) and autumn (last week of September - mid November).

To test the hypothesis that zooplankton body size, macrophyte cover and nutrients are associated with chlorophyll *a* levels in the ponds, we related phytoplankton (log (chl*a*) biomass across the seasons with zooplankton body size, submerged macrophyte cover (averaged across the lake), the concentration of total nitrogen and total phosphorus) using Pearson’s correlations. We performed this analysis for all data for each week separately, so that we could also assess seasonal differences in the degree to which zooplankton, macrophytes and nutrients were associated with variation in turbidity in the study ponds.

We then applied stepwise multiple regressions to identify the variables that best explained the variation in phytoplankton biomass in spring and summer of 2014 and 2015. The best regression model was selected using a selection procedure based on the second-order Akaike Information Criterion (Aho et al., 2014). Collinearity among explanatory variables was investigated using the Variance Inflation Factor (VIF). Only explanatory variables with VIF values not exceeding 3 were considered in the model, which is below the threshold (O'Brien, 2007). Statistical analysis were carried out in R using the "vegan", "MASS", and "car" packages (R Development Core Team, 2016). The chlorophyll *a* data were log transformed to fulfill the assumptions of parametric testing.

Results

Phytoplankton biomass within and across ponds and years

The set of investigated ponds varied strongly in overall phytoplankton biomass (Figure 1; Figure 2). Mean phytoplankton biomass in the studied ponds varied from 4.73 – 332.6 µg/L. The average phytoplankton biomass across all ponds was lowest in spring and highest in summer in both years (29.6µg/L and 39.5µg/L, respectively, in 2014; 40.6µg/L and 62.2 µg/L, respectively, in 2015; Figure SI2 b&c).

If we categorize the ponds as turbid or clear-water based on the 20 µg/L chlorophyll *a* criterion, we see that three ponds remained in a clear-water state and five ponds stayed in the turbid state over the entire study period (Figure 1; Figure 2; Figure SI1). Eight ponds showed strong variation in phytoplankton biomass over time ("dynamic") in both years, and nine ponds changed behavior across years: three ponds shifted from a clear-water state in 2014 to a more dynamic behavior in 2015, five ponds shifted from a dynamic behavior in 2014 to a turbid state in 2015, and one pond shifted from a turbid state in 2014 into a more dynamic behavior in 2015 (Figure SI1).

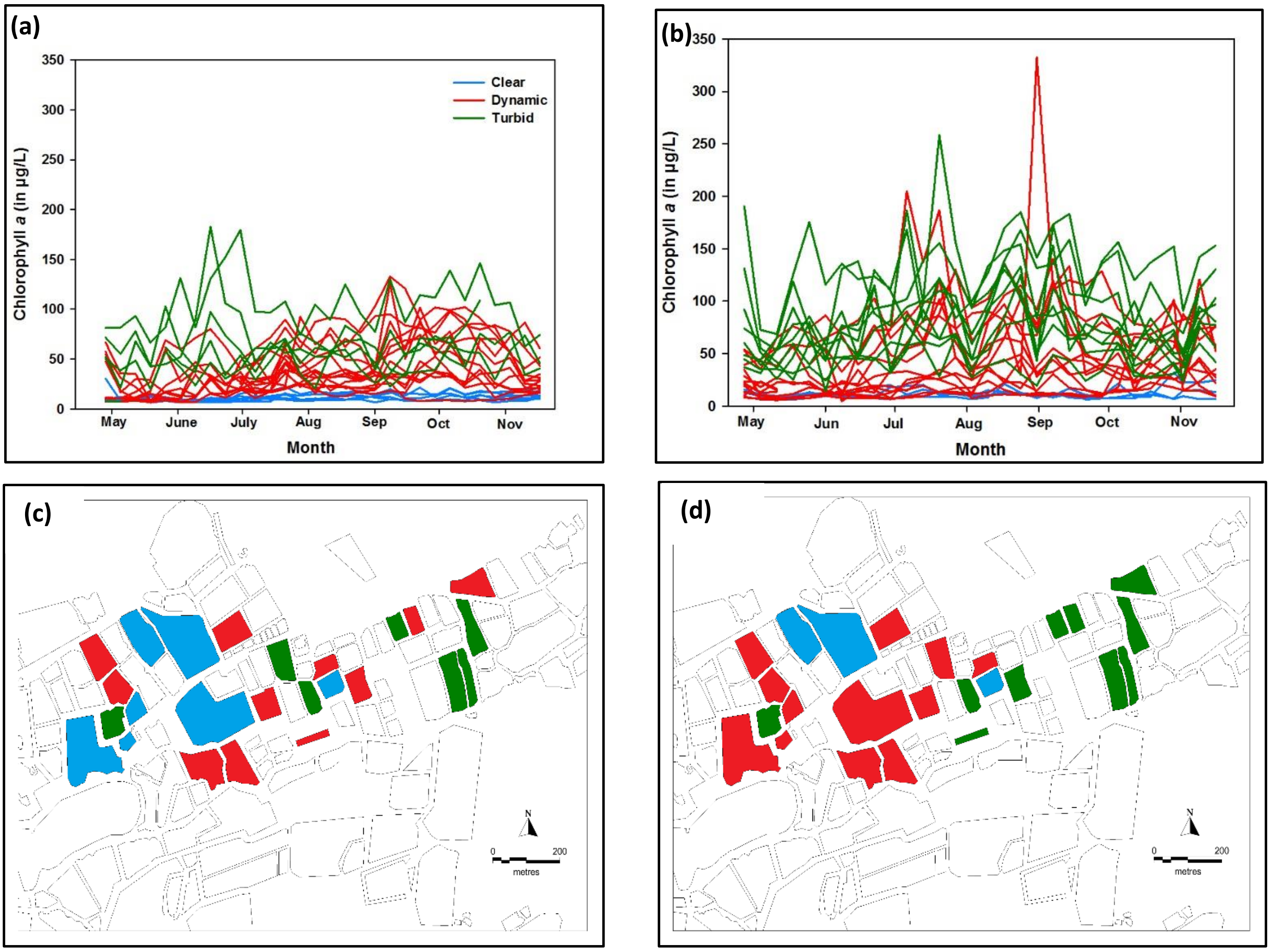

Figure 2. Phytoplankton biomass during the growth season for each pond in (a) 2014 and (b) 2015. Colors represent the different categories of ponds (Blue = clear-water ponds, with chlorophyll *a* concentrations consistently below 20 µg/L; green = turbid ponds, with chlorophyll *a* levels consistently above 20 µg/L; red = dynamic ponds). Each line represents a different pond. Lower panels provide an aerial map with the different investigated ponds and the category to which they belong in (c) 2014 and (d) 2015.

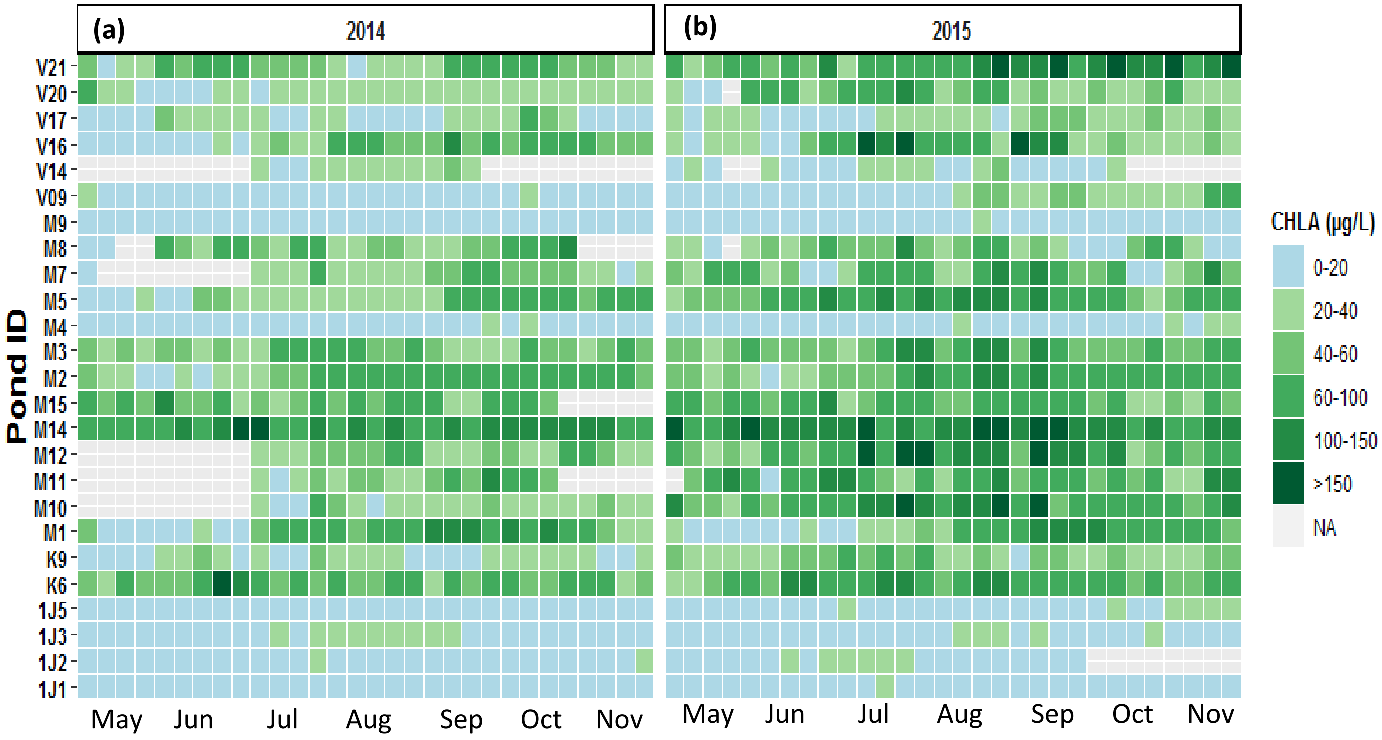

Figure 3. Heat map for chlorophyll *a* concentration measured in a set of 25 interconnected ponds in (a) 2014 and (b) 2015. NA (gray patches) represent missing values, because the ponds were drained or too low in water to reach it so that no measurement could be carried out. For the codes identifying the ponds, see Figure 1).

On average phytoplankton biomass was significantly different between the two study years (paired t-test = -4.2001, $p < 0.001$). Phytoplankton biomass in 2015 was on average higher than in 2014 (Figure SI2 a). Yet, overall the differences among ponds were consistent across years, with a significant positive correlation between the annual pond average phytoplankton biomass in 2014 and the annual pond average phytoplankton biomass in 2015 when the data are paired by pond ID (Pearson correlation, $R = 0.85$, $p < 0.001$) (Figure 4a).

The coefficient of variation (CV) in phytoplankton biomass varied substantially among ponds in both years (Figure SI3 a). The CV of phytoplankton biomass in 2014 was not significantly correlated to the

CV of 2015 (R = 0.31, p = 0.13) (Figure 4b). We observed a significant overall difference in variation in chlorophyll *a* across weeks between both years (Wilcoxon test, V-value = 82, p = 0.03) (Figure SI2 c), with variation within years and ponds being on average higher in 2015 than in 2014.

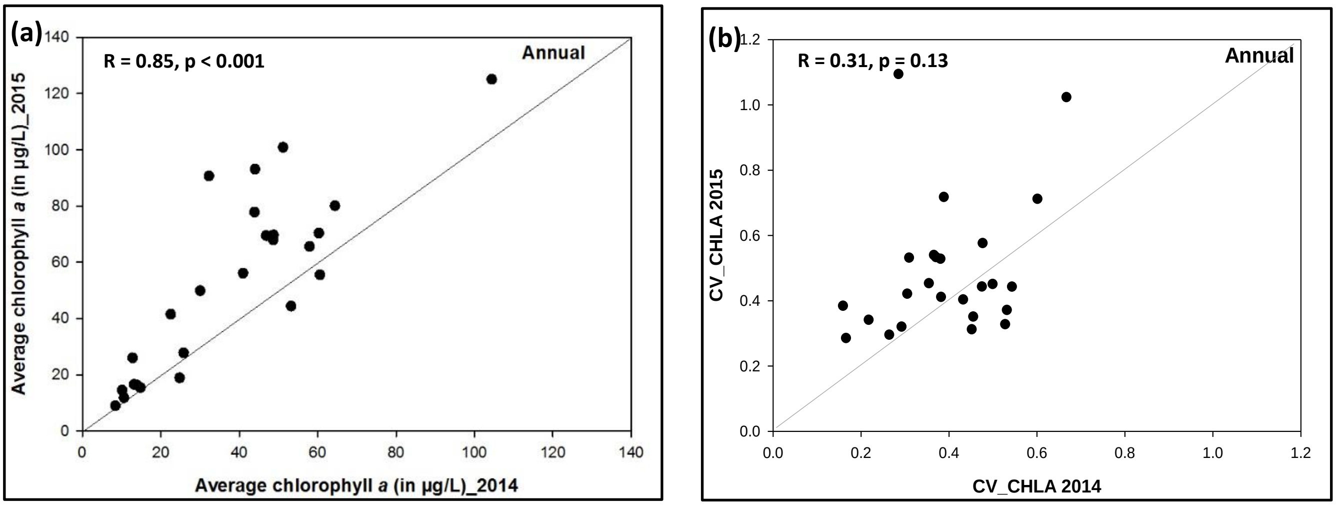

Figure 4. Pearson correlation plots visualizing the association of the (a) annual average phytoplankton biomass and (b) annual average coefficients of variation in chlorophyll *a* across the growth season between the two study years. Lines indicate the 1:1 isocline.

We also observed differences in average phytoplankton biomass when comparing the three seasons for each year separately (linear mixed-effect model, 2014: $F_{(2,42)}$ = 7.466, p < 0.01, post hoc Tukey test, summer versus spring: p = 0.02; autumn versus spring: p < 0.01; 2015: $F_{(2,46)}$ = 9.387, p < 0.01, post hoc Tukey test, summer versus spring: p < 0.01; all other comparisons p>0.05; Figure SI2 b). There was a significant positive correlation between average phytoplankton biomass of 2014 and 2015 across ponds when analyzed for each season separately (Pearson correlation; spring: R = 0.93, p < 0.001; summer: R = 0.75, p < 0.001; autumn: R = 0.69, p < 0.001; Figure SI4 a-c).

In 2014, the linear mixed-effect model showed that the CV in phytoplankton biomass was significantly different between seasons ($F_{(2,42)}$, p < 0.001; post hoc Tukey test, spring versus summer: p < 0.001;

spring versus autumn: $p < 0.001$, Figure SI2 d), while we did not find significant differences in CV among seasons in 2015. There were no significant correlations between CVs in the two study years across ponds when tested for each of the seasons separately (spring: $R = 0.44$, $p = 0.05$; summer: $R = 0.09$, $p = 0.67$; autumn: $R =- 0.29$, $p = 0.32$) (Figure SI4 d-f).

Mechanisms underpinning differences in phytoplankton biomass across ponds

We observed considerable variation in macrophyte cover, zooplankton body size, and nitrogen and phosphorus concentrations among ponds and years (Figure SI5). Macrophyte cover ranged from 0 to 95% (score: 1 to 5). A total of 29 cladoceran species were encountered in the entire set of samples. *Bosmina* was the most abundant taxon. *Daphnia magna* was only found in a limited number of ponds (in 7 of the 25 ponds and in 8 of the 75 samples in 2014; in 2 of the 25 ponds and in 3 of the 75 samples in 2015). The weighted average zooplankton body size ranged from 0.22 to 1.69 mm over the entire set of samples (Figure SI5b). The investigated ponds can be classified as mesotrophic to highly eutrophic based on the average concentration of total nitrogen (TN: 0.7-6.79mg/L; Figure SI5c) and total phosphorus (TP: 0.05-4.56mg/L; Figure SI5d). There were significant correlations of total nitrogen concentrations ($R = 0.44$, $p = 0.03$), total phosphorus concentrations ($R = 0.82$, $p < 0.01$), average macrophyte cover ($R = 0.64$, $p < 0.01$), and average zooplankton body size ($R = 0.58$, $p < 0.01$) across the study ponds between 2014 and 2015 (Figure SI6).

As a first exploration of how macrophyte cover is related to phytoplankton biomass, we show in Figure 5 Pearson's correlation coefficients for the association between macrophyte cover and the phytoplankton biomass in the same ponds for each week, both for 2014 (Figure 5a) and 2015 (Figure 5b). We also performed these analyses for macrophyte cover averaged over the spring (last week of April to third week of June) and summer (last week of June to third week of September), i.e. quantifying the association between phytoplankton biomass at each week with average macrophyte cover in spring (Figure 5c) and summer (Figure 5d) with data paired by pond ID. We find a clear tendency for a negative association between macrophyte cover and phytoplankton biomass. For the weekly associations these are only significant during a few weeks in summer in 2014 and nearly the entire summer season in 2015. Negative associations are especially strong and consistent between phytoplankton biomass and macrophyte cover averaged over the summer, both in 2014 (Figure 5c) and in 2015 (Figure 5d). In the analyses using average macrophyte cover data over spring and summer, the association of phytoplankton biomass across lakes sampled at weekly intervals was significant during the whole year when using summer averages of macrophyte cover, but only during the spring period when using spring averages of macrophyte cover. Macrophyte cover in the summer is more strongly associated with phytoplankton biomass in the spring than macrophyte cover in the spring (compare Figure 5c with 5a and Figure 5d with 5b).

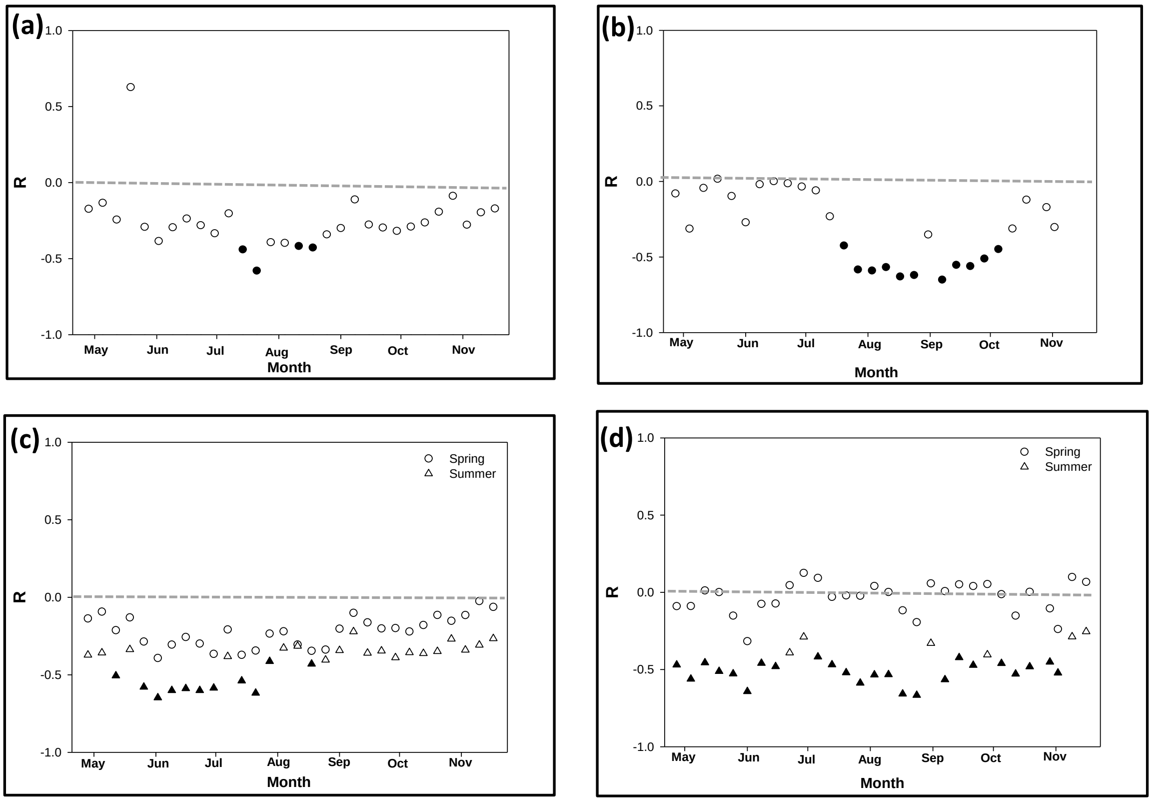

Figure 5. R-values of Pearson correlations between weekly phytoplankton biomass (log(chla) and submerged macrophyte cover during the same week over the entire growth season of 2014 (a) and 2015 (b), and between weekly phytoplankton biomass and macrophyte cover averaged over the spring (last week of April to third week of June)and summer (last week of June to third week of September) for 2014 (c) and 2015 (d). Filled symbols denote significant correlations (p<0.05).

A similar analysis for zooplankton body size, which was quantified only three times in each growing season, reveals that zooplankton body size in spring but not in summer is significantly negatively associated with phytoplankton biomass across the growing season (Figure 6, Figure SI7).

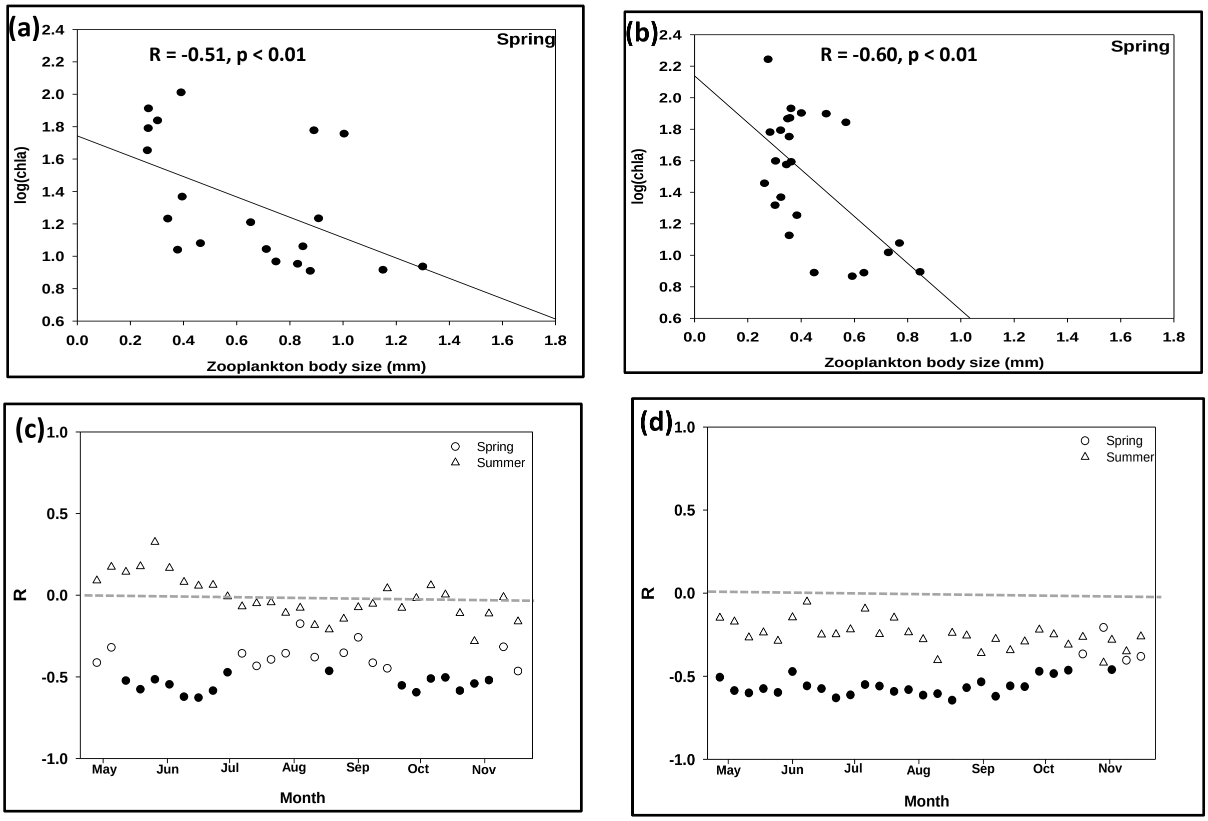

Figure 6. Pearson correlations between the phytoplankton biomass (log(chla)) in a given pond and the average zooplankton body size in the same pond during the spring sampling moment of zooplankton in 2014 (26/05/2014) (a) and 2015 (25/05/2015) (b), and value of the association between weekly phytoplankton biomass (log(chla) and zooplankton body size in spring and summer in 2014 (c) and 2015 (d). Filled symbols denote significant correlations ($p<0.05$).

A multiple regression using nutrient concentrations, zooplankton body size in spring and macrophyte cover in summer revealed that phytoplankton biomass in spring was negatively associated with spring zooplankton body size and positively associated with total nitrogen in both 2014 and 2015 (Table 1).

In both years, phytoplankton biomass in summer was negatively associated with summer macrophyte cover. In 2015, phytoplankton biomass in summer was also positively associated with total nitrogen. Total phosphorus did not show significant associations with phytoplankton biomass in any of the years, although there was a clear tendency for an association for summer chlorophyll *a* in 2015 (see also Figure SI8 for simple correlations).

Table 1. Multiple regression models for spring and summer phytoplankton biomass in 2014 and 2015. Environmental variables that were considered as explanatory values are zooplankton body size in spring, macrophyte cover in summer, and total nitrogen and total phosphorus concentrations. Significant p-values are shown in bold.

	Model and source	**Estimate**	**Std Error**	**t-value**	**p-value**
2014	**Chlorophyll *a* _Spring (R^2adj = 0.58 ,$F_{(2,15)=}$ 12.58 , $p < 0.01$)**				
	Zooplankton body size _spring	-0.4014	0.1715	-2.340	**0.03**
	Total Nitrogen	0.1352	0.0402	3.365	**0.01**
	Chlorophyll *a* _summer (R^2adj = 0.33 ,$F_{(2,15)=}$ 5.184 , p=0.02)				
	Submerged macrophyte cover_summer	-0.1478	0.0572	-2.585	**0.02**
	Zooplankton body size _spring	-0.4222	0.2076	-2.034	0.06
2015	**Chlorophyll *a* _Spring (R^2adj = 0.50 ,$F_{(2,21)=}$ 12.63 , $p < 0.01$)**				
	Zooplankton body size _spring	-0.9750	0.3265	-2.987	**0.01**
	Total Nitrogen	0.0950	0.0353	2.696	**0.01**
	Chlorophyll *a* _summer (R^2adj = 0.42 ,$F_{(3,21)=}$ 6.887 , $p < 0.01$)				
	Submerged macrophyte cover_summer	-0.2067	0.0812	-2.545	**0.02**
	Total Nitrogen	0.1591	0.0530	3.000	**0.01**
	Total Phosphorus	-0.1553	0.0734	-2.115	0.05

Discussion

We observed a substantial variation in overall phytoplankton biomass (chlorophyll *a* concentration) among ponds and years. The study systems, even though they are interconnected and share the same water source, strongly differed in their behavior in terms of being permanently turbid, permanently in the clear-water state, or shifting between both (Figure 2). In both years the majority of ponds showed one or more shift from one state to another (Figure 2, Figure 3). The differences between clear-water ponds and dynamic ones are in most cases strongest during summer, whereas the differences between dynamic and turbid ponds are most striking in spring.

There was an overall positive association between chlorophyll *a* concentrations across ponds in the two years, but their variation (quantified as CV) was not correlated. This suggests that there is some repeatability in the state in which ponds are, but that their tendency to show shifts in chlorophyll *a* concentration throughout the season can vary among years. We observed a higher degree of variation in phytoplankton biomass in 2015 compared to 2014.

Of the 25 lakes, 16 lakes did not change their behavior among years. Three lakes remained in the clear-water state, five remained turbid, and eight showed state shifts in both years. Nine lakes changed their behavior among years. Overall, this implies that in total only eight out of the 25 lakes did not show a pronounced change in chlorophyll *a* concentration during the study period. This shows that, even though we only monitored in the growing season (April – November) and studied a set of interconnected systems that share the same water source, there were substantial dynamics in chlorophyll *a* concentration, both within as well as among lakes. Using our criteria that consistency of state (eight lakes) and repeatable seasonal patterns (eight lakes) might not reflect true regime shifts but rather associations with strong environmental gradients or change, our data suggest that true regime shifts might have occurred in the nine ponds that showed a change in behavior across years. We conclude that variation in pond state is high, even in a set of interconnected and similar ponds as studied here, that part of this variation is likely linked to strong environmental (e.g. food web linked) gradients, but also that a substantial part of this variation might be linked to regime shifts.

We observed a significant negative relationship between phytoplankton biomass and macrophyte cover in summer (Figure 5) and zooplankton body size during spring (Figure 6) in both years (Table 1). The negative association between phytoplankton and macrophyte cover is in line with the suggested mechanisms underpinning stability of the two alternative stable states (Scheffer et al., 1993) and are in line with earlier research such as the study of Norlin et al. (2005), who found that the occurrence of low chlorophyll *a* concentrations was tightly linked to macrophyte cover in shallow lakes. The negative association between submerged macrophytes and phytoplankton biomass during summer can result from competition for nutrients between macrophytes and phytoplankton, macrophytes serving as a refuge for large-bodied zooplankton species that graze on phytoplankton, and the production of allelopathic substances by macrophytes that inhibit the growth of phytoplankton (Timms & Moss, 1984; Hamilton & Mitchell, 1996; Jeppesen et al., 1997; Gross, 2003; Vanderstukken et al., 2014). Several studies have used macrophytes as a remediation tool to restore eutrophied shallow lakes (Zhang et al., 2010; Pan et al., 2011), and submerged macrophytes can stabilize the clear-water state in shallow lakes (Scheffer et al., 1993; Bal et al., 2011). Our observation of a strong negative relationship between phytoplankton biomass and zooplankton body size in spring similarly is in line with the suggested mechanisms underpinning stability of the two alternative stable states (Scheffer et al., 1993) and is likely linked to the high grazing efficiency of large-bodied cladoceran species (Carpenter et al., 2001; Lampert et al., 2007; Verreydt et al., 2012; Gianuca et al., 2016). Our results are also in agreement with findings from multiple enclosure experiments (e.g. Sommer et al., 2003; Stibor et al., 2004; Sommer & Sommer, 2006). We observed a large range in mean cladoceran body size (0.22 – 1.69mm) in our study system, comparable with the range displayed by studies in which zooplankton body size was a significant predictor of chlorophyll *a* (Pace, 1984). The observation that the association between zooplankton body size and phytoplankton biomass only holds for the zooplankton body size distribution in spring, whereas the relationship with macrophyte cover is strongest for macrophyte data in summer, reinforces the suggestion of causal patterns. Once there is a good macrophyte cover in summer suppressing algae through competition and allelopathy, zooplankton densities may be suppressed because of a lack in food. This can explain why zooplankton body size in summer is not a good predictor of phytoplankton biomass. Conversely, zooplankton body size in spring might be crucial in allowing macrophytes to develop, as they cause a top-down control of phytoplankton during the period that macrophytes have to establish themselves after winter and need to do that in competition for light with phytoplankton.

We also detected a positive association between phytoplankton biomass and total nitrogen in our systems (Table 1), in line with an earlier study on this pond system Lemmens et al. (2018). This is striking, because all ponds share the same water source and thus initially tend to have the same water quality. We did not observe a significant relationship with total phosphorus concentrations. The latter might reflect the fact that the N:P ratios in all systems are lower than Redfield ratios (16:1; Sterner & Elser, 2002). There are several studies that have demonstrated the occurrence of nitrogen limitation (Elser et al., 2007; Xu et al., 2010; Harpole et al., 2011) in freshwater systems. Given that all ponds share the same water source, the large range in nutrient concentrations that we observe in the ponds might be a consequence of their different ecology and history. Large differences in fish biomass and differences in histories of pond management might have created a gradient in nutrient concentrations in the sediment (Lemmens et al., 2013).

We conclude that the 25 interconnected study ponds differ strongly in their phytoplankton biomass, and that these differences are associated with differences in nitrogen concentration, macrophyte cover in summer and zooplankton body size in spring. These differences among ponds result in repeatable differences in their state, i.e. whether they are in the clear-water or turbid state. Yet, many ponds shift from one state to another, and nine out of the 25 ponds show a change in dynamics across years. Our results on this set of interconnected ponds suggest that alternative stable states occur, that a spatial survey showing that these two states co-occur in a landscape does not necessarily reflect the occurrence of regime shifts, that regime shifts are nevertheless quite common, and that zooplankton grazing and macrophyte cover are important determinants of phytoplankton biomass and may be crucial in determining the dynamics underlying regime shifts.

Supplementary information to chapter 1

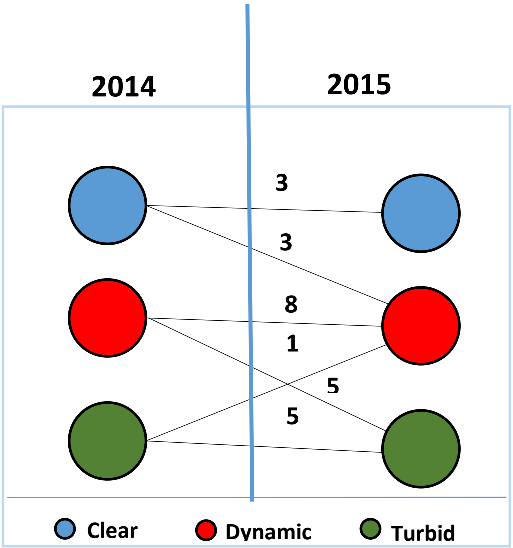

Figure SI1. Summary scheme of the frequencies of ponds in the turbid, dynamic and clear-water state in 2014 and 2015. Arrows with numbers indicate the number of ponds that changed their behavior in the two years.

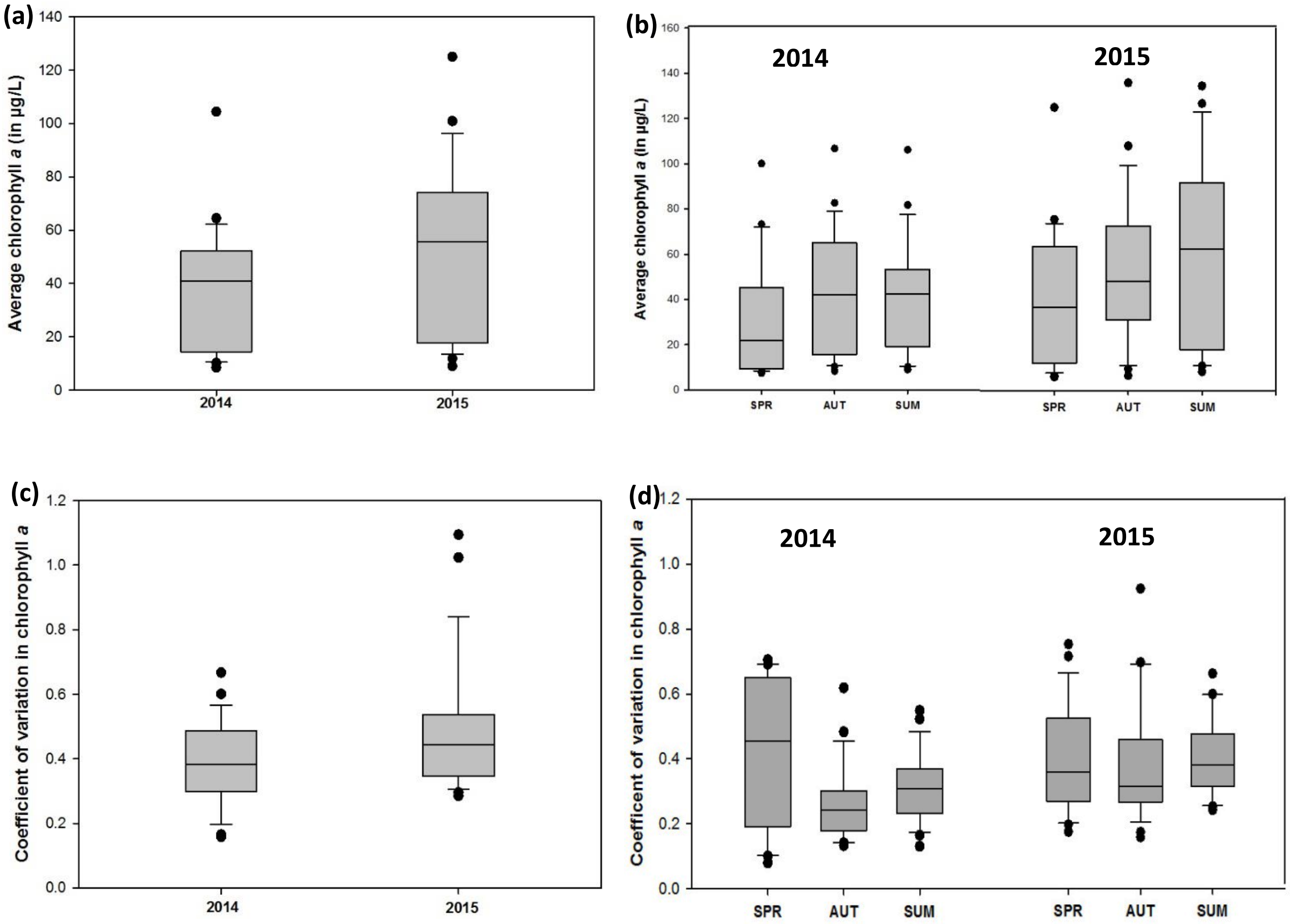

Figure SI2. Box plots of (a) annual and seasonal (spring, summer and autumn) phytoplankton biomass averaged across all ponds (b) for 2014 and 2015, and of (c) annual and seasonal coefficients of variation in chlorophyll-*a* (d) for 2014 and 2015.

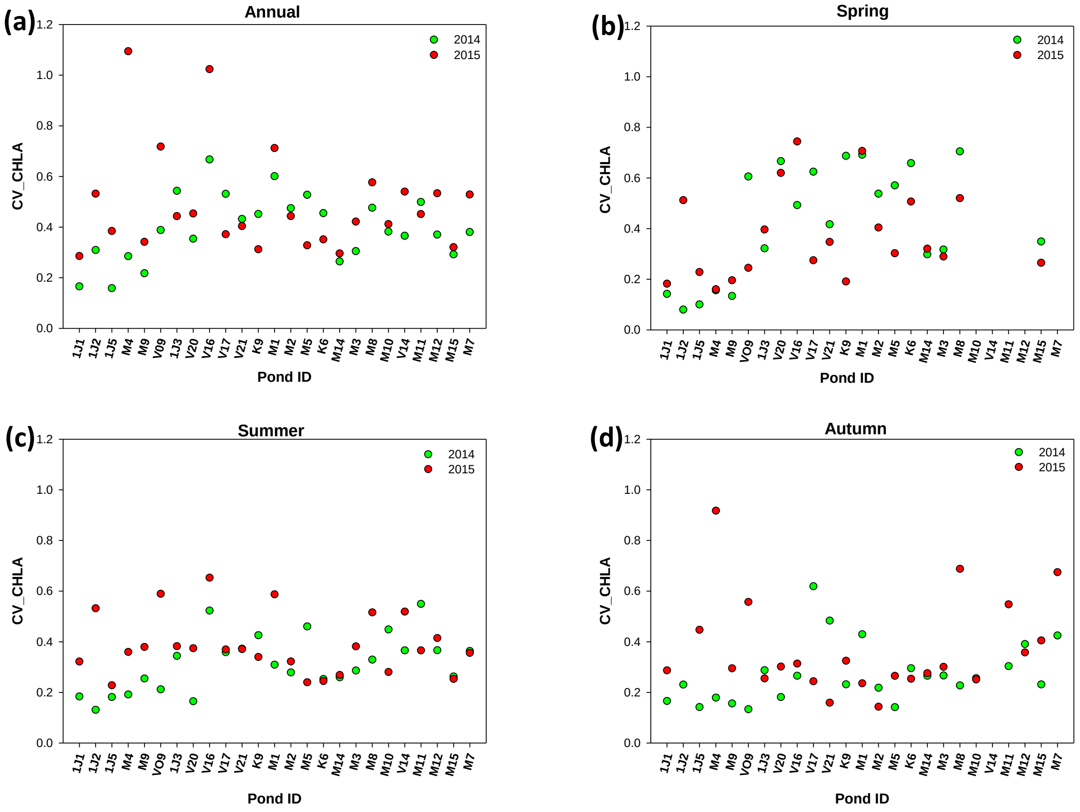

Figure SI3. Coefficient of variation in chlorophyll *a* for each pond (a) across the year and during (b) spring, (c) summer and (d) autumn in 2014 and 2015.

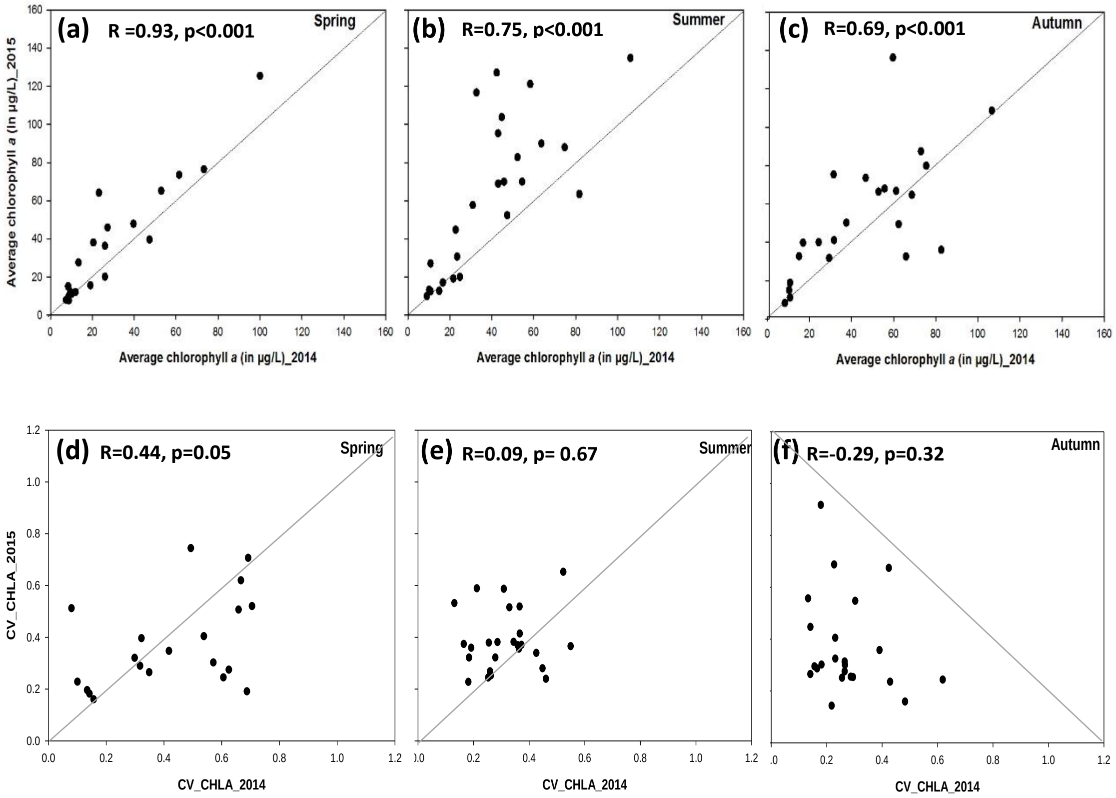

Figure SI4. Pearson correlation plots visualizing the association of the (a) average spring (b), average summer, and (c) average autumn phytoplankton biomass, and Pearson correlation plots visualizing the association of the (d) average spring (e), average summer, and (f) average autumn coefficients of variation in chlorophyll *a* between the two study years. Lines indicate the 1:1 isocline.

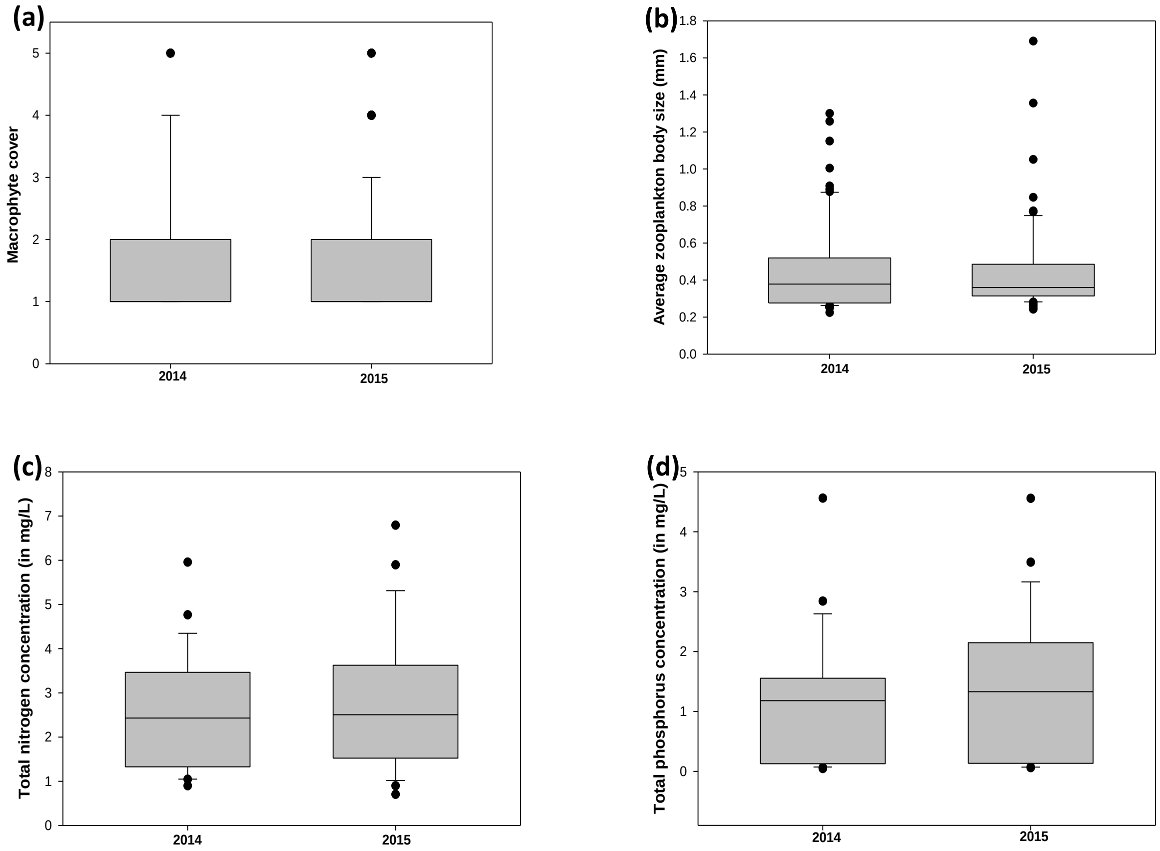

Figure SI5. Box plots of annual averages for (a) macrophyte cover, (b) zooplankton body size, (c) total nitrogen concentrations, and total (d) phosphorus concentrations during the two study years.

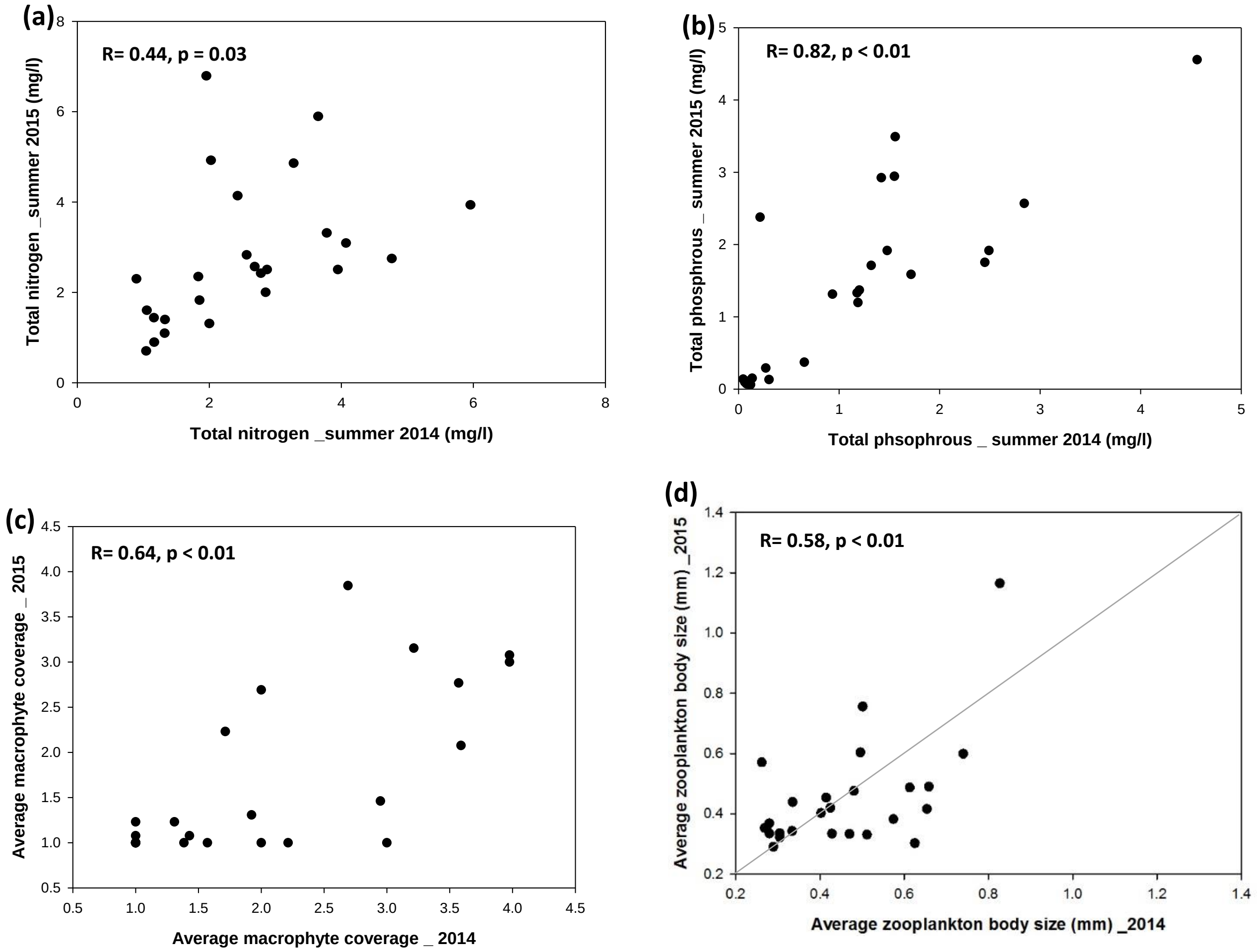

Figure SI6. Pearson's correlation between (a) total nitrogen concentration, (b) total phosphorus concentration, (c), average macrophyte cover and (d) average zooplankton body size between 2014 and 2015. Lines indicate the 1:1 isocline.

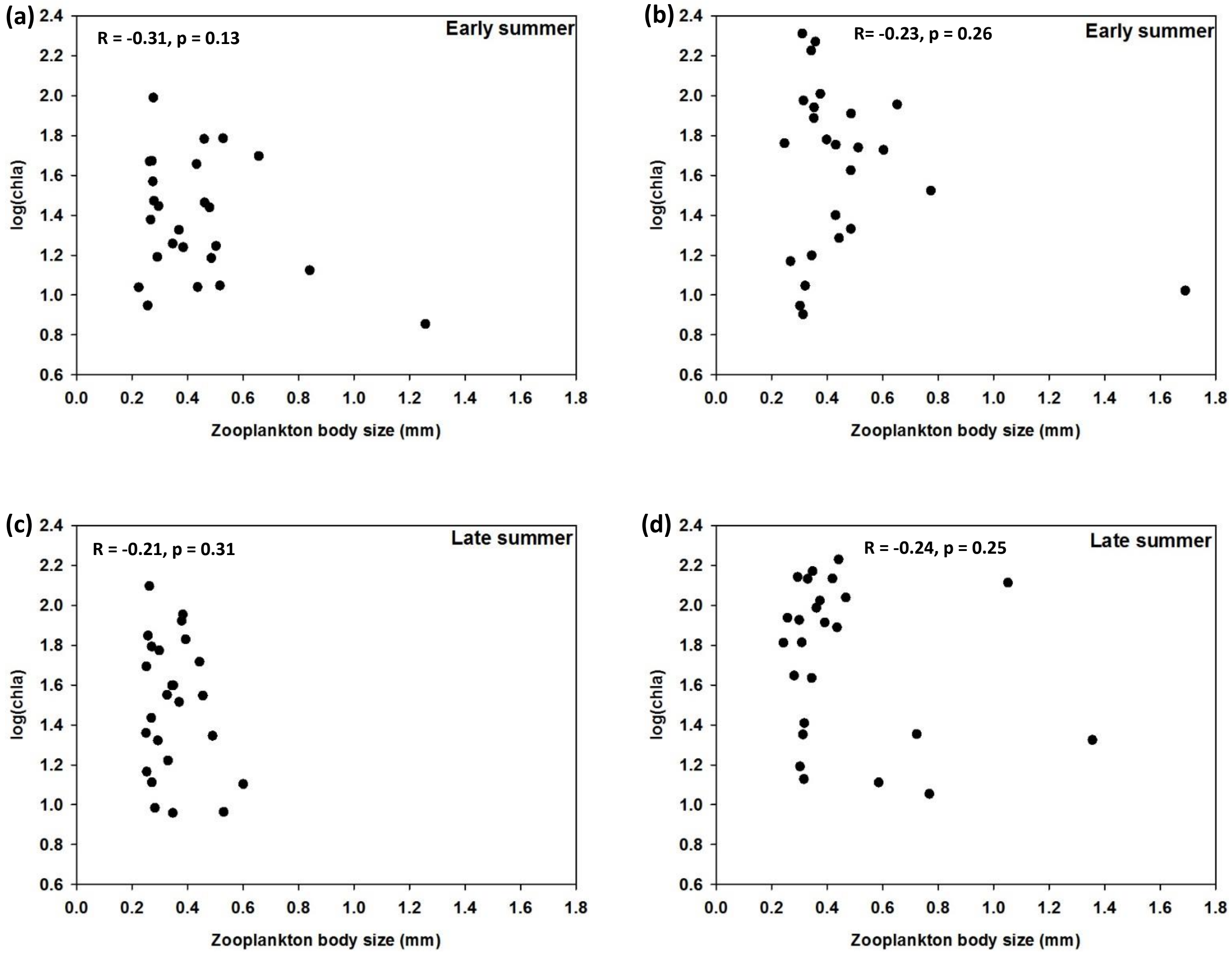

Figure SI7. Pearson correlations between phytoplankton biomass (log(chla)) and average zooplankton body size in (a-c) 2014 and (b-d) 2015 for the sampling moment early summer (first week of July) and late summer (third week of August) for which zooplankton samples were taken.

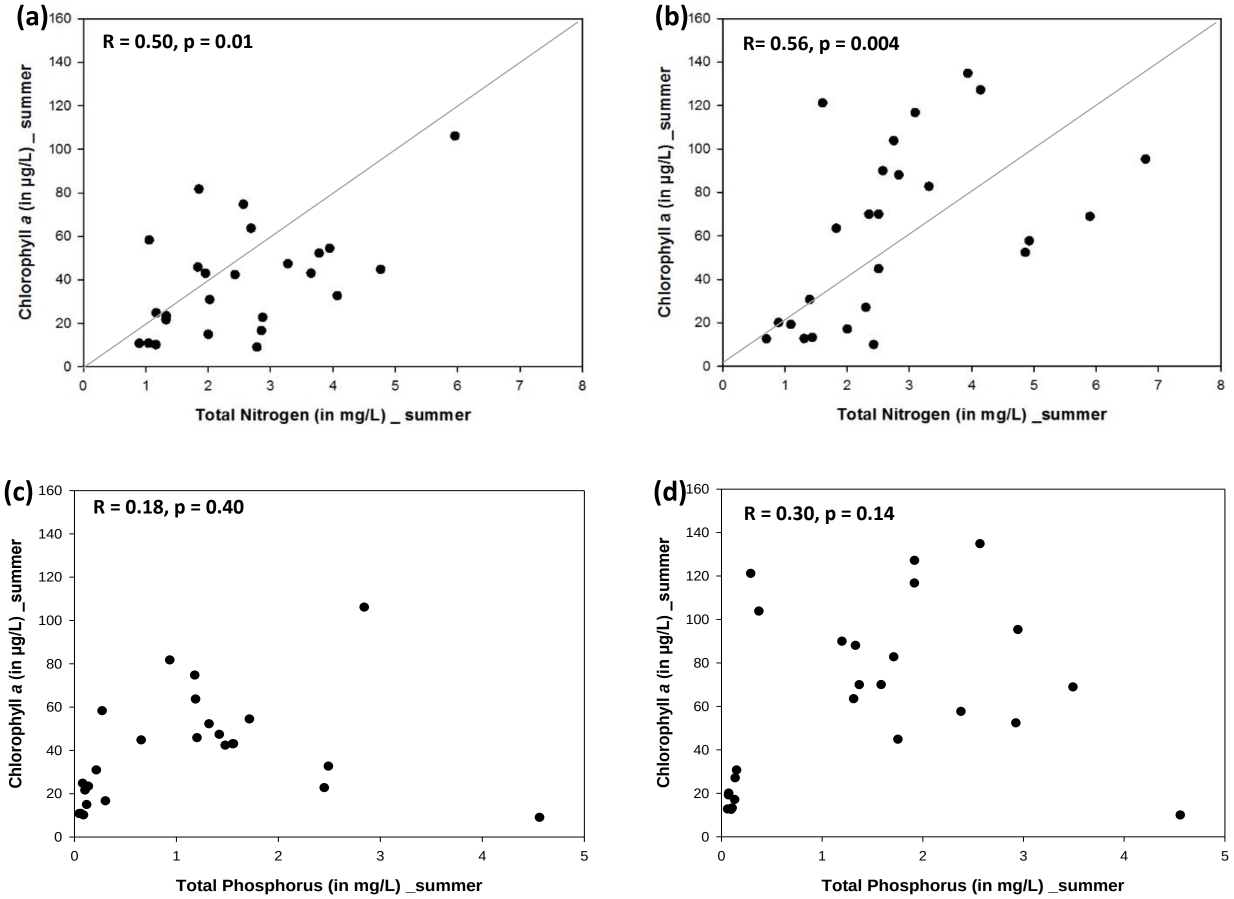

Figure SI8. Pearson's correlation between chlorophyll *a* concentration and nutrients in 2014 (a and c) and 2015 (b and d), for total nitrogen (TN; panels a-b) and total phosphorus (TP; panels c-d).

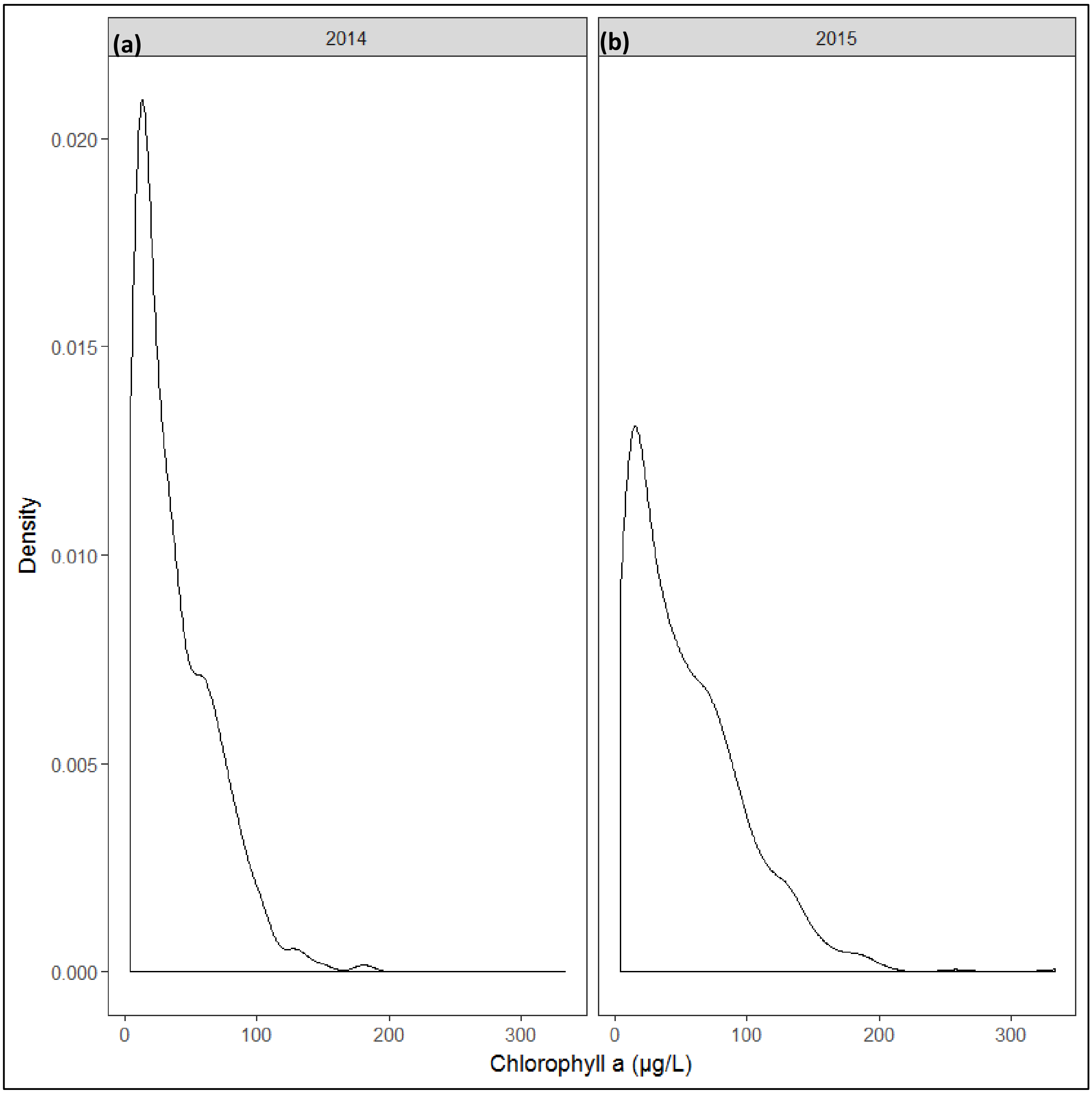

Figure SI9. Frequency distribution of phytoplankton biomass measured in the total dataset of 25 interconnected ponds sampled at weekly intervals in 2014 (a) and 2015 (b).

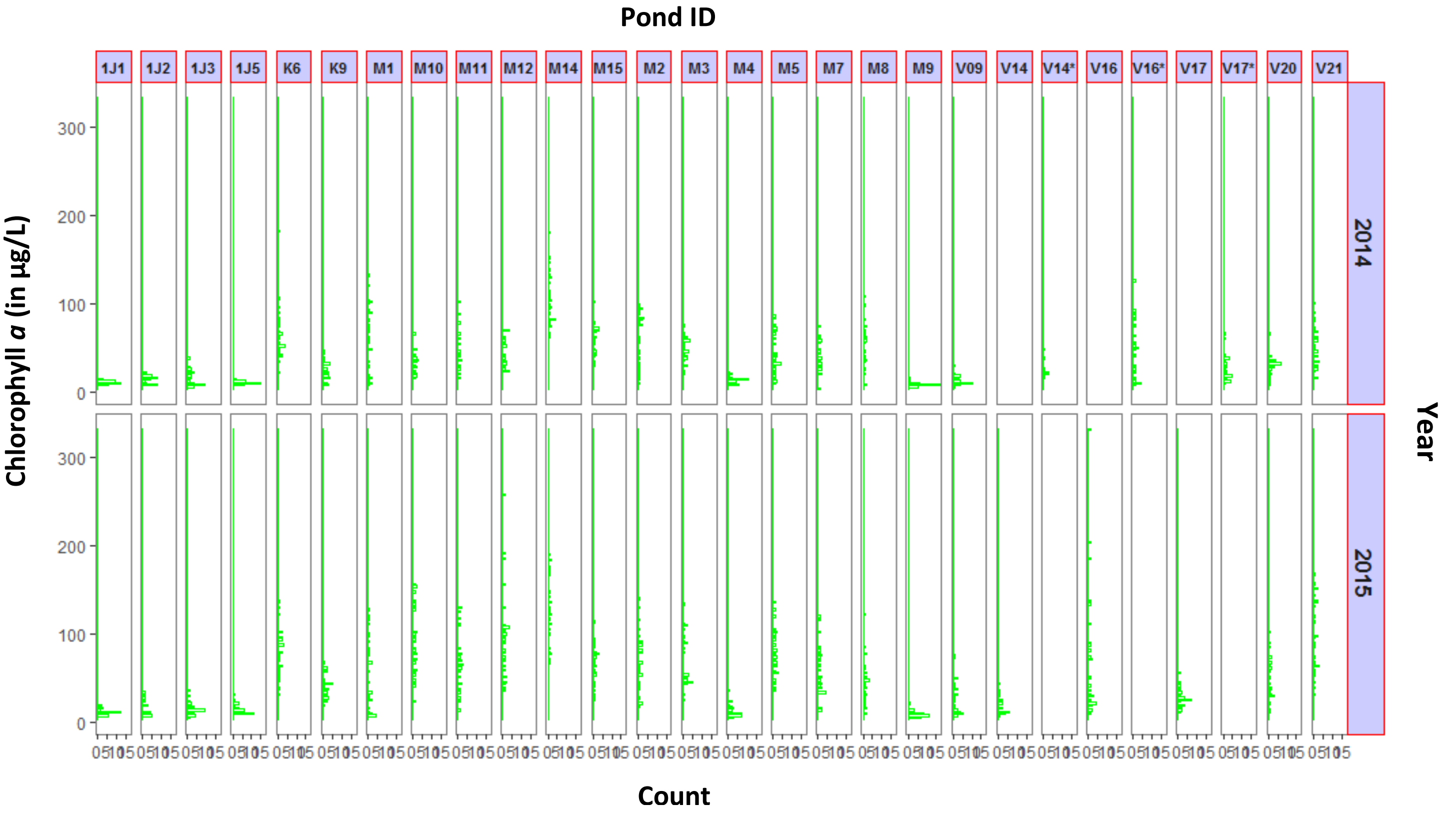

Figure SI10. Frequency distributions for phytoplankton biomass for each pond separately, in 2014 and 2015. For Pond ID see (Figure 1).

Table SI1. Multiple regression models for spring and summer coefficient of variation in chlorophyll a in 2014 and 2015. Environmental variables that were considered as explanatory values are zooplankton body size in spring, macrophyte cover in summer, and total nitrogen and total phosphorus concentrations. Significant p-values are shown in bold.

	Model and source	Estimate	Std Error	t-value	p-value
2014	**CV_CHLA _Spring (R^2adj = 0.20 ,$F_{(3,15)}$= 2.46 , p = 0.10)**				
	Zooplankton body size _spring	-0.4490	0.1707	-2.631	**0.02**
	Total Nitrogen	-0.0700	0.0445	-1.573	0.14
	Submerged macrophyte cover_spring	-0.033	0.0539	-0.614	0.55
	CV_CHLA _summer (R^2adj = -0.12 ,$F_{(3,15)}$= 0.34 , p=0.79)				
	Submerged macrophyte cover_summer	-0.0145	0.0274	-0.529	0.60
	Total Phosphorus	0.0033	0.0269	0.124	0.90
	Zooplankton body size _spring	-0.0569	0.0857	-0.663	0.52
2015	**CV_CHLA _Spring (R^2adj =- 0.02,$F_{(2,20)}$= 0.80 , p=0.46)**				
	Zooplankton body size _spring	-0.1194	0.2493	-0.479	0.64
	Total Nitrogen	0.0239	0.0253	0.943	0.36
	CV_CHLA_summer (R^2adj = -0.08 ,$F_{(3,19)}$= 0.49, p=0.69)				
	Submerged macrophyte cover_summer	0.0430	0.0436	0.986	0.34
	Total Phosphorus	0.0049	0.0277	0.180	0.86
	Zooplankton body size _spring	0.0127	0.1646	0.077	0.94

References

Bal, K., Struyf, E., Vereecken, H., Viaene, P., De Doncker, L., de Deckere, E., . . . Meire, P. (2011). How do macrophyte distribution patterns affect hydraulic resistances? *Ecological Engineering, 37*(3), 529-533.

Bestelmeyer, B. T., Ellison, A. M., Fraser, W. R., Gorman, K. B., Holbrook, S. J., Laney, C. M., . . . Rassweiler, A. (2011). Analysis of abrupt transitions in ecological systems. *Ecosphere, 2*(12), 1-26.

Carpenter, S. R., & Brock, W. A. (2006). Rising variance: A leading indicator of ecological transition. *Ecology Letters, 9*(3), 311-318.

Carpenter, S. R., Cole, J. J., Hodgson, J. R., Kitchell, J. F., Pace, M. L., Bade, D., . . . Schindler, D. E. (2001). Trophic cascades, nutrients, and lake productivity: Whole-lake experiments. *Ecological Monographs, 71*(2), 163-186.

Carpenter, S. R., Cole, J. J., Pace, M. L., Batt, R., Brock, W. A., Cline, T., . . . Seekell, D. A. (2011). Early warnings of regime shifts: A whole-ecosystem experiment. *science, 332*(6033), 1079-1082.

Carpenter, S. R., Cottingham, K. L., & Schindler, D. E. (1992). Biotic feedbacks in lake phosphorus cycles. *Trends Ecol Evol, 7*(10), 332-336. doi: 10.1016/0169-5347(92)90125-u

Carpenter, S. R., Kinne, O., & Wieser, W. (2003). *Regime shifts in lake ecosystems: Pattern and variation* (Vol. 15): International Ecology Institute Oldendorf/Luhe.

Carpenter, S. R., & Lodge, D. M. (1986). Effects of submersed macrophytes on ecosystem processes. *Aquatic Botany, 26*, 341-370.

De Bie, T., De Meester, L., Brendonck, L., Martens, K., Goddeeris, B., Ercken, D., . . . Gucht, K. (2012). Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. *Ecology Letters, 15*(7), 740-747.

Donohue, I., Hillebrand, H., Montoya, J. M., Petchey, O. L., Pimm, S. L., Fowler, M. S., . . . McClean, D. (2016). Navigating the complexity of ecological stability. *Ecology Letters, 19*(9), 1172-1185.

Donohue, I., Petchey, O. L., Montoya, J. M., Jackson, A. L., McNally, L., Viana, M., . . . Emmerson, M. C. (2013). On the dimensionality of ecological stability. *Ecology Letters, 16*(4), 421-429.

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., . . . Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters, 10*(12), 1135-1142.

Flössner, D. (2000). *Die haplopoda und cladocera*. Mitteleuropas Leiden: Backhuys Publishers.

Foley, J. A., Coe, M. T., Scheffer, M., & Wang, G. (2003). Regime shifts in the sahara and sahel: Interactions between ecological and climatic systems in northern africa. *Ecosystems, 6*(6), 524-532.

Free, G., Little, R., Tierney, D., Donnelly, K., & Caroni, R. (2006). A reference based typology and ecological assessment system for irish lakes-preliminary investigations. *Environmental Protection Agency, Wexford.*, 266.

Gianuca, A. T., Pantel, J. H., & De Meester, L. (2016). Disentangling the effect of body size and phylogenetic distances on zooplankton top-down control of algae. *Proceedings. Biological sciences / The Royal Society, 283*(1828). doi: 10.1098/rspb.2016.0487

Gross, E. M. (2003). Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences, 22*(3-4), 313-339.

Hamilton, D. P., & Mitchell, S. F. (1996). An empirical model for sediment resuspension in shallow lakes. *Hydrobiologia, 317*(3), 209-220.

Harpole, W. S., Ngai, J. T., Cleland, E. E., Seabloom, E. W., Borer, E. T., Bracken, M. E. S., . . . Shurin, J. B. (2011). Nutrient co-limitation of primary producer communities. *Ecology Letters, 14*(9), 852-862.

Holling, C. S. (1973). Resilience and stability of ecological systems. *Annual Review of Ecology and Systematics, 4*(1), 1-23.

Irvine, K., Moss, B., & Stansfield, J. (1990). The potential of artificial refugia for maintaining a community of large-bodied cladocera against fish predation in a shallow eutrophic lake *Biomanipulation tool for water management* (pp. 379-389): Springer.

Jeppesen, E., Jensen, J. P., Søndergaard, M., Lauridsen, T., Pedersen, L. J., & Jensen, L. (1997). Top-down control in freshwater lakes: The role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia, 342*, 151-164.

Jiao, Y. (2009). Regime shift in marine ecosystems and implications for fisheries management, a review. *Reviews in Fish Biology and Fisheries, 19*(2), 177-191.

Kéfi, S., Dakos, V., Scheffer, M., Van Nes, E. H., & Rietkerk, M. (2013). Early warning signals also precede non-catastrophic transitions. *Oikos, 122*(5), 641-648.

Kéfi, S., Holmgren, M, & Scheffer, M. (2016). When can positive interactions cause alternative stable states in ecosystems? *Functional Ecology, 30*(1), 88-97.

Koroleff, F. (1970). Determination of total phosphorus in natural waters by means of persulphate oxidation. *An. Interlab. Rep*(3).

Lampert, W., & Sommer, U. (2007). *Limnoecology: The ecology of lakes and streams*: Oxford university press.

Lemmens, P., Declerck, S. A. J., Tuytens, K., Vanderstukken, M., & De Meester, L. (2018). Bottom-up effects on biomass versus top-down effects on identity: A multiple-lake fish community manipulation experiment. *Ecosystems, 21*(1), 166-177. doi: 10.1007/s10021-017-0144-x

Lemmens, P., Mergeay, J., De Bie, T., Van Wichelen, J., De Meester, L., & Declerck, S. A. (2013). How to maximally support local and regional biodiversity in applied conservation? Insights from pond management. *PLoS One, 8*(8), e72538. doi: 10.1371/journal.pone.0072538

Loreau, M., & Mazancourt, C. (2013). Biodiversity and ecosystem stability: A synthesis of underlying mechanisms. *Ecology Letters, 16*(s1), 106-115.

May, R. M. (1977). Thresholds and breakpoints in ecosystems with a multiplicity of stable states. *Nature, 269*(5628), 471-477.

Norlin, J., Bayley, S. E., & Ross, L. (2005). Submerged macrophytes, zooplankton and the predominance of low-over high-chlorophyll states in western boreal, shallow-water wetlands. *Freshwater Biology, 50*(5), 868-881.

O'Brien, R. M. (2007). A caution regarding rules of thumb for variance inflation factors. [journal article]. *Quality & Quantity, 41*(5), 673-690. doi: 10.1007/s11135-006-9018-6

Pan, G., Yang, B., Wang, D., Chen, H., Tian, Bing-hui, Zhang, Mu-lan, . . . Chen, J. (2011). In-lake algal bloom removal and submerged vegetation restoration using modified local soils. *Ecological Engineering, 37*(2), 302-308.

Petraitis, P. (2013). *Multiple stable states in natural ecosystems*: OUP Oxford.

Poikane, S., Portielje, R., Berg, M., Phillips, G., Brucet, S., Carvalho, L., . . . Van Wichelen, J. (2014). Defining ecologically relevant water quality targets for lakes in europe. *Journal of Applied Ecology, 51*(3), 592-602. doi: doi:10.1111/1365-2664.12228

R Development Core Team. (2016). A language and environment for statistical computing. Vienna, Austria: Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Scheffer, M. (1998). *Ecology of shallow lakes*. London, UK: Chapman and Hall.

Scheffer, M., Bascompte, J., Brock, W. A., Brovkin, V., Carpenter, S. R., Dakos, V., . . . Sugihara, G. (2009). Early-warning signals for critical transitions. *Nature, 461*(7260), 53-59.

Scheffer, M., Carpenter, S., Foley, J. A., Folke, C., & Walker, B. (2001). Catastrophic shifts in ecosystems. *Nature, 413*(6856), 591-596.

Scheffer, M., Hosper, S.H., Meijer, M.L., Moss, B., & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution, 8*(8), 275-279.

Scheffer, M., & van Nes, E. H. (2007). Shallow lakes theory revisited: Various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia, 584*(1), 455-466.

Schmitz, O. J. (2004). Perturbation and abrupt shift in trophic control of biodiversity and productivity. *Ecology Letters, 7*(5), 403-409.

Schröder, A., Persson, L., & De Roos, A. M. (2005). Direct experimental evidence for alternative stable states: A review. *Oikos, 110*(1), 3-19.

Sommer, U., & Sommer, F. (2006). Cladocerans versus copepods: The cause of contrasting top–down controls on freshwater and marine phytoplankton. *Oecologia, 147*(2), 183-194.

Sommer, U., Sommer, F., Santer, B., Zöllner, E., Jürgens, K., Jamieson, C., . . . Gocke, K. (2003). Daphnia versus copepod impact on summer phytoplankton: Functional compensation at both trophic levels. *Oecologia, 135*(4), 639-647.

Søndergaard, M., Jeppesen, E., Lauridsen, T. L., Skov, C., Van Nes, E. H., Roijackers, R., . . . Portielje, R. O. B. (2007). Lake restoration: Successes, failures and long-term effects. *Journal of Applied Ecology, 44*(6), 1095-1105.

Søndergaard, M., Johansson, L. S., Lauridsen, T. L., Jørgensen, T. B., Liboriussen, L., & Jeppesen, E. (2010). Submerged macrophytes as indicators of the ecological quality of lakes. *Freshwater Biology, 55*(4), 893-908.

Sterner, R. W., & Elser, J. J. (2002). *Ecological stoichiometry: The biology of elements from molecules to the biosphere*: Princeton University Press.

Stibor, H., Vadstein, O., Diehl, S., Gelzleichter, A., Hansen, T., Hantzsche, F., . . . Peters, C. (2004). Copepods act as a switch between alternative trophic cascades in marine pelagic food webs. *Ecology Letters, 7*(4), 321-328.

Tessier, A. J., Leibold, M. A., & Tsao, J. (2000). A fundamental trade-off in resource exploitation by daphnia and consequences to plankton communities. *Ecology, 81*(3), 826-841.

Timms, R. M., & Moss, B. (1984). Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. *Limnology and Oceanography, 29*(3), 472-486.

Van Donk, E., Grimm, M. P., Gulati, R. D., & Breteler, J.P.G.K.(1990). Whole-lake food-web manipulation as a means to study community interactions in a small ecosystem. *Hydrobiologia, 200*(1), 275-289.

Vanderstukken, M., Declerck, S. A. J., Decaestecker, E, & Muylaert, K. (2014). Long-term allelopathic control of phytoplankton by the submerged macrophyte elodea nuttallii. *Freshwater Biology, 59*(5), 930-941.

Verreydt, D., De Meester, L., Decaestecker, E., Villena, M. J., Van Der Gucht, K., Vannormelingen, P., . . . Declerck, S. (2012). Dispersal-mediated trophic interactions can generate apparent patterns of dispersal limitation in aquatic metacommunities. *Ecology Letters, 15*(3), 218-226.

Worm, B., Barbier, E. B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., . . . Palumbi, S. R. (2006). Impacts of biodiversity loss on ocean ecosystem services. *science, 314*(5800), 787-790.

Xu, H., Paerl, H. W., Qin, B., Zhu, G., & Gaoa, G. (2010). Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic lake taihu, china. *Limnology and Oceanography, 55*(1), 420-432.

Zhang, Shi-yang, Liu, Ai-fen, Ma, Jian-min, Zhou, Qiao-hong, Xu, D., Cheng, Shui-ping, . . . Wu, Zhen-bin. (2010). Changes in physicochemical and biological factors during regime shifts in a restoration demonstration of macrophytes in a small hypereutrophic chinese lake. *Ecological Engineering, 36*(12), 1611-1619.

Chapter 2: Year-to-year variation in environmental conditions and zooplankton community composition in a set of interconnected ponds

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Abstract

Understanding how communities are assembled and vary in space and time is the key goal of community ecology. In shallow lakes, there is evidence for both sorting of species in response to environmental gradients and for the existence of alternative stable states that under similar abiotic conditions show pronounced differences linked to food web structure. In the present study, we monitored zooplankton community composition and environmental variables in a set of 25 interconnected fish ponds during three consecutive years to assess variation in space and time and its link to changes in environmental conditions and fish stock management. We show that in all three years fish stock management, involving a change in food web structure, is the main determinant of zooplankton metacommunity structure as well as for local environmental conditions. Pond ID was associated with the first PCA axis of environmental variation, explaining 49 % of variation in environmental conditions, while changes among years were associated with the second PCA axis, capturing 12 % of the environmental variation. The pattern of variation in zooplankton community composition was more erratic and not systematically linked to the first or second PCA axis. Based on our results, we conclude that environmental conditions and zooplankton community composition are rather consistent within ponds among years in this set of interconnected ponds. In addition, biotic interactions seem to a more important for structuring this regional zooplankton metacommunity than the abiotic environment.

Introduction

Understanding the mechanisms underpinning temporal and spatial variation in community characteristics is a central goal of community ecology (Vellend, 2010; Leibold et al., 2018). Ecological communities assemble and change over time through a combination of four major processes, being dispersal, selection, speciation and stochastic fluctuations (Vellend, 2010; Leibold et al., 2018). These processes can act independently of each other, but they may also play interactively and simultaneously at multiple spatial and temporal scales (Cooper, 2007; Vellend, 2010).

In a niche-dominated perspective, the degree of variability in community composition is expected to depend on environmental conditions (Cadotte et al., 2006; Violle & Jiang, 2009). One can make a distinction between the abiotic (physical and chemical) characteristics of the habitat, and the biotic environment and associated biotic interactions that are themselves in part the result of the abiotic environment but might provide key environmental gradients to the focal community (Bengtsson, Baillie, & Lawton, 1997). Relationships between the structure of biological communities and environmental factors have been found in many types of ecosystems (e.g. Melo, 2009; Ng et al., 2010), including shallow lakes and ponds (Cottenie, Michels, et al., 2003; Souffreau et al., 2018).

Shallow lakes are also prime examples of systems that exhibit alternative stable states, including a turbid phytoplankton dominated state and a clear-water state with profound submerged aquatic vegetation (Scheffer et al., 1993; Scheffer et al., 2001). The turbid and clear-water state are each stabilized by feed-back loops that involve biotic interactions and are determined by competitive interactions between macrophytes and phytoplankton and by trophic cascades (Scheffer et al., 1993; Scheffer, 1998). The environmental conditions that affect freshwater zooplankton communities are particularly well studied (Pulliam, 1988; Cottenie & De Meester, 2003) and provide striking examples of the strength of local environmental drivers of community composition (Carpenter, 2012). These patterns are generally clear because lakes represent discrete "islands" for zooplankton. Although zooplankton has relatively good dispersal capacities (Havel & Shurin, 2004; Kramer, Sarnelle, & Knapp, 2008; Frisch et al., 2012), the number of individuals dispersing among habitats is low compared to internal dynamics of community change, resulting in strong patterns of niche-based sorting (De Bie et al., 2012). Abiotic factors driving variation in freshwater zooplankton communities among lakes include water chemistry (e.g., nutrient concentrations, pH, conductivity) and turbidity (Johannsson, Mills, & O'Gorman, 1991). Biotic drivers can involve both bottom-up factors involving resources and top-down factors such as predation (Carpenter, Kitchell, & Hodgson, 1985; McQueen, Post, & Mills, 1986; Vanni, 1987; Lampert & Sommer, 2007; Lemmens et al., 2018). Predation by fish is generally considered a common and important driver of among lake variation in zooplankton community composition. Zooplankton communities in lakes with high fish predation are typically dominated by small-bodied species, because large-bodied species are more vulnerable to visual predators (Brooks & Dodson, 1965; Jeppesen et al., 2003; Brucet et al., 2010). Zooplankton communities in lakes with no or low fish predation are often dominated by larger bodied zooplankton as they are stronger competitors (Lampert et al., 2007). Many studies have documented the importance of trophic cascades in structuring zooplankton communities in lakes (Carpenter et al., 1985; Carpenter & Kitchell, 1996; Jeppesen et al., 1997; Scheffer, 1998; Lemmens et al., 2018).

Understanding the temporal and spatial variation in communities is also important to assess the value of snapshot surveys to our understanding of community assembly as well as to assess to what extent patterns in space can be used to predict changes in response to environmental changes in time. One approach to assess how variation in space and time relate to each other is carrying out repeated surveys through time. In the context of shallow lakes, repeated surveys in time also allow to assess the stability of the system and its associated zooplankton community.

The present study aims to investigate the year-to-year variation in local environmental conditions and zooplankton community composition in a set of 25 regionally clustered interconnected ponds during three consecutive years. We relate zooplankton community composition to major environmental variables and fish stock management across years and ponds to assess the repeatability of the patterns.

Focusing on a set of regionally clustered interconnected ponds allows us to ignore speciation as an important process that might structure communities (Vellend, 2010). Working on interconnected systems explicitly tests for the importance of environmental drivers in a context providing good connectivity, so excluding dispersal limitation as a structuring factor (Cottenie, Michels, et al., 2003). It also provides a setting where differences in environmental conditions are not driven by differences in water quality (as all ponds share the same water source), but rather reflects internal ecosystem structuring linked to biotic interactions such as trophic cascades and physical structure offered by macrophytes (Cottenie, Michels, et al., 2003). Our specific objectives are to (1) relate variation in zooplankton community composition across ponds and years to fish stock management and variation in major local environmental pond conditions; (2) explore to what extent zooplankton community composition is determined by the same drivers across years; and (3) determine whether changes in fish stock management mediate changes in local environmental pond conditions and zooplankton community composition.

Materials and methods

Study area and sampling

The study was carried out in “Vijvergebied Midden-Limburg”, located in the north-eastern part of Belgium (50° 59’ 00.92” N; 5° 19’ 55.85” O) (Figure 1). The region comprises approximately 4000 hectares dry and wet heats, forest, and reed vegetation, and it includes more than 1000 interconnected shallow man-made ponds (Lemmens et al., 2013). All these ponds share a common water source (Roosterbeek) and have historically been managed for purposes of fish farming. Because of its high international value for biodiversity conservation, the region is currently designated as Nature 2000 site and protected by the EU Birds Directive and the Habitats Directive.

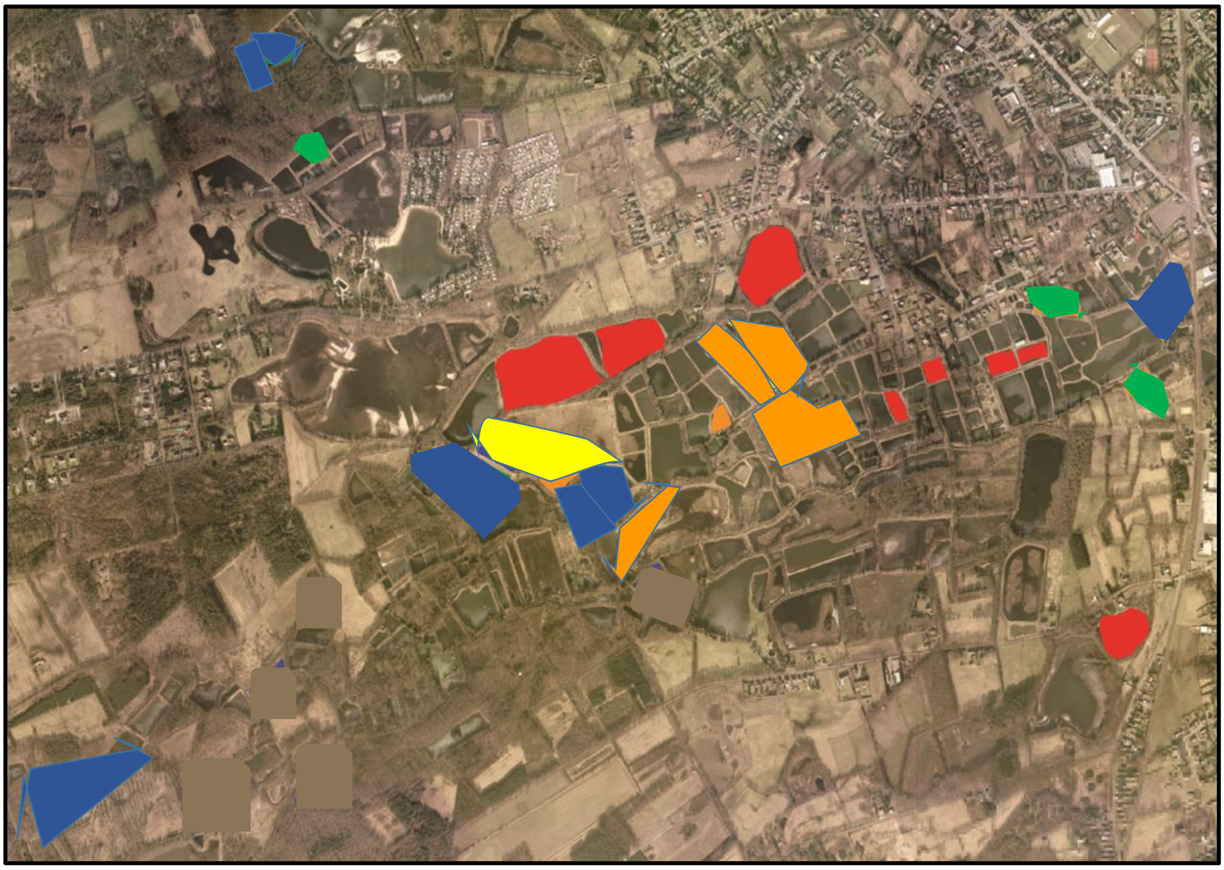

Figure 1. Overview of a part of “Vijvergebied Midden –Limburg” with the studied ponds shown in color. Different colors represent different fish stock types (CF: red, EXT: orange, JF: green, NF: blue, NM: yellow, see also Table 1).

We randomly selected a set of 25 ponds representing different fish stock types (see Table 1). Depending on the applied fish stock management, the selected ponds have different fish densities and thus differ with respect to fish predation intensity.

Table 1**.** Description of the different fish stock types.

Management type	Purpose	Fish stock management	Frequency of drainage
No fish (NF)	To create fishless ponds (mainly for amphibian conservation	No fish stocking. Nets are placed on the inlets to avoid immigration of fish.	Ponds are drained annually in autumn and refilled in early spring
Juveniles (JF)	commercial farming of juvenile fish	Stocking with fish fry (ide [*Leuciscus idus*] and Common caro [*Cyprinus carpio*]) in late spring. Overall fish density is relatively low. Fish is harvested in autumn.	Ponds are annually drained in autumn and are refilled in spring in order to allow development of lush emergent vegetation for juveniles.
No management (NM)	No specific purpose	No fish stock management. Fish can freely move in and out the ponds via rivulets.	Last drainage more than ten years ago.
Extensive (EXT)	To maintain extensive fish farming practices that historically resulted in high conservation values	Ponds were drained, refilled and initially stocked with adult rudd (*Rutilus rutilus*) tench (*Tinca tinca*) and pike (*Esox lucius*) (total 40 kg ha^{-1}). Fish can freely move in and out of the ponds.	Ponds are occasionally drained approximately every five years, but irregularly spaced in time.
Carp (CF)	Commercial semi - intensive farming (mainly common carp, 1000 kg ha^{-1} year^{-1}	Pond are stocked with 1000 kg ha $^{-1}$ of fish in spring. Use of artificial feeds (ca. 1400 kg ha^{-1} year $^{-1}$) to increase fish production	Annual or bi-annual winter drainage to harvest fish

(from Lemmens et al., 2015)

Some ponds (n=4, see also Table SI1) changed fish stock management over the study period, which allows us to relate changes in fish stock type to changes in environmental conditions and zooplankton community composition. The investigated ponds were located close to each other to assure that they share the same regional zooplankton species pool. Major local environmental variables and zooplankton community composition were determined for each pond once a year in August during three consecutive years (2013, 2014 and 2015). Earlier investigations revealed the absence of a clear relation between fish stock management and pond morphology (Lemmens et al., 2013).

Zooplankton communities were sampled quantitatively in the littoral and pelagic zone of each pond. Depth integrated water samples (25L) were collected with a tube sampler (length 1.2m; diameter 75mm) at five randomly chosen locations in both mesohabitats (littoral and pelagic). Samples from both mesohabitats were pooled together and 40L from this combined sample was filtered through a conical plankton net (mesh size 64µm). Zooplankton samples were preserved with glucose-saturated formaldehyde (4% concentration). Cladocerans were identified to species level following Flössner (2000) and counted, except for the genera *Chydorus* and *Ceriodaphnia* that were identified to genus level. *Daphnia galeata* and *Daphnia longispina* were considered as one taxon (*Daphnia* complex) since they both belong to the subgenus Hyalodaphnia (Petrusek, Hobæk, et al., 2008), and hybrids between both taxa are frequently observed (Petrusek, Seda, et al., 2008). Copepods were classified into two major groups, cyclopoids and calanoids, and counted.

Water transparency was determined with a sneller tube (Louette & De Meester, 2005). Conductivity and pH were measured using standard electrodes (Hach multimeter). Water samples for the measurement of total phosphorus (TP) and total nitrogen (TN) concentrations were taken from the pooled water sample taken with a tube sampler and were stored cool in the field. In the laboratory, these samples were frozen at -20˚C until further analysis. TP and TN concentrations were quantified using a Technicon Autoanalyzer II (Technicon, Tarrytown, New York, USA) after alkaline persulphate digestion (Koroleff, 1970). The amount of suspended solids in the water column was determined gravimetrically by filtering a known volume of pond water through a dry pre-weighed glass fiber filter (Whatmann, GF/F). Phytoplankton biomass and the density of cyanobacteria were estimated from depth integrating pelagic water samples through *in vivo* measurement of chlorophyll *a* and phycocyanine concentrations, respectively, using a hand-held fluorometer (Aquafluor, Turner Design, Sunnyvale, CA). The percentage of pond surface covered with submerged and emergent vegetation was estimated visually during each sampling occasion.

Data analysis

The variation in environmental characteristics and zooplankton community composition in relation to fish stock type, pond identity (Pond ID) and sampling year (Year) was visualized using ordination plots of Principal Component Analyses (PCA). Variation partitioning analyses were used to formally test for the shared and unique contributions of fish stock type, Pond ID and Year on variation in local environmental pond characteristics and zooplankton community composition. Variation partitioning allows to partition the total amount of variation explained by a statistical model into unique and shared contributions of sets of explanatory variables (Peres-Neto et al., 2006; Borcard, Gillet, & Legendre, 2011). The significance of the models was evaluated using Monte Carlo permutations (n=999).

Similarity in environmental conditions and zooplankton community composition within ponds between years was investigated by comparing PCA ordinations from different years through multiple Procrustes analyses. Procrustes analysis allows to compare two sets of site scores from two separate ordinations (Legendre, 2012) by scaling, rotating and dilating one ordination solution to match the other ordination(Lisboa et al., 2014). The significance of the Procrustean fits was tested with a Monte Carlo statistic using PROTEST (Jackson, 1995; Peres-Neto et al., 2006). The residuals of the Procrustes analyses, which are an inverse measure of congruency between site scores of two ordinations, were used to explore differences in similarity in environment characteristics and in zooplankton community between years for different fish stock types and for ponds that changed fish stock type between years.

Procrustes analyses and subsequent randomization tests were also used to investigate the association of zooplankton community structure with local environmental pond characteristics for each year separately. Additional variation partitioning analyses were used to test for the relative importance of the shared and unique contributions of fish stock type, Pond ID and local environment on variation in zooplankton community composition.

Prior to statistical analyses, all environmental variables, except pH, were logarithmically transformed to improve assumptions of normality. Zooplankton abundance data were Hellinger transformed

(Legendre & Gallagher, 2001). All statistical analyses were done in R (version 3.4.4) using the ‘vegan’ package (Oksanen et al., 2013).

Results

The investigated ponds ranged widely in local environmental conditions (Table 2). The entire set of ponds clearly represents a gradient from a turbid, phytoplankton dominated state to a clear water state with profound submerged aquatic vegetation. The first two axes of the PCA ordination plot jointly explained more than 60% of the variation in local environmental conditions between ponds across years (Figure 2A).

The first PCA axis comprised 49% of the environmental variation and was closely associated with turbidity related variables, including nutrient concentrations, phytoplankton biomass, water transparency and the percentage of coverage with submerged aquatic vegetation (Table SI2). The second PCA axis explained considerably less environmental variation (12%) and tended to be more associated with water temperature, pH and conductivity. Carp ponds differentiated from other fish stock types by high concentrations of chlorophyll *a*, phycocyanin and nutrients (Figure SI1). Differences in environmental conditions were less pronounced for other fish stock types, but ponds without fish and ponds with juvenile fish tended to have higher coverage with submerged vegetation and higher water transparency (Figure SI1).

The first and second axes of the PCA ordination plot based on zooplankton community composition jointly explained 63% of the compositional variation between ponds across years (Figure 2B). The first axis was positively associated with cyclopoid copepods and had a negative association with *B. coregoni*. The second axis seemed positively associated with the genus *Ceriodaphnia*, and to lesser extent also with calanoid copepods (Table SI3). Also here, carp ponds seemed to differ from other fish stock types by the higher abundances of relative small bodied cladocerans (*Moina sp.* and *Bosmina sp.*) and cyclopoid copepods in these ponds. A similar pattern of variation in local environment and zooplankton community composition between ponds could also be observed for each year separately (Figure SI2.)

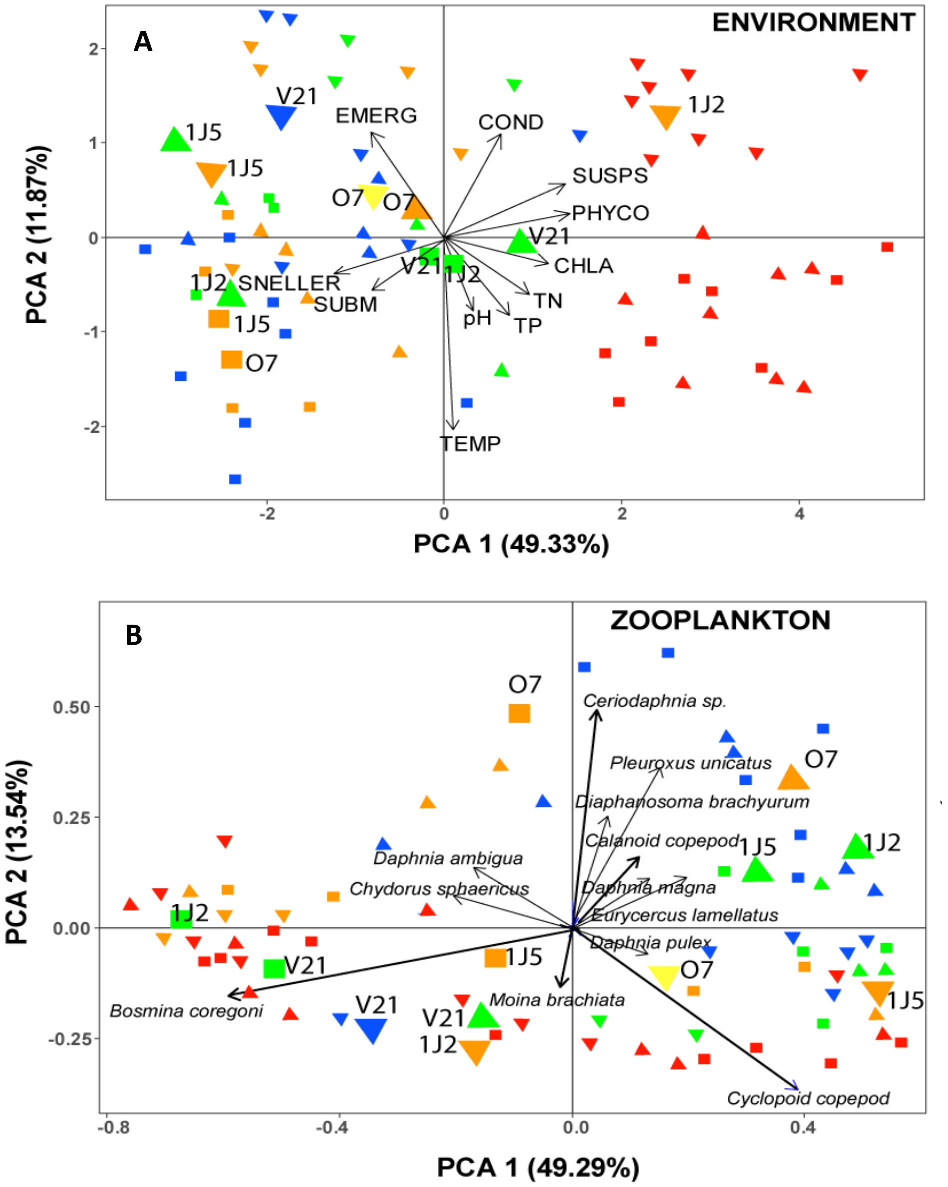

Figure 2. Ordination plot of a Principal Component Analysis visualizing (A) the association between pond identity (Pond ID), year and fish stock type with local environmental conditions, and (B) the association between the association between pond identity, year and fish stock type with zooplankton community composition. Black arrows represent environmental variables or zooplankton taxa. Small symbols show the position of each pond in all three studied years (▽2013, □ 2014, and △ 2015). Large symbols show the change in fish stock types. Different colors represent different fish stock types (CF: red, EXT: orange, JF: green, NF: blue, NM: yellow, see also Table 1).

Table 2. Median (minimum, maximum) values for the different environmental variables as quantified for all investigated ponds in summer 2013, 2014 and 2015.

Environmental variables	2013	2014	2015
Water transparency (cm)	17 (8-38)	24 (12-46)	22 (13-41)
Suspended solids (mg/L)	39.9 (4.08-209)	15.12 (5.12-90.60)	14.8 (0.86-67.27)
Water temperature (°C)	18.95 (16.2-21.55)	23 (19.4-26.6)	23.9 (20.8-28.2)
pH	7.83 (7.47-9.5)	8.25 (7.5-10.5)	8.05 (2.93-9.5)
Total nitrogen (mg/L)	1.855 (0.03-7.29)	1.89 (0.9-6.05)	2.30 (0.70-4.54)
Total phosphorus (mg/L)	0.25 (0.08-0.76)	0.19 (0.04-3.61)	0.22 (0.04-2.4)
Conductivity (µS/cm)	383 (267-466)	308 (224-392)	407 (246-461)
Chlorophyll a (µg/L)	20.34 (8.05-93.6)	18.0 (9.7-150.8)	23.5 (8.9-161.8)
Phycocyanine (RFU)	2.1 (0.6-18.9)	0.9 (0.4-18.2)	1.9 (0.4-8.8)
Submerged vegetation cover (%)	3 (0-70)	15 (0-80)	10 (0-95)
Emergent vegetation cover (%)	15 (0-80)	15 (0-80)	10 (0-80)

Closer inspection of the plots revealed that the pond scores for PCA axis 1 for environmental conditions remained consistent across years for a given pond (Figure 3), which implies that environmental conditions are rather consistent among years, that ponds consistently differ in the local environmental conditions that they provide, and that variation among ponds is larger than among years. For PCA axis 2, variation was more strongly linked to time than to pond identity. For zooplankton community composition, variation along PCA1 and PCA2 was less consistently linked to pond identity or time than for environmental variation (Figure 3).

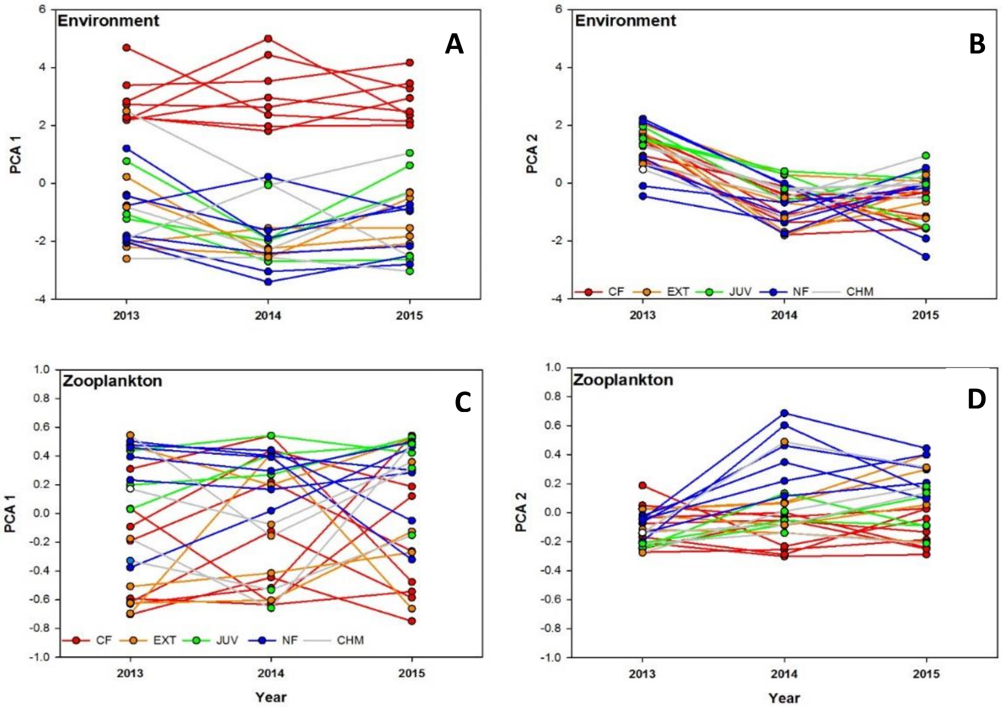

Figure 3. PCA scores of all 25 ponds for PCA axis 1 (A and C) and PCA axis 2 (B and D) for environmental variables (plot A and B) and zooplankton community composition (C and D) in all three studied years. The plot shows how PCA scores change from year to year. Colors of lines represent different fish stock management types.

Procrustes analyses and subsequent randomization tests confirmed strong similarity in local environmental conditions and zooplankton community composition within ponds among years (Table 3). Congruency in environmental and zooplankton community between years did not differ systematically between different fish stock types, nor did we find clear changes in congruency of environmental and zooplankton community structure for ponds that changed fish stock type between years (Figure SI3).

Table 3. Results of Procrustes analyses and subsequent randomization tests testing the concordance of PCA ordinations based on environmental variables and zooplankton community composition among different years.

		r	m²	P
Environmental characteristics				
	2013-2014	0.709	0.497	0.001
	2014-2015	0.728	0.524	0.001
	2013-2015	0.690	0.470	0.001
Zooplankton community composition				
	2013-2014	0.610	0.629	0.002
	2014-2015	0.498	0.752	0.068
	2013-2015	0.560	0.687	0.004

Variation partitioning analysis revealed that variation in local environmental conditions between ponds was significantly explained by fish stock type and Pond ID, whereas sampling year did not significantly explain any variation in local environmental pond conditions (Figure 4A). Fish stock type overall explained 26.73% of the environmental variation between ponds, but the largest proportion of this variation was shared with Pond ID (21%). In addition, a relative large proportion of environmental variation between ponds across years was uniquely explained by Pond ID (19.9%). A variation partitioning analysis with fish stock type, Pond ID and Year as explanatory variables and zooplankton community composition as response variable yielded very similar results (Figure 4B). Fish stock type and Pond ID both had an overall significant effect on compositional variation in zooplankton community between ponds across years (overall proportion of explained variation 11.72% and 26.31% respectively), while sampling year did not. Pond ID also had a unique significant effect on zooplankton community composition.

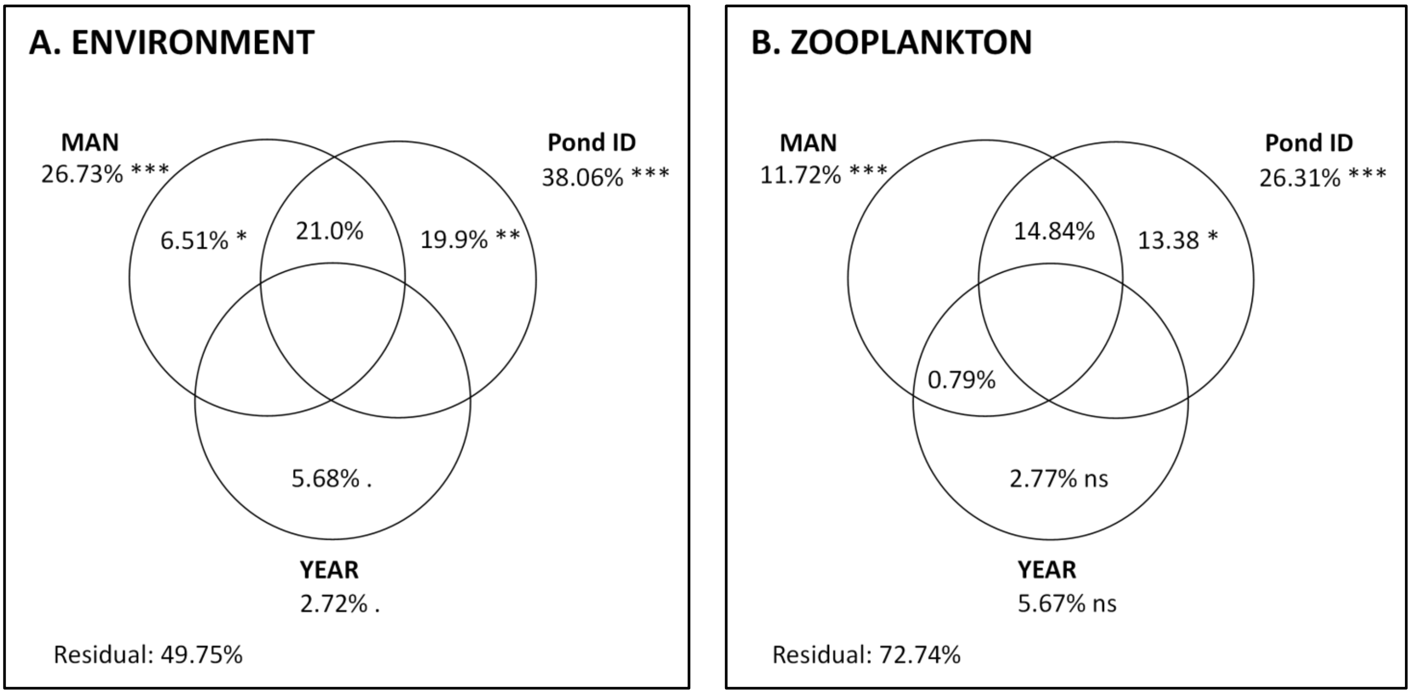

Figure 4. Venn diagrams showing the results of a variation partitioning analyses with (A) local environmental conditions, and (B) zooplankton community composition as response variables. Fish stock management, Pond ID and sampling year were defined as explanatory variables in both analyses. The significance levels are indicated: '*' p<0.05; '**' p<0.01; '***' p<0.001; and 'ns' not significant. Note that the significance of shared fractions cannot be tested.

An additional variation partitioning analysis testing for the effect of fish stock type, Pond ID and local environment on zooplankton community composition using the entire set of samples from three sampling years revealed no significant unique effect of environment on variation in zooplankton community composition between ponds (Figure SI4). In contrast, Procrustes analyses with randomization tests revealed a significant concordance between local environmental conditions and zooplankton community composition in 2013 and 2015, but not for 2014 (Table 3).

Discussion

In this study of year-to-year variation in environmental conditions and zooplankton community composition in a set of 25 interconnected ponds, we observed some variation among years, but also repeatable differences among systems linked to fish stock type. This implies that environmental conditions and zooplankton community composition are rather consistent among years, that ponds consistently differ in the local environmental conditions and zooplankton community composition, and that variation in environmental conditions and zooplankton community composition among ponds is larger than variation among years. Differences in environment and zooplankton community structure between ponds across years were largely driven by fish stock type and Pond ID.

In this set of interconnected ponds that share the same water source, one expects the basic chemical properties to be similar. Yet, environmental conditions varied substantially among ponds. We argue that these differences are largely a consequence of differences in fish stock, which is reflected by the fact that fish stock type indeed explains a considerable amount of variation in environmental conditions in our variation partitioning analysis. Fish stock management to a large extent involves manipulation of the fish community and thus food web structure of the system. Our results therefore reflect an important impact of food web structure on pond systems, and are as such in line with the vast literature on the effect of fish on the structure and the functioning of shallow lakes and ponds (Carpenter et al., 1996; Tátrai et al., 1997; Vanni, 2002; Cottenie & De Meester, 2004) including trophic cascades and its use for shallow lake restoration (Carpenter et al., 1985; Carpenter et al., 1996; Jeppesen et al., 2007; Lampert et al., 2007). Indeed, it is well known that fishes do not only affect their prey communities, but can also have profound effects on ecosystem structure and local environmental conditions. In our study, carp ponds were characterized by higher phytoplankton biomass, higher nutrient concentrations and lower coverage with submerged aquatic vegetation. This observation is well in line with earlier investigations on the impact of fish stock management on pond ecosystem structure (Lemmens et al., 2013; Lemmens et al., 2015) and very likely results from a combination of multiple mechanisms. First, the high densities of fish in these ponds increase predation pressure on zooplankton, which mediates a shift towards smaller bodied zooplankton species and individuals that are less efficient in grazing on phytoplankton (Brooks et al., 1965). Secondly, high densities of benthic fish (common carp) also promote resuspension of sediments (Havens, 1991; Breukelaar et al., 1994) and algae (Roozen et al., 2007) through benthic foraging behavior, which enhances water turbidity and internal eutrophication (Breukelaar et al., 1994) that further promote phytoplankton growth and prevent the development of submerged vegetation (Zambrano, Scheffer, & Martínez-Ramos, 2001). The absence of profound difference in environmental differences between other fish stock types might be explained by the absence of strong differences in fish density between these types, and is also in agreement with earlier observations of Lemmens et al. (2013).

We can also conclude that zooplankton metacommunity structure in the pond system was consistent across years. Even though both environmental conditions and zooplankton community structure varied from year to year, the main structuring factor was similar in all three years. There was an interesting difference in how fish stock management impacted environmental conditions and zooplankton community structure. With respect to local environmental conditions, we observe for the first PCA axis a gradual change with decreasing intensity of fish stocking, ranging from very intensive carp culture to the fishless ponds, but with the strongest contrast between intensive carp culture and the other ponds. For zooplankton, the step-wise change is rather associated with the very low fish density management (juvenile fish culture) or the absence of fish. Environmental conditions might indeed reflect the intensity of management and fish culture, whereas for zooplankton community structure we might rather see a direct response to intensity of fish predation pressure.

The significant unique effect of Pond ID on local environmental conditions and zooplankton community composition might suggest that unmeasured environmental variables, pond history, or geographical location of the ponds also affect variation in measured environmental conditions and zooplankton community structure in the set of investigated ponds. Our data do not allow us to identify the exact mechanism, but we can exclude the importance of geographical pond location since an additional variation partitioning analysis including spatial factors as explanatory variable set indicate that geographical location of the ponds did not add significant explanatory power (see supplementary information, Figure SI5). While we observed a large gradient in environmental conditions (e.g. from 0.04 to 3.61 mg/L total phosphorus, 8.05 to 161.82 µg/L for chlorophyll *a*, and 0 to 95% for macrophyte cover), these environmental gradients in water chemistry, phytoplankton density and macrophyte cover did not clearly explain a significant amount of variation in zooplankton community composition in our data. In contrast, fish pond type explained a significant part of the variation in zooplankton community composition between ponds. We therefore conclude that biotic interactions are more important structuring factors of zooplankton communities than abiotic gradients in the studied set of interconnected ponds sharing the same water source and being geographically very close to each other (Carpenter et al., 1985; Carpenter et al., 1996; Scheffer, 1998).

The observed absence of a clear effect of environmental conditions on zooplankton community composition is in line with an earlier investigation in the same study region by Lemmens et al. (2015), and the importance of biotic interactions on zooplankton community composition in interconnected ponds as previously also been demonstrated (Cottenie et al. 2001, Lemmens et al., 2013, 2015, 2018). Nevertheless, our results contrast with a vast body of studies reporting a profound link between environmental conditions and zooplankton community characteristics (Arnott & Vanni, 1993; Jeppesen et al., 2000; Cottenie et al., 2001; Dodson et al., 2008). Two important limitations of our study should be taken into account when interpreting these results. First, the present study considered only a yearly snapshot of the local environmental conditions and the zooplankton community composition and did not take into account any seasonal dynamics, which thus assumes synchrony in environmental variation among ponds. Secondly, it might be that some unmeasured environmental variables affected the zooplankton community in our study ponds. Although we included key environmental variables known to be important variables in shallow lakes (Scheffer, 1998), and which have previously been identified as major drivers of variation in zooplankton community structure in ponds (Cottenie et al., 2001; Cottenie, Michels, et al., 2003), the relative high importance of Pond ID and the absence of a clear spatial effect on variation in zooplankton community structure to some extent suggest that unmeasured variables might have been important. While an annual snapshot sampling might have prevented us in detecting a clear link between local environmental variables and zooplankton community composition, our sampling approach was well suited to test for overall differences between ponds and fish stock types across years.

Although we found strong evidence for biotic structuring of pond local environmental conditions and zooplankton community composition that was largely mediated by differences in fish stock type, we did not observe systematic changes in environment and zooplankton community structure when ponds changed fish stock management. This is surprising given the profound link of fish stock type with environment and zooplankton, but might be related to the fact that the largest differences in environment and zooplankton were observed between carp ponds and other fish stock types, while our data set only has ponds that changed from extensive to juveniles, from no fish to juveniles, and from no management to extensive fish stock. As a consequence, changes in the structure and the functioning of the pond system after changes in fish stock type might thus not have been very profound and directed. In addition, we only had a very limited number of ponds that changed fish stock type. As a consequence, we might not have been able to filter out potential additional factors determining changes in local environmental pond conditions and zooplankton community composition after changing fish stock type, such as for example pond history.

We conclude that environmental conditions and zooplankton community composition in the investigated set of ponds are consistent among years and that ponds consistently differ in local environmental conditions and zooplankton community structure. Biotic interactions seem to a more important for structuring the regional zooplankton metacommunity than the abiotic environment in this regional set of interconnected shallow ponds that share the same water source.

References

Arnott, S. E., & Vanni, M. J. (1993). Zooplankton assemblages in fishless bog lakes: Influence of biotic and abiotic factors. *Ecology, 74*(8), 2361-2380.

Bengtsson, J., Baillie, S. R., & Lawton, J. (1997). Community variability increases with time. *Oikos*, 249-256.

Borcard, D., Gillet, F., & Legendre, P. (2011). *Numerical ecology with r,* . New York. : springer.

Breukelaar, A. W., Lammens, E. H.R.R., Breteler, J. G.P. K., & Tatrai, I. (1994). Effects of benthivorous bream (abramis brama) and carp (cyprinus carpio) on sediment resuspension and concentrations of nutrients and chlorophyll a. *Freshwater Biology, 32*(1), 113-121.

Brooks, J. L., & Dodson, S. I. (1965). Predation, body size, and composition of plankton. *Science, 150*(3692), 28-35.

Brucet, S., Boix, D., Quintana, X. D., Jensen, E., Nathansen, L. W., Trochine, C., . . . Jeppesena, E. (2010). Factors influencing zooplankton size structure at contrasting temperatures in coastal shallow lakes: Implications for effects of climate change. *Limnology and Oceanography, 55*(4), 1697-1711.

Cadotte, M., xa, William, Associate Editor: Michel, Loreau, & Editor: Jonathan, B. Losos. (2006). Dispersal and species diversity. *The American Naturalist, 167*(6), 913-924. doi: 10.1086/504850

Carpenter, S. R. (2012). *Complex interactions in lake communities*: Springer Science & Business Media.

Carpenter, S. R., & Kitchell, J. F. (1996). *The trophic cascade in lakes*: Cambridge University Press.

Carpenter, S. R., Kitchell, J. F., & Hodgson, J. R. (1985). Cascading trophic interactions and lake productivity. *Bioscience, 35*(10), 634-639.

Cooper, G. J. (2007). *The science of the struggle for existence: On the foundations of ecology*: Cambridge University Press.

Cottenie, K., & De Meester, L. (2003). Connectivity and cladoceran species richness in a metacommunity of shallow lakes. *Freshwater Biology, 48*(5), 823-832.

Cottenie, K., & De Meester, L. (2004). Metacommunity structure: Synergy of biotic interactions as selective agents and dispersal as fuel. *Ecology, 85*(1), 114-119.

Cottenie, K., Michels, E., Nuytten, N., & De Meester, L. (2003). Zooplankton metacommunity structure: Regional vs. Local processes in highly interconnected ponds. *Ecology, 84*(4), 991-1000.

Cottenie, K., Nuytten, N., Michels, E., & De Meester, L. (2001). Zooplankton community structure and environmental conditions in a set of interconnected ponds. *Hydrobiologia, 442*(1-3), 339-350.

De Bie, T., De Meester, L., Brendonck, L., Martens, K., Goddeeris, B., Ercken, D., . . . Gucht, K. (2012). Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. *Ecology Letters, 15*(7), 740-747.

Dodson, S. I., Newman, A. L., Will-Wolf, S., Alexander, M. L., Woodford, M. P., & Van Egeren, S. (2008). The relationship between zooplankton community structure and lake characteristics in temperate lakes (northern wisconsin, USA). *Journal of Plankton Research, 31*(1), 93-100.

Flössner, D. (2000). *Die haplopoda und cladocera*. Mitteleuropas Leiden: Backhuys Publishers.

Frisch, D., Cottenie, K., Badosa, A., & Green, A. J. (2012). Strong spatial influence on colonization rates in a pioneer zooplankton metacommunity. *PloS one, 7*(7), e40205.

Havel, J. E., & Shurin, J. B. (2004). Mechanisms, effects, and scales of dispersal in freshwater zooplankton. *Limnology and Oceanography, 49*(4part2), 1229-1238.

Havens, K. E. (1991). Fish-induced sediment resuspension: Effects on phytoplankton biomass and community structure in a shallow hypereutrophic lake. *Journal of Plankton Research, 13*(6), 1163-1176.

Jackson, D. A. (1995). Protest: A procrustean randomization test of community environment concordance. *Ecoscience, 2*(3), 297-303.

Jeppesen, E., Jensen, J. P., Jensen, C., Faafeng, B., Hessen, D. O., Søndergaard, M., . . . Christoffersen, K. (2003). The impact of nutrient state and lake depth on top-down control in the pelagic zone of lakes: A study of 466 lakes from the temperate zone to the arctic. *Ecosystems, 6*(4), 313-325.

Jeppesen, E., Jensen, J. P., Søndergaard, M., Lauridsen, T., Pedersen, L. J., & Jensen, L. (1997). Top-down control in freshwater lakes: The role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia, 342*, 151-164.

Jeppesen, E., Meerhoff, M., Jacobsen, B. A., Hansen, R. S., Søndergaard, M., Jensen, J. P., . . . Branco, C. W. C. (2007). Restoration of shallow lakes by nutrient control and biomanipulation—the successful strategy varies with lake size and climate. *Hydrobiologia, 581*(1), 269-285.

Jeppesen, E., Peder, J. J., SØndergaard, M., Lauridsen, T., & Landkildehus, F. (2000). Trophic structure, species richness and biodiversity in danish lakes: Changes along a phosphorus gradient. *Freshwater Biology, 45*(2), 201-218.

Johannsson, O. E., Mills, E. L., & O'Gorman, R. (1991). Changes in the nearshore and offshore zooplankton communities in lake ontario: 1981–88. *Canadian Journal of Fisheries and Aquatic Sciences, 48*(8), 1546-1557.

Koroleff, F. (1970). Determination of total phosphorus in natural waters by means of persulphate oxidation. *An. Interlab. Rep*(3).

Kramer, A. M., Sarnelle, O., & Knapp, R. A. (2008). Allee effect limits colonization success of sexually reproducing zooplankton. *Ecology, 89*(10), 2760-2769.

Lampert, W., & Sommer, U. (2007). *Limnoecology: The ecology of lakes and streams*: Oxford university press.

Legendre, P., & Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia, 129*(2), 271-280.

Legendre, P., Legendre, L.F.J.,. (2012). *Numerical ecology* (3rd Edn ed.).

Leibold, M. A., Chase, J. M., Levin, S. A., & Horn, H. S. (2018). *Metacommunity ecology, volume 59*: Princeton University Press.

Lemmens, P., Declerck, S. A. J., Tuytens, K., Vanderstukken, M., & De Meester, L. (2018). Bottom-up effects on biomass versus top-down effects on identity: A multiple-lake fish community manipulation experiment. *Ecosystems, 21*(1), 166-177. doi: 10.1007/s10021-017-0144-x

Lemmens, P., Mergeay, J., De Bie, T., Van Wichelen, J., De Meester, L., & Declerck, S. A. (2013). How to maximally support local and regional biodiversity in applied conservation? Insights from pond management. *PLoS One, 8*(8), e72538. doi: 10.1371/journal.pone.0072538

Lemmens, P., Mergeay, J., Van Wichelen, J., De Meester, L., & Declerck, S. A. J. (2015). The impact of conservation management on the community composition of multiple organism groups in eutrophic interconnected man-made ponds. *PloS one, 10*(9), e0139371.

Lisboa, F. J. G., Peres-Neto, P. R., Chaer, G. M., da Conceição Jesus, E., Mitchell, R. J., Chapman, S. J., & Berbara, R. L. L. (2014). Much beyond mantel: Bringing procrustes association metric to the plant and soil ecologist's toolbox. *PloS one, 9*(6), e101238.

Louette, G., & De Meester, L. (2005). High dispersal capacity of cladoceran zooplankton in newly founded communities. *Ecology, 86*(2), 353-359.

McQueen, D. J., Post, J. R., & Mills, E. L. (1986). Trophic relationships in freshwater pelagic ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences, 43*(8), 1571-1581.

Melo, A. S. (2009). Explaining dissimilarities in macroinvertebrate assemblages among stream sites using environmental variables. *Zoologia (Curitiba), 26*(1), 79-84.

Ng, Haig-They, da Motta Marques, D., Jeppesen, E., & Søndergaard, M. (2010). Bacterioplankton in the littoral and pelagic zones of subtropical shallow lakes. *Hydrobiologia, 646*(1), 311-326.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, , P., Minchin, P.R., , O'hara, R.B., Simpson, G.L., . . . Oksanen, M.J. (2013). Community ecology package (Version 2(9)).

Peres-Neto, P. R., Legendre, P., Dray, S., & Borcard, D. (2006). Variation partitioning of species data matrices: Estimation and comparison of fractions. *Ecology, 87*(10), 2614-2625.

Petrusek, A., Hobæk, A., Nilssen, J. P., Skage, M., ČErný, M.Brede, N., & Schwenk, K. (2008). A taxonomic reappraisal of the european daphnia longispina complex (crustacea, cladocera, anomopoda). *Zoologica Scripta, 37*(5), 507-519.

Petrusek, A., Seda, J., Macháček, J., Ruthová, Š., & Šmilauer, P. (2008). Daphnia hybridization along ecological gradients in pelagic environments: The potential for the presence of hybrid zones in plankton. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 363*(1505), 2931-2941.

Pulliam, H. R. (1988). Sources, sinks, and population regulation. *The American Naturalist, 132*(5), 652-661.

Roozen, F. C. J. M., Lürling, M., Vlek, H., Van Der Pouw Kraan, E. A. J., Ibelings, B. A. S. W., & Scheffer, M. (2007). Resuspension of algal cells by benthivorous fish boosts phytoplankton biomass and alters community structure in shallow lakes. *Freshwater Biology, 52*(6), 977-987.

Scheffer, M. (1998). *Ecology of shallow lakes*. London: Chapman and Hall.

Scheffer, M., Carpenter, S., Foley, J. A., Folke, C., & Walker, B. (2001). Catastrophic shifts in ecosystems. *Nature, 413*(6856), 591-596.

Scheffer, M., Hosper, S. H, Meijer, M. L, Moss, B., & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution, 8*(8), 275-279.

Souffreau, C, Busschaert, P, Denis, C, Van Wichelen, J, Lievens, B, Vyverman, W, & De Meester, L. (2018). A comparative hierarchical analysis of bacterioplankton and biofilm metacommunity structure in an interconnected pond system. *Environmental Microbiology, 20*(3), 1271-1282. doi: doi:10.1111/1462-2920.14073

Tátrai, I., Oláh, J., Paulovits, G., Mátyás, K., Kawiecka, B. J., & Pekár, F. (1997). Changes in the lower trophic levels as a consequence of the level of fish manipulation in the ponds. *Internationale Revue der gesamten Hydrobiologie und Hydrographie, 82*(2), 213-224.

Vanni, M. J. (1987). Effects of nutrients and zooplankton size on the structure of a phytoplankton community. *Ecology, 68*(3), 624-635.

Vanni, M. J. (2002). Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics, 33*(1), 341-370.

Vellend, M. (2010). Conceptual synthesis in community ecology. *The Quarterly review of biology, 85*(2), 183-206.

Violle, C., & Jiang, L. (2009). Towards a trait-based quantification of species niche. *Journal of Plant Ecology, 2*(2), 87-93.

Zambrano, L., Scheffer, M., & Martínez-Ramos, M. (2001). Catastrophic response of lakes to benthivorous fish introduction. *Oikos, 94*(2), 344-350.

Supplementary information to chapter 2

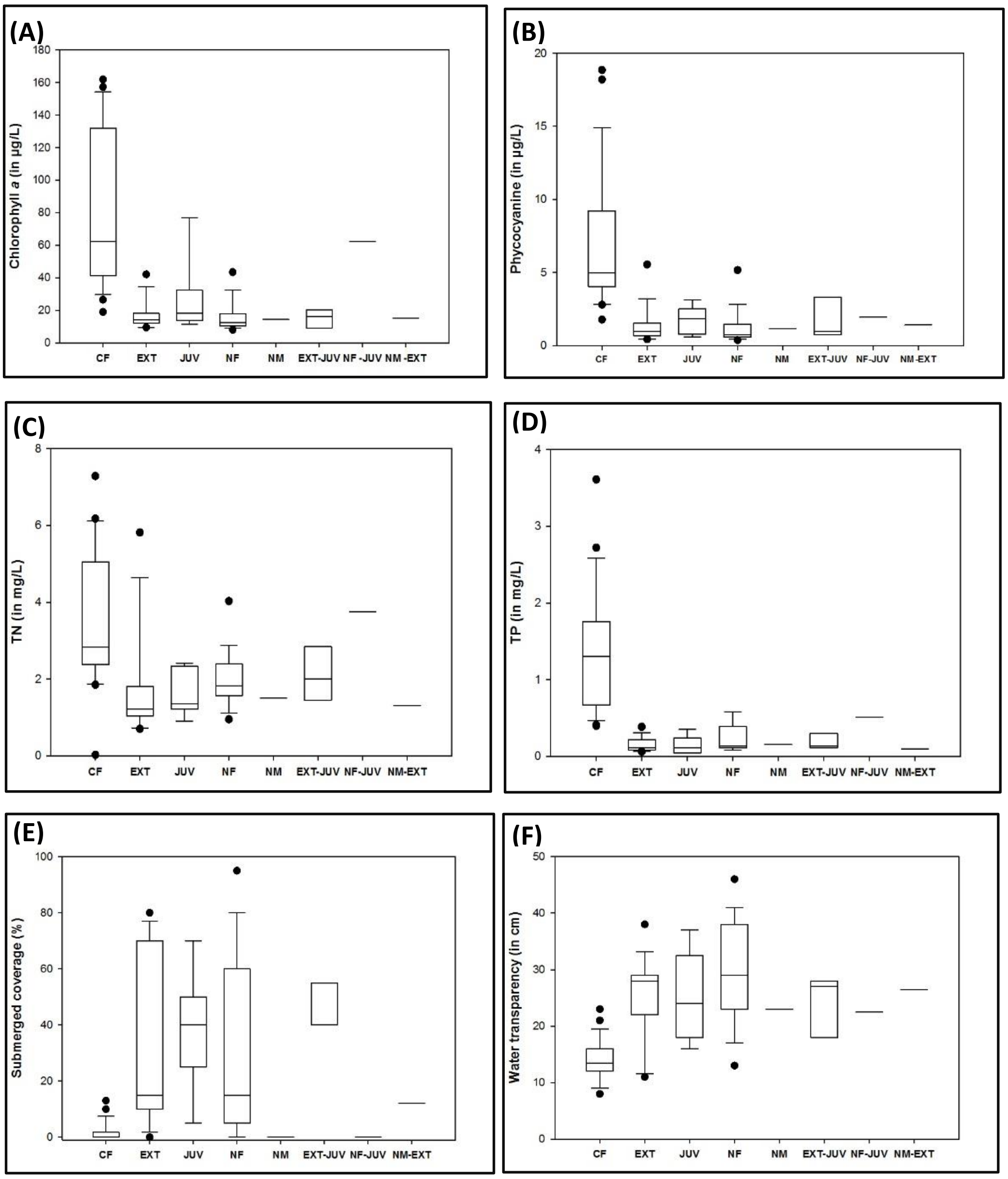

Figure SI1. Box plots with the median of different local environmental conditions in relation to pond management type. (A) Chlorophyll *a*, (B) Phycocyanin, (C) Total Nitrogen (TN), (D) Total Phosphorus (TP), (E) percentage of submerged vegetation, (F) Water transparency.

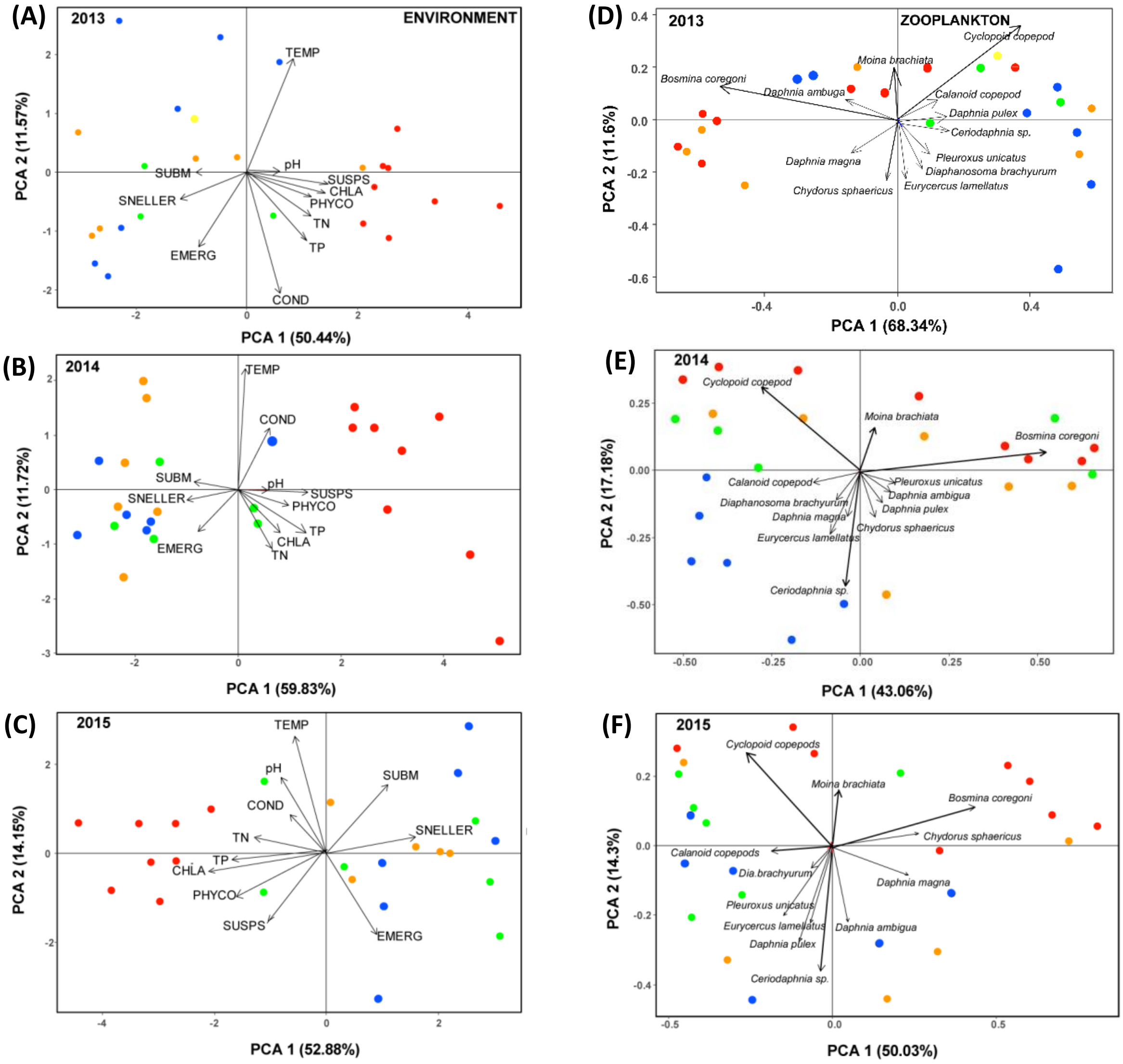

Figure SI2. PCA ordinations based on local environment conditions for each year (A-C), and the same set of ordinations based on zooplankton community composition (D-F). Black arrows represent environmental variables or zooplankton taxa. Different colors represent different fish stock types (CF: red, EXT: orange, JF: green, NF: blue, NM: yellow, see also Table SI1).

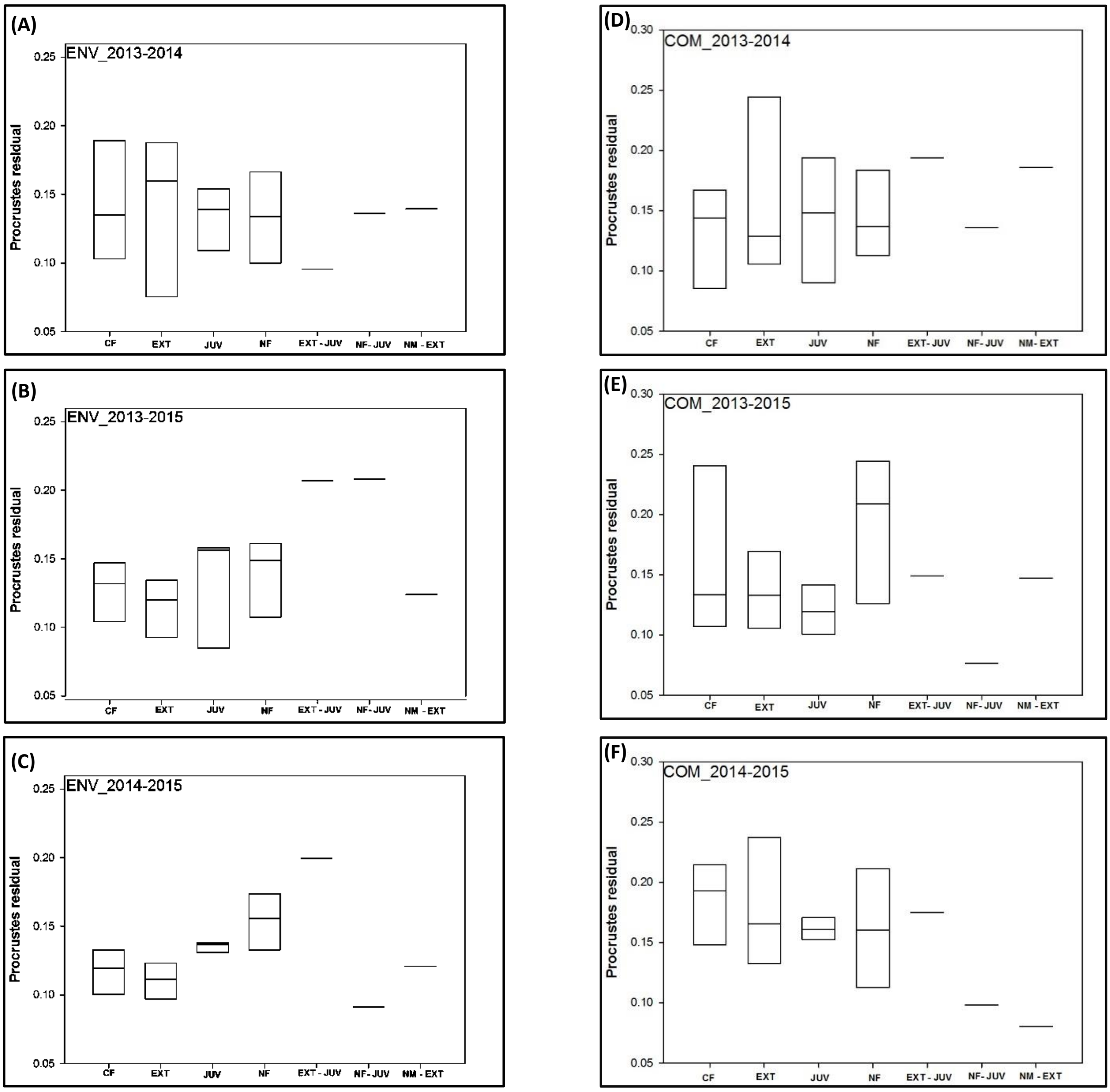

Figure SI3. Boxplots of the residuals of the procrustes analyses for each fish stock type and the ponds that changes fish stock type depending on local (A-C) local environmental conditions, and (D-F).

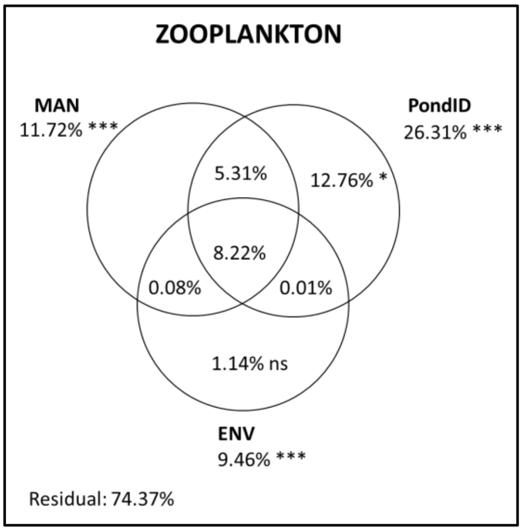

Figure SI4. Venn diagrams showing the results of a variation partitioning analyses with zooplankton community composition as response variables and fish stock management, PondID and environment as explanatory variables for all years combined. ‘*’ p<0.05, ‘***’ p<0.001, ‘ns’ not significant. Note that the significance of shared fractions cannot be tested.

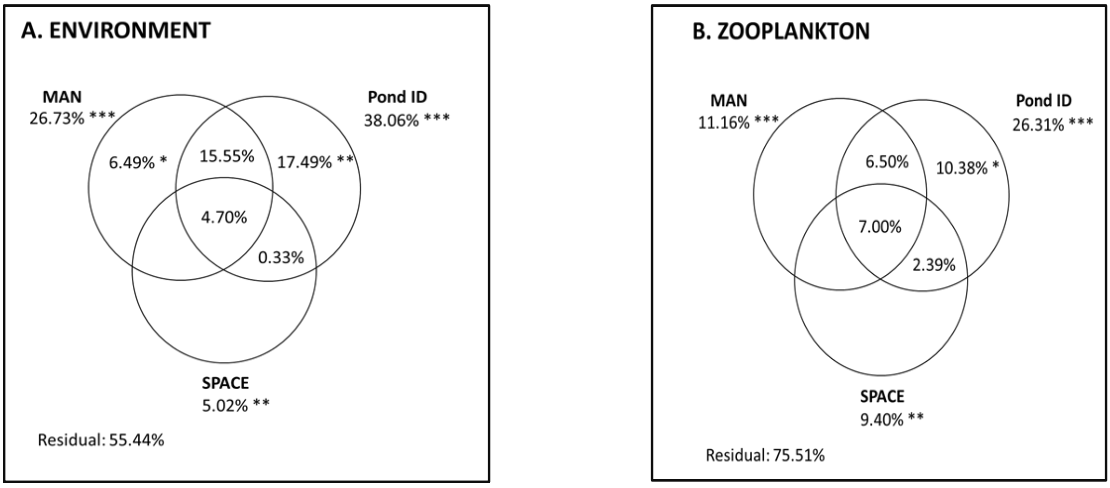

Figure SI5: Venn diagrams showing the results of a variation partitioning analyses with (A) local environmental conditions, and (B) zooplankton community composition as response variables and fish stock management, PondID and space year as explanatory variables. ‘*’ p<0.05, ‘***’ p<0.001, ‘ns’ not significant. Note that the significance of shared fractions cannot be tested.

Table SI1. Fish stock types for the investigated ponds during the three study years. Note that ponds changes fish stock type (indicated with ‘*’)

Pond ID	2013	2014	2015
1J1	Extensive	Extensive	Extensive
1J2*	Extensive	Juveniles	Juveniles
1J5*	Extensive	Extensive	Juveniles
1J7	Juveniles	Juveniles	Juveniles
BK1	No fish	No fish	No fish
BK2	No fish	No fish	No fish
BK6	No fish	No fish	No fish
BKN1	No fish	No fish	No fish
K1	Carp	Carp	Carp
K11	Carp	Carp	Carp
K2	Carp	Carp	Carp
K3	Carp	Carp	Carp
K4	Carp	Carp	Carp
K5	Carp	Carp	Carp
K6	Carp	Carp	Carp
K9	Carp	Carp	Carp
O1	No fish	No fish	No fish
O2	No fish	No fish	No fish
O3	Extensive	Extensive	Extensive
O4	Extensive	Extensive	Extensive
O7*	No management	Extensive	Extensive
V09	Juveniles	Juveniles	Juveniles
V1	Juveniles	Juveniles	Juveniles
V21*	No fish	Juveniles	Juveniles
V4	Extensive	Extensive	Extensive

Table SI2. Factor loadings of the different environmental variables with the first and second axis of the PCA ordination plot based on local environmental variables.

	all years		2013		2014		2015	
	PCA1	PCA2	PCA1	PCA2	PCA1	PCA2	PCA1	PCA2
pH	0.178925	-0.24712	0.160302	-0.00385	0.208936	-0.00284	-0.19341	0.408928
water transparency	-0.3789	-0.17847	-0.38689	-0.14737	-0.35805	-0.06888	0.381593	0.08714
suspended solids	0.350256	0.189706	0.333211	-0.04566	0.347419	-0.01971	-0.37222	-0.1893
temperature	0.037193	-0.77105	0.20965	0.562612	0.050351	0.803283	-0.136	0.628573
total nitrogen	0.298912	-0.06835	0.249453	-0.09098	0.321599	-0.20842	-0.29186	0.007745
total phosphorus	0.352358	-0.21012	0.372505	-0.19496	0.357637	-0.10344	-0.37788	-0.05963
conductivity	0.184821	0.28227	0.192192	-0.66219	0.223992	0.40587	-0.15571	0.207655
chla	0.370749	-0.05626	0.377556	-0.05588	0.346669	-0.20138	-0.36724	-0.08943
phycocyanine	0.386295	0.10409	0.350638	-0.081	0.360168	-0.10027	-0.39528	-0.09503
submerged vegetation	-0.30692	-0.16212	-0.29686	0.00209	-0.3095	0.052272	0.264012	0.370281
emergent vegetation	-0.26799	0.328312	-0.27944	-0.40642	-0.27878	-0.27818	0.216582	-0.43974

Table SI3. Factor loadings for each zooplankton taxon with the first and second axis of the PCA ordination plot based on zooplankton community composition.

	all years combined		2013		2014		2015	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Acroperus harpae	0.01383	0.039532	0.001789	0.001068	-0.02833	-0.04999	-0.01372	-0.01121
Alona costata	0.015453	-0.00866	0.050543	-0.18405	0.001895	0.00806	-0.00139	-0.01421
Alonella exigua	0.015035	0.029862	0.008171	-0.02038	-0.03968	-0.04512	-0.00357	-0.00061
Alona guttata	0.033057	-0.02943	0.077883	-0.30067	-0.03938	0.030587	0.006535	-0.00067
Alonella nana	0.006665	-0.00215	0.021617	-0.05391	8.67E-19	-1.1E-16	0	-2.8E-17
Alona quadrangula	0.019804	-0.02345	0.061026	-0.27809	-0.00233	0.000373	-0.00596	0.005884
Alona rectangula	0.005775	0.00459	0.020225	-0.04024	0.013666	0.021578	-0.00893	0.029334
Bosmina coregoni	**-0.82379**	**-0.20679**	**-0.78778**	**0.262457**	**0.854252**	**0.097746**	**0.802355**	**0.094868**
Calanoid copepod	**0.104345**	**0.237724**	**0.02429**	**0.041729**	**-0.16425**	**-0.20516**	**-0.12245**	**-0.01296**
Camptocercus rectirostris	0.011778	0.000632	0.038106	-0.23066	-4.1E-25	-1.7E-21	-0.00155	-0.01001
Ceriodaphnia sp.	**0.058311**	**0.675892**	0.006588	-0.12543	-0.06824	-0.70233	-0.0865	-0.87774
Chydorus sphaericus	-0.04575	0.06077	-0.04307	-0.34608	0.022296	-0.00663	0.050656	0.023163
Cyclopoid copepod	**0.53399**	**-0.50601**	**0.572587**	**0.558446**	**-0.45372**	**0.506422**	**-0.54241**	**0.32642**
Daphnia ambigua	-0.02516	0.020425	0.009247	0.003987	0.02395	0.004077	0.070024	-0.05654
Daphnia cucullata	-0.00667	-0.01874	0.023266	0.018167	0.016096	0.005972	0.023935	0.007968
Daphnia magna	0.001094	0.087208	-2.8E-45	6.16E-33	-0.01177	-0.01888	0.02532	-0.20508
Daphnia obtusa	0.000763	-0.00054	0.002529	0.00151	-3.4E-41	0	-3.5E-46	-2.4E-35
Daphnia pulex	0.030862	0.078783	0.010497	0.041914	-0.10359	-0.17149	8.55E-50	-2.4E-38
Daphnia complex	0.00829	-0.03087	0.038024	0.009993	0	0	0	-2.3E-41
Diaphanosoma brachyurum	**0.083408**	**0.347989**	**0.02735**	**0.042424**	**-0.06027**	**-0.27892**	**-0.15675**	**-0.15644**
Eurycercus lamellatus	0.027301	0.041397	0.04755	-0.11952	-0.03706	-0.06523	-0.00398	-0.03436
Graptoleberis testudinaria	0.031529	0.016779	0.064518	-0.32188	-0.03933	-0.03701	-0.00413	0.001437
Ilyocryptus sordidus	0.000538	-0.00318	0	0	0.000562	0.005978	-0.00085	0.004648
Kurzia latissima	0.001601	0.004223	0.002529	0.00151	0	0	-0.0022	-0.01416
Leptodora kinditii	-0.00245	-0.01224	0	0	-0.00253	0.014611	0.014682	0.02525
Leydigia leydigi	-0.00033	0.010358	0	0	-0.00018	-0.02027	0.002231	0.002656
Macrothrix rosea	0.000859	0.000837	0	0	0	0	-0.00239	0.001442
Megafenestra aurita	0	0	0	0	0	0	0	0
Moina brachiata	**-0.02878**	**-0.18457**	**-0.0154**	**0.142446**	**0.066485**	**0.261269**	**0.02816**	**0.187261**
Pleuroxus aduncus	0.026766	0.02653	0.027215	-0.00856	-0.02547	-0.02747	-0.0274	-0.00793
Pleuroxus denticulatus	0.026707	0.007258	0.065346	-0.14827	-0.01785	-0.00418	-0.00442	-0.04753
Pleuroxus truncatus	0.009437	0.003691	0.023086	-0.07132	0.000606	-0.01233	-0.00776	-0.00908
Pleuroxus unicatus	0.010183	0.018315	0.008679	-0.00266	-0.02648	-0.04184	-0.00097	-0.00352
Polyphemus pediculus	0.013955	0.065436	0	0	-0.04162	-0.08544	-0.00231	-0.04031
Scapholeberis mucronata	0.016794	0.001844	0.03719	-0.0057	0.000755	-0.0091	-0.00831	0.023019
Scapholeberis rammneri	0.004011	-0.01067	0.013731	0.029565	0	0	0.000657	0.006358
Sida crystallina	0.019307	-0.00564	0.044633	-0.1061	0.000677	0.003164	-0.0155	-0.01883
Simocephalus serrulatus	0.039955	-0.03824	0.119423	-0.19508	0	0	-0.01191	0.008442
Simocephalus vetulus	0.046533	0.014348	0.035594	-0.05187	-0.04532	-0.01881	-0.05981	-0.00661

Chapter 3: Temporal variability of phytoplankton biomass in a set of shallow lakes

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[Unpublished manuscript]

Abstract

The occurrence of regime shifts in ecosystems is increasingly reported and receives growing attention. Regime shifts are especially important because they involve major changes in ecosystem structure and functioning, which may undermine the provisioning of ecosystem services. Ponds and shallow lakes are characterized by the occurrence of two distinct alternative stable states; a clear-water state with profound submerged vegetation and a turbid, phytoplankton dominated state with few or no submerged vegetation. We here intensively monitored chlorophyll *a* and phycocyanin concentrations in four interconnected shallow fish ponds during the summer growth season (June-October) of two consecutive years (2016 and 2017) using data loggers. Our results show considerable temporal variation in phytoplankton biomass within and across years, but this variation was not consistent across years and systems. We also show a strong positive association between chlorophyll *a* and phycocyanin concentrations. Using existing tools to detect early warning signals for regime shifts, we found an indication of a regime shift in one pond in 2016.

Introduction

The majority of dynamic system is characterized by the occurrence of multiple alternative stable states that are stabilized by a set of positive and negative feed-back mechanisms (Van Donk et al., 1990; Scheffer et al., 1993; Kéfi, Holmgren, & Scheffer, 2016). There currently is a rapidly growing interest in the seemingly sudden shifts of ecosystems from one state to another. State shifts occur when an ecosystem passes its boundaries of resilience for a certain environmental driver (Scheffer et al., 2001; Folk et al., 2004). The occurrence of alternative stable states and regime shifts is not only important in science, but is also highly relevant for policy and sustainable environmental management. Regime shifts are an abrupt nonlinear change in ecosystem state (Scheffer et al., 2001; Carpenter & Kinne, 2003; Biggs, Carpenter, & Brock, 2009). Regime shifts typically results in a fundamental reorganization of ecosystem structure and functioning (Carpenter et al., 2011). This might severely undermine the provisioning of major ecosystem services, and often results in considerable social and economic costs.

The occurrence of alternative stable states and regime shifts has historically been best investigated in shallow lakes and ponds (Carpenter, Kitchell, & Hodgson, 1985; Scheffer et al., 1993; Kéfi et al., 2013). Indeed, many shallow lakes and ponds are characterized by the occurrence of two alternative stable states, (1) a clear water state with low phytoplankton density and high coverage with submerged macrophyte, and (2) a turbid water state with high phytoplankton density and the absence of profound aquatic vegetation (Scheffer et al., 1993).

Understanding the processes underlying regime shifts is generally is not straightforward since the strength of the interaction between multiple feedback mechanisms can vary considerably in time and space, and the extent to which they control stable states tends to be ecosystem specific (Mulderij, Van Nes, & Van Donk, 2007; Scheffer & van Nes, 2007; Sayer et al., 2010). An increasing number of scientific studies is currently directed towards finding generic early warning signals or leading indicators that allow to predict when ecosystems are close to their tipping point at which they shift from one state into another (Carpenter et al., 2014; Spears et al., 2017). A tipping point corresponds to a critical level an external condition (for example nutrient inflow in a shallow lake) where critical transitions of the system will occur (Lenton et al., 2008; Scheffer et al., 2009).

Multiple statistical methods for detecting changes in ecological stability have been developed (Carpenter et al., 2011; Dakos et al., 2012; Lenton et al., 2012). Generic early warning signals (EWI's) are statistical metrics that are increasingly used to quantify the loss of temporal or spatial resilience. Such tools allow to detect early warning signals that indicate when a system is reaching its critical threshold at which a major state shift might occur (Scheffer et al., 2009). Several EWIs are related to a critical slowing down, which is a typical characteristic of dynamic systems that are close at a threshold beyond which a catastrophic regime shift likely occurs (Van Nes & Scheffer, 2007). As the system approaches such a threshold, the return rate to the equilibrium after a relative small perturbation is slow, so that the system tends to become more similar to its own past, resulting in an increase in autocorrelation in time series data (Ives, 1995). For example, an increase in autocorrelation in phytoplankton biomass due to critical slowing down seems to precede the transition from a clear-water state to turbid-water state in shallow lakes and ponds (Dakos et al., 2012).

Laboratory and field experiments suggest that generic early warning indicators may indeed predict critical transitions of real ecosystems (Drake & Griffen, 2010; Carpenter et al., 2011; Dakos et al., 2012; Wang et al., 2012). An important application of these methods is their potential real-time use as warnings of increased risk of upcoming transitions (Carpenter & Brock, 2006; Scheffer et al., 2009). To date, the performance of these methods has largely been tested using experimental data and simulations (Carpenter et al., 2014; Spears et al., 2017), while studies based on empirical monitoring of ecosystems are still very scarce.

In shallow lakes and ponds, the interaction between phytoplankton and submerged macrophytes plays a pivotal role in the dynamics and functioning of the food web. Macrophytes promote the clear water state by stabilizing lake sediments, by increasing sedimentation rate of particles, by mediating food web interactions as well as by suppression of phytoplankton growth through competition for resources and allelopathy (Søndergaard & Moss, 1998; Burks et al., 2002; Gross, 2003; Vanderstukken et al., 2014) whereas phytoplankton promotes turbid water. Changes in phytoplankton biomass dynamics might thus be highly indicative for changes in biotic and abiotic conditions in lake ecosystems (Dokulil, 1993; Reynolds, 2006).

The main objective of the present study is to explore differences in temporal and spatial dynamics of plankton biomass in a set of shallow ponds. We specifically aim to explore the existence of early warning signals for regime shifts in shallow ponds. For this purpose, we engaged in an intensive monitoring of phytoplankton biomass dynamics in a set of 4 shallow ponds over the summer growth season of two years. We quantify differences in phytoplankton dynamics among ponds and among years. We screen for sudden regime shifts in the investigated ponds using extensive time-series data and existing statistical tools for detecting early warning signals for critical state shifts.

Material and Methods

Study area and sampling

The study was carried out in "Vijvergebied Midden-Limburg", located in the north-eastern part of Belgium (50° 59' 00.92" N; 5° 19' 55.85" O). Vijvergebied Midden-Limburg comprises a total area of 4000 ha and consists more than 1000 interconnected shallow ponds, dry and wet heats, forest and reed vegetation (Lemmens et al., 2013). All ponds share a common water source (Roosterbeek). Although most ponds are of anthropogenic origin and have historically been managed for purposes of fish farming, the region now is a biodiversity hotspot of European importance. Vijvergebied Midden-Limburg is designated as Natura 2000 site and also protected by the EU Birds directive and the Habitats directive. Fish farming is still an important local practice, but the majority of ponds is currently property of the Flemish Government (Agency for Nature and Forest) and managed for purposes of biodiversity conservation (see Lemmens et al., 2013 for more details).

We selected 4 shallow ponds that behaved differently in terms of phytoplankton biomass dynamics as quantified during an earlier investigation in 2015 (see chapter 1). The concentration of chlorophyll *a* and phycocyanin in these selected ponds were monitored at 15 minutes intervals during the summer growth season (June-October) of 2016 and 2017using data loggers (EXO2 sonde, YSI Incorporated, Yellow Spring, USA) that were positioned in the pelagic zone of each ponds. Chlorophyll *a* and phycocyanin concentrations were used as a proxy of phytoplankton and cyanobacteria biomass respectively. Both are important components of the plankton community in shallow lakes and ponds (Carpenter et al., 1985; Elliott, Jones, & Thackeray, 2006; Schindler et al., 2008; Huisman et al., 2018), but to some extent can show different responses and dynamics due to their ecological differences (Reynolds, 2006; Whitton & Potts, 2007). We explicitly monitored during the summer growth season as this is the time period in which we expect strong phytoplankton community dynamics and potential regime shifts.

Data analysis

We tested for consistency across ponds and years for both phytoplankton and cyanobacteria biomass dynamics using Pearson’s correlations based on daily averages of chlorophyll *a* and phycocyanin concentration. Data on chlorophyll *a* and phycocyanin were log transformed prior to any statistical analyses in order to improve assumptions of normality and homoscedasticity. Screening for early warning signals of regime shifts in the set of investigated ponds was done using the generic early warning indicator tool outlined by Dakos et al. (2012; see also Scheffer et al., 2009; Wang et al., 2012; Carpenter et al., 2014; Spears et al., 2017). Here, we used data from the 15 minutes interval measurements to avoid problems with limiting time series data as these tools are data demanding and demand relative long time series. A rolling time window of 5 % of the length of the time series was used. All time series data were detrended using Gaussian kernel filtering prior to analysis. Statistical analysis were carried out in R (v.3.4.3) using the “vegan”, “MASS”, “car”, and “earlywarnings” packages (R Development Core Team, 2016).

Results

The concentration of chlorophyll *a* and phycocyanin showed strong temporal variation within and across ponds during both years (overall range across time and ponds 1.29- 866 µg/L and 0.05 - 188 µg/L for chlorophyll *a* and phycocyanin respectively) (Figure 1). Annual means of chlorophyll *a* for the studied ponds varied between 42.5 and 238.3 μg/L. The mean concentration of phycocyanin varied considerably (across ponds over the entire study period (between 1.98 and 34.56 μg/L).

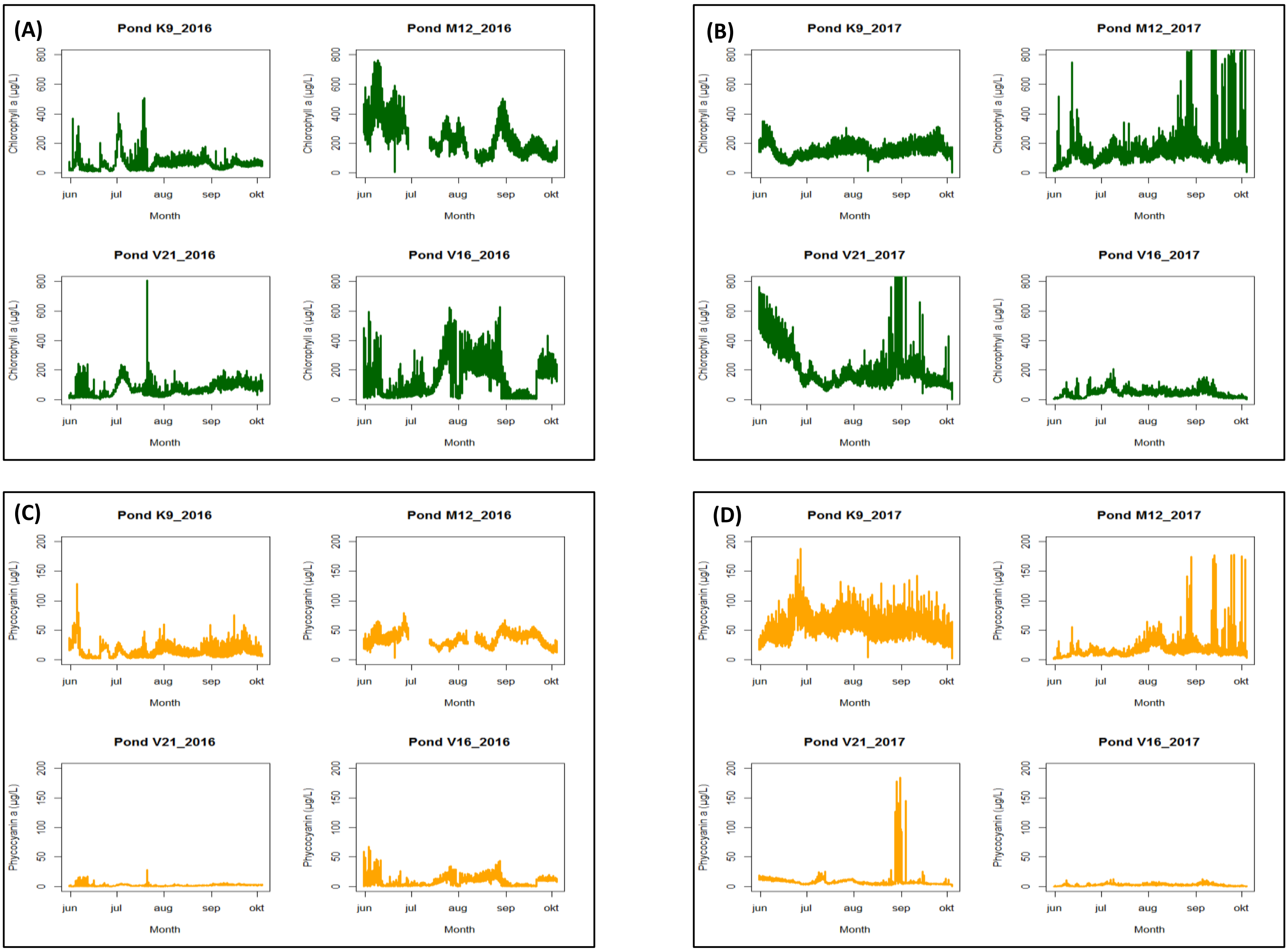

Figure 1. The concentration of chlorophyll *a* and phycocyanin as quantified in each pond over the summer growth season in 2016 (panel A and C) and 2017 (panel B and D).

Pearson correlations showed that the correlation in chlorophyll *a* between ponds within and across years was overall very low and did not show profound consistency. For chlorophyll *a* concentrations in pond K9 and pond V16 seem to be positively associated in 2016, while we observe a negative association in chlorophyll *a* concentration between pond M2 and pond V21 (Figure 2). A similar result was obtained for the concentration of phycocyanin in 2016 (Figure 3). In contrast, we observed a significant positive correlation for phycocyanin between pond K9 and pond M12 as well as between pond K9 and pond V16 in 2017.

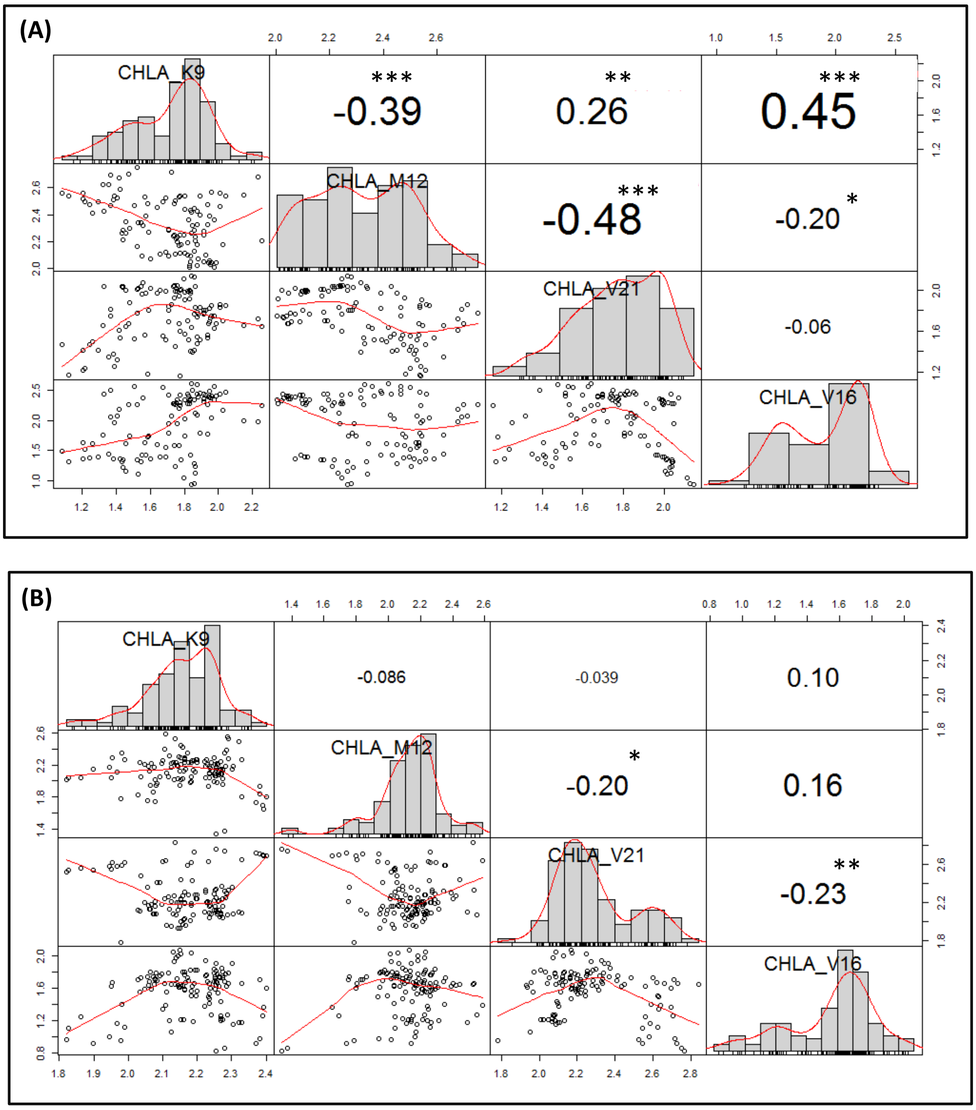

Figure 2. Pearson correlation matrices plots visualizing the association of the annual chlorophyll *a* (log(chla) among ponds in (A) 2016 and (B) 2017. Significant p-values are shown in asterisks. CHLA_K9 = daily average chlorophyll *a* concentration (log(chla) at Pond K9, CHLA_M12 = daily average chlorophyll *a* concentration (log(chla) at Pond M12, CHLA_V21 = daily average chlorophyll *a* concentration (log(chla) at Pond V21, and CHLA_V16 = daily average chlorophyll *a* concentration (log(chla) at Pond V16.

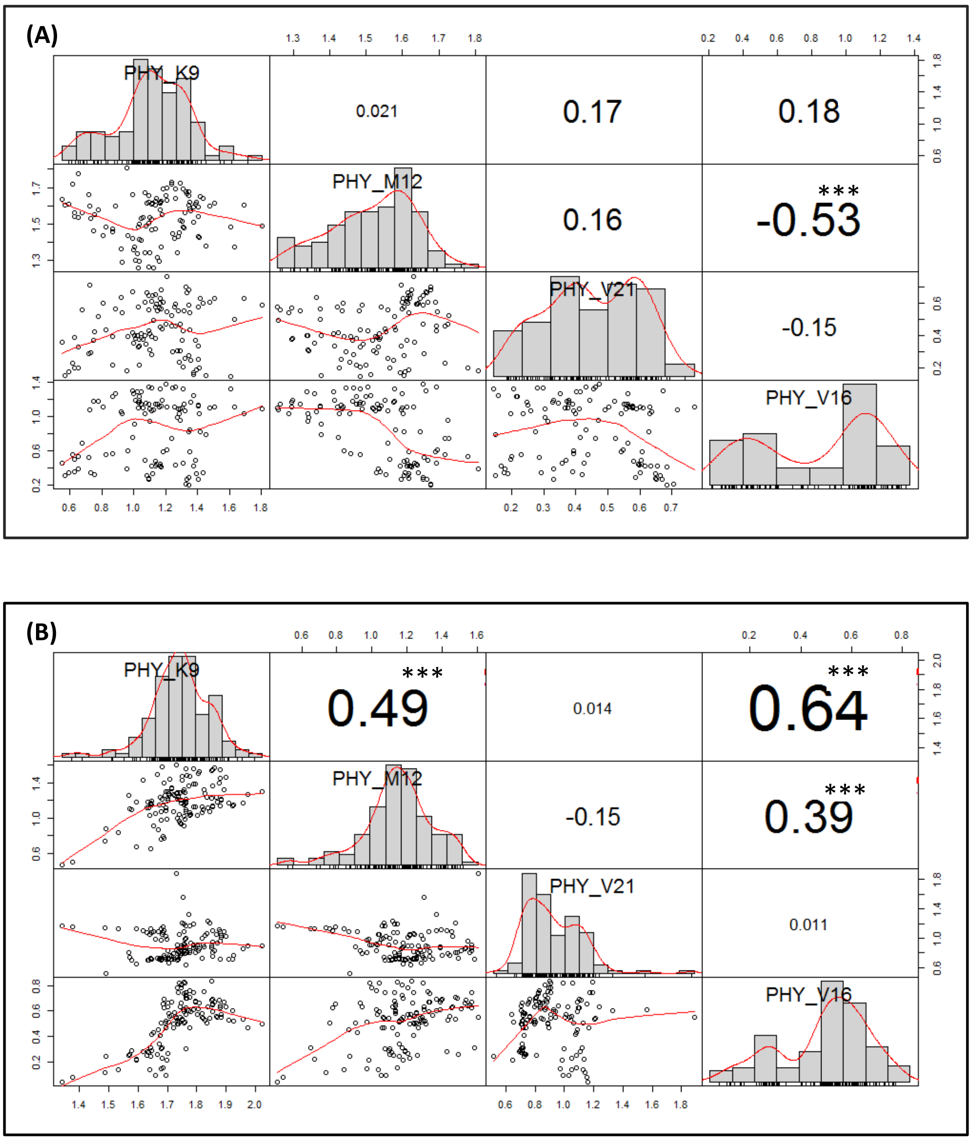

Figure 3. Pearson correlation matrices plots visualizing the association of the annual phycocyanin (log(phyco) among ponds in (A) 2016 and (B) 2017. Significant p-values are shown in asterisks. PHY_K9 = daily average phycocyanin concentration (log(phyco) at Pond K9, PHY_M12 = daily average phycocyanin concentration (log(phyco) at Pond M12, PHY_V21 = daily average phycocyanin concentration (log(phyco) at Pond V21, and PHY_V16 = daily average phycocyanin concentration (log(phyco) at Pond V16.

For all studied systems we did not observed a strong significant correlation in chlorophyll *a* and phycocyanin between the two study periods (Figure 4 A&B).

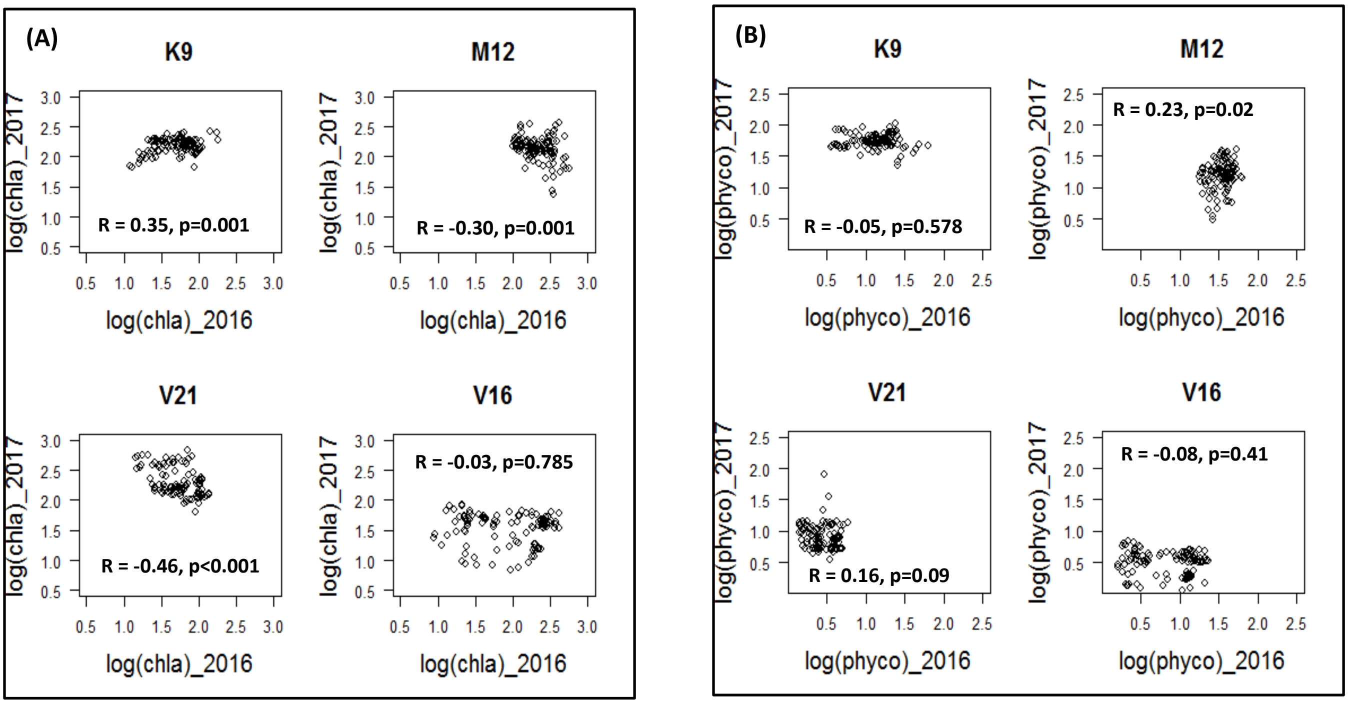

Figure 4. Pearson correlation plots visualizing the association of the annual phytoplankton biomass, (A) annual chlorophyll *a*, (B) annual phycocyanin concentration for each pond between the two study years.

An overall positive association between chlorophyll *a* and phycocyanin concentration was observed for all ponds in 2016 (R = 0.66 and $p < 0.001$; R = 0.47 and $p < 0.01$; R = 0.87 and $p < 0.001$; R = 0.97 and $p < 0.001$, for pond K9, M12, V21, and V16 respectively) (Figure 5 A). A similar pattern was observed in 2017 for most investigated ponds, except for pond K9 (R = -0.05 and p = 0.584; R = 0.78 and $p < 0.001$; R = 0.63 and $p < 0.001$; R = 0.96 and $p < 0.001$, for pond K9, M12, V21, and V16 respectively) (Figure 5 B).

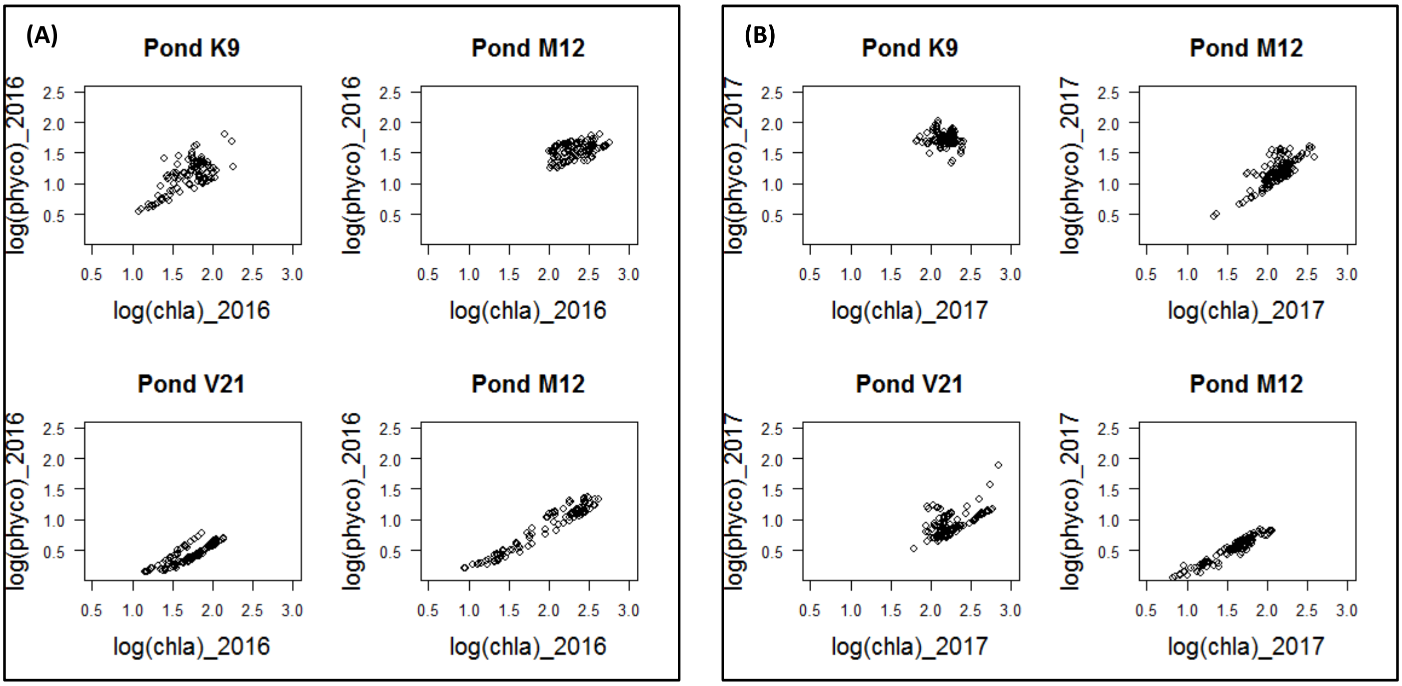

Figure 5. Pearson’s correlation between chlorophyll *a* and phycocyanin concentration for each pond during the summer growth season in 2016 (A) and 2017 (B).

The screening for early warning signals of regime shifts using the criterion of a strong increase in temporal autocorrelation in time series data showed a clear signal for a regime shift in one pond (V16) in 2016 on both chlorophyll *a* and phycocyanin (Figure 6 A; Figure 7A). Based on our analyses, other ponds only showed a gradual change in chlorophyll *a* and phycocyanin (Figure 6 A; Figure 7A). In 2017, we did not find an early warning signal for a regime shift in any of the studied ponds (Figure 6 B; Figure 7 B).

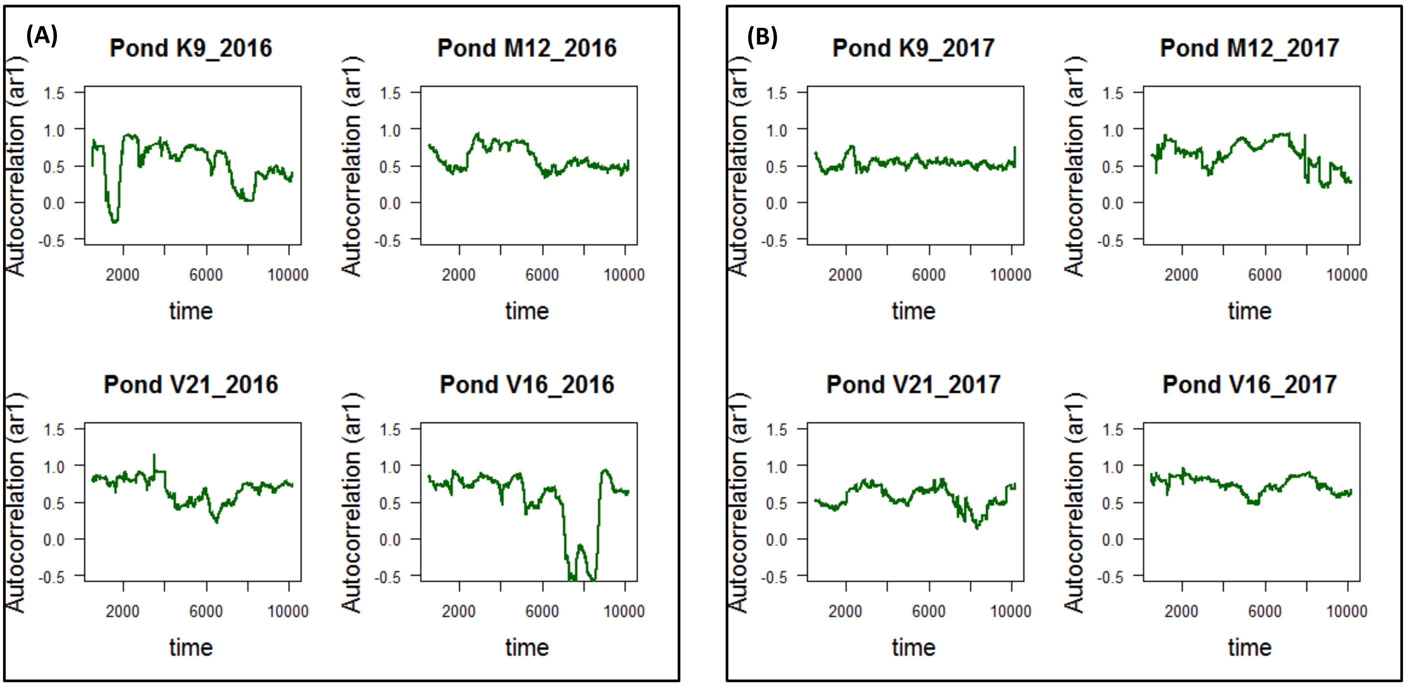

Figure 6. Plots showing the autocorrelation in chlorophyll *a* time series data for each pond separately for 2016 and 2017 (panel A and B respectively). A rolling time window of 5% of the length of the time series was used.

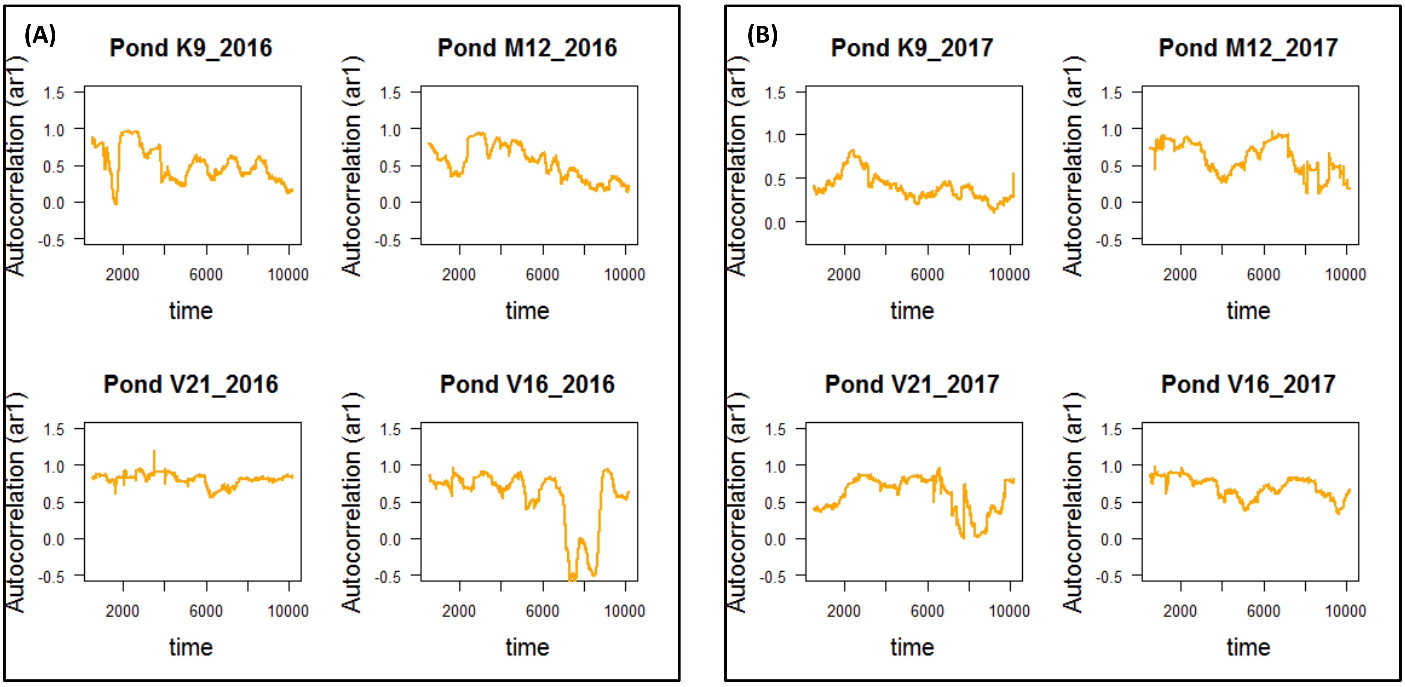

Figure 7. Plots showing the autocorrelation in phycocyanin time series data for each pond separately for 2016 and 2017 (panel A and B respectively). A rolling time window of 5% of the length of the time series was used.

Discussion

Our investigation of four shallow ponds over a time period of two subsequent summer growth seasons revealed strong temporal variation in phytoplankton biomass within and across years. Phytoplankton biomass dynamics in the investigated ponds seem to differ strongly between ponds and between years. Interestingly, we did not observe a clear consistency through time in both chlorophyll *a* and phycocyanin concentration. Our analyses show that the concentration of chlorophyll *a* and phycocyanin is largely correlated. Based on published methods to detect early warning signals for regime shift, we found evidence for a regime shift in one pond in 2016.

The lack of a clear consistency through time in phytoplankton biomass is to some extent surprising. However, this can be explained by the fact that the blooms are in different time periods. This is more evident, for example in 2016, M12 is the pond that it was bloom in June and July and apparently become less bloom in August, and there is another pond V16 having an immense bloom in August (Figure 1A). Our observation suggests that other factors drive phytoplankton biomass dynamics (Cottenie et al., 2003; Lemmens et al., 2013; Lemmens et al., 2018). The substantial variation in phytoplankton dynamics can be linked to food web structure associated to pond management.

The investigated ponds showed different phytoplankton biomass dynamics within and between years. This is in line with previous investigations by Lemmens et al. (2018). Another explanation for differential phytoplankton biomass dynamics might be related to variation in zooplankton grazing pressure between ponds and across years. The investigated ponds differed in fish community density and might thus have different zooplankton community characteristics. This might affect the overall phytoplankton biomass, but can definitely also impact the phytoplankton dynamics. Differences in coverage with submerged macrophytes between ponds can also result in differential in phytoplankton dynamics. Indeed, submerged macrophytes can affect phytoplankton through competition for nutrients, serving as a refuge for large-bodied zooplankton species that graze on phytoplankton, and the production of allelopathic substances that inhibit the growth of phytoplankton (Timms & Moss, 1984; Hamilton & Mitchell, 1996; Jeppesen et al., 1997; Gross, 2003; Vanderstukken et al., 2014).

We found some indications for an early warning signal in one pond in 2016 using previously published methods for detecting generic early warning signals. Overall, it seems that the directionality and extent of changes in chlorophyll *a* vary strongly within ponds over the summer growth season of both years, and it seems that changes in chlorophyll *a* are rather gradual than sudden. However, if we apply a strict criterion outlined by Dakos et al. (2012; see also Scheffer et al., 2009; Wang et al., 2012; Carpenter et al., 2014; Spears et al., 2017), strong increase in temporal autocorrelation, we have actually one case of regime shift in state. In pond V16, we observed about 25 transitions in chlorophyll *a* in 2016 and two of those are indicative for regime shifts. We observed considerable changes in phytoplankton biomass in our study systems within and across years. However, these changes seem to be not dramatic, but rather gradual. This might be linked to changes in fish predation intensity or changes in environmental conditions, such as nutrient availability. Our results are actually in lined with the statements made by Scheffer et al. (2001); regime shifts are intrinsically related to resilience. So, if you have systems that are resilient, the environmental changes could already be going on and the system frantically kept in the same state and suddenly they shift.

References

Biggs, R., Carpenter, S. R., & Brock, W. A. (2009). Turning back from the brink: Detecting an impending regime shift in time to avert it. *Proceedings of the national Academy of Sciences, 106*(3), 826-831. doi: 10.1073/pnas.0811729106

Burks, R. L., Lodge, D. M., Jeppesen, E., & Lauridsen, T. L. (2002). Diel horizontal migration of zooplankton: Costs and benefits of inhabiting the littoral. *Freshwater Biology, 47*(3), 343-365.

Carpenter, S. R., & Brock, W. A. (2006). Rising variance: A leading indicator of ecological transition. *Ecology Letters, 9*(3), 311-318.

Carpenter, S. R., Brock, W. A., Cole, J. J., & Pace, M. L. (2014). A new approach for rapid detection of nearby thresholds in ecosystem time series. *Oikos, 123*(3), 290-297.

Carpenter, S. R., Cole, J. J., Pace, M. L., Batt, R., Brock, W. A., Cline, T., . . . Seekell, D. A. (2011). Early warnings of regime shifts: A whole-ecosystem experiment. *science, 332*(6033), 1079-1082.

Carpenter, S. R., & Kinne, O. (2003). *Regime shifts in lake ecosystems: Pattern and variation* (Vol. 15): International Ecology Institute Oldendorf/Luhe.

Carpenter, S. R., Kitchell, J. F., & Hodgson, J. R. (1985). Cascading trophic interactions and lake productivity. *Bioscience, 35*(10), 634-639.

Cottenie, K., Michels, E., Nuytten, N., & De Meester, L. (2003). Zooplankton metacommunity structure: Regional vs. Local processes in highly interconnected ponds. *Ecology, 84*(4), 991-1000.

Dakos, V., Carpenter, S. R., Brock, W. A., Ellison, A. M., Guttal, V., Ives, A. R., . . . van Nes, E. H. (2012). Methods for detecting early warnings of critical transitions in time series illustrated using simulated ecological data. *PloS one, 7*(7), e41010.

Dokulil, M.T. (1993). Long-term response of phytoplankton population dynamics to oligotrophication in mondsee, austria. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen, 25*(1), 657-661.

Drake, J. M., & Griffen, B. D. (2010). Early warning signals of extinction in deteriorating environments. *Nature, 467*(7314), 456.

Elliott, J. A., Jones, I. D., & Thackeray, S. J. (2006). Testing the sensitivity of phytoplankton communities to changes in water temperature and nutrient load, in a temperate lake. *Hydrobiologia, 559*(1), 401-411.

Folk, C., Carpenter, S., Walker, B., Scheffer, M., Elmqvist, T, Gunderson, L., & Holling, C. S. (2004). Regime shifts, resilience, and biodiversity in ecosystem management. *Annual Review of Ecology, Evolution, and Systematics, 35*(1), 557-581. doi: 10.1146/annurev.ecolsys.35.021103.105711

Gross, E. M. (2003). Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences, 22*(3-4), 313-339.

Hamilton, D. P., & Mitchell, S. F. (1996). An empirical model for sediment resuspension in shallow lakes. *Hydrobiologia, 317*(3), 209-220.

Huisman, J., Codd, G. A., Paerl, H. W., Ibelings, B. W., Verspagen, J. M. H., & Visser, P. M. (2018). Cyanobacterial blooms. *Nature Reviews Microbiology, 16*, 471-483.

Ives, A. R. (1995). Measuring resilience in stochastic systems. *Ecological Monographs, 65*(2), 217-233.

Jeppesen, E., Jensen, J. P., Søndergaard, M., Lauridsen, T., Pedersen, L. J., & Jensen, L. (1997). Top-down control in freshwater lakes: The role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia, 342*, 151-164.

Kéfi, S., Dakos, V., Scheffer, M., Van Nes, E. H., & Rietkerk, M. (2013). Early warning signals also precede non-catastrophic transitions. *Oikos, 122*(5), 641-648.

Kéfi, S., Holmgren, M, & Scheffer, M. (2016). When can positive interactions cause alternative stable states in ecosystems? *Functional Ecology, 30*(1), 88-97.

Lemmens, P., Declerck, S. A. J., Tuytens, K., Vanderstukken, M., & De Meester, L. (2018). Bottom-up effects on biomass versus top-down effects on identity: A multiple-lake fish community manipulation experiment. *Ecosystems, 21*(1), 166-177. doi: 10.1007/s10021-017-0144-x

Lemmens, P., Mergeay, J., De Bie, T., Van Wichelen, J., De Meester, L., & Declerck, S. A. (2013). How to maximally support local and regional biodiversity in applied conservation? Insights from pond management. *PLoS One, 8*(8), e72538. doi: 10.1371/journal.pone.0072538

Lenton, T. M., Held, H., Kriegler, E., Hall, J. W., Lucht, W., Rahmstorf, S., & Schellnhuber, H. J. (2008). Tipping elements in the earth's climate system. *Proceedings of the national Academy of Sciences, 105*(6), 1786-1793.

Lenton, T. M., Livina, V. N., Dakos, V., & Scheffer, M. (2012). Climate bifurcation during the last deglaciation? *Climate of the Past, 8*(4), 1127-1139.

Mulderij, G., Van Nes, E. H., & Van Donk, E. (2007). Macrophyte–phytoplankton interactions: The relative importance of allelopathy versus other factors. *Ecological Modelling, 204*(1-2), 85-92.

R Development Core Team. (2016). A language and environment for statistical computing. Vienna, Austria: Foundation for Statistical Computing. Retrieved from http://www.R-project.org

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

Sayer, C. D., Burgess, A. M. Y., Kari, K., Davidson, T. A., Peglar, S., Yang, H., & Rose, N. (2010). Long-term dynamics of submerged macrophytes and algae in a small and shallow, eutrophic lake: Implications for the stability of macrophyte-dominance. *Freshwater Biology, 55*(3), 565-583.

Scheffer, M., Bascompte, J., Brock, W. A., Brovkin, V., Carpenter, S. R., Dakos, V., . . . Sugihara, G. (2009). Early-warning signals for critical transitions. *Nature, 461*(7260), 53-59.

Scheffer, M., Carpenter, S., Foley, J. A., Folke, C., & Walker, B. (2001). Catastrophic shifts in ecosystems. *Nature, 413*(6856), 591-596.

Scheffer, M., Hosper, S.H., Meijer, M.L., Moss, B., & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution, 8*(8), 275-279.

Scheffer, M., & van Nes, E. H. (2007). Shallow lakes theory revisited: Various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia, 584*(1), 455-466.

Schindler, D. W., Hecky, R. E., Findlay, D. L., Stainton, M. P., Parker, B. R., Paterson, M. J., . . . Kasian, S. E. M. (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proceedings of the national Academy of Sciences, 105*(32), 11254-11258.

Søndergaard, M., & Moss, B. (1998). Impact of submerged macrophytes on phytoplankton in shallow freshwater lakes *The structuring role of submerged macrophytes in lakes* (pp. 115-132): Springer.

Spears, B. M., Futter, M. N., Jeppesen, E., Huser, B. J., Ives, S., Davidson, T. A, . . . Carvalho, L. (2017). Ecological resilience in lakes and the conjunction fallacy. *Nature ecology & evolution, 1*(11), 1616.

Timms, R. M., & Moss, B. (1984). Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. *Limnology and Oceanography, 29*(3), 472-486.

Van Donk, E., Grimm, M. P., Gulati, R. D., & Breteler, J.P.G.K. (1990). Whole-lake food-web manipulation as a means to study community interactions in a small ecosystem. *Hydrobiologia, 200*(1), 275-289.

Van Nes, E. H., & Scheffer, M. (2007). Slow recovery from perturbations as a generic indicator of a nearby catastrophic shift. *The American Naturalist, 169*(6), 738-747.

Vanderstukken, M., Declerck, S. A. J., Decaestecker, E, & Muylaert, K. (2014). Long-term allelopathic control of phytoplankton by the submerged macrophyte elodea nuttallii. *Freshwater Biology, 59*(5), 930-941.

Wang, R., Dearing, J. A., Langdon, P. G., Zhang, E, Yang, X., Dakos, V., & Scheffer, M. (2012). Flickering gives early warning signals of a critical transition to a eutrophic lake state. *Nature, 492*(7429), 419.

Whitton, B. A., & Potts, M. (2007). *The ecology of cyanobacteria: Their diversity in time and space*: Springer Science & Business Media.

Chapter 4: Rapid evolution leads to differential population dynamics and top-down control in resurrected *Daphnia* populations

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Abstract

There is growing evidence of rapid genetic adaptation of natural populations to environmental change, opening the perspective that evolutionary trait change may subsequently impact ecological processes such as population dynamics, community composition and ecosystem functioning. To study such eco-evolutionary feedbacks in natural populations, however, requires samples across time. Here we capitalize on a resurrection ecology study that documented rapid and adaptive evolution in a natural population of the water flea *Daphnia magna* in response to strong changes in predation pressure by fish, and carry out a follow-up mesocosm experiment to test whether the observed genetic changes influence population dynamics and top-down control of phytoplankton. We inoculated populations of the water flea *D. magna* derived from three time periods of the same natural population known to have genetically adapted to changes in predation pressure in replicate mesocosms, and monitored both *Daphnia* population densities and phytoplankton biomass in the presence and absence of fish. Our results revealed differences in population dynamics and top-down control of algae between mesocosms harboring populations from the time period before, during and after a peak in fish predation pressure caused by human fish stocking. The differences, however, deviated from our a priori expectations. An S-map approach on time series revealed that the interactions between adults and juveniles strongly impacted the dynamics of populations and their top-down control on algae in the mesocosms, and that the strength of these interactions was modulated by rapid evolution as it occurred in nature. Our study provides an example of an evolutionary response that fundamentally alters the processes structuring population dynamics and impacts ecosystem features.

Introduction

Ecological and evolutionary dynamics have long been considered as largely uncoupled and independent processes. More recently, it has become increasingly clear that both processes are strongly intertwined and can occur on the same time scales (Ellner, Geber, & Hairston, 2011; Hairston, Ellner, Geber, Yoshida, & Fox, 2005; Hendry, 2016; Schoener, 2011; Whitham et al., 2006). An increasing number of studies unequivocally demonstrate the existence of important feedbacks between evolutionary change and ecological dynamics (Bassar et al., 2010; Matthews et al., 2011; Pantel, Duvivier, & De Meester, 2015). For example, genetic diversity can profoundly alter population, community, and ecosystem characteristics (Crutsinger et al., 2006; Johnson, Vellend, & Stinchcombe, 2009; Whitham et al., 2006). Evolutionary trait change can mediate changes in population dynamics, community composition (Bassar et al., 2010; Matthews et al., 2011; Pantel et al., 2015; Terhorst, Lennon, & Lau, 2014; Urban et al., 2008) and ecosystem functions (Fussmann, Loreau, & Abrams, 2007). However, few of the studies so far report feedbacks of evolution that has been shown to have occurred in nature in a well-defined time frame.

Predation by fish is an important determinant of variation in zooplankton and phytoplankton community characteristics in many lakes and ponds (Carpenter et al., 2001; Jeppesen et al., 2003). Fish are efficient predators that may not only affect the biomass, but also the qualitative characteristics of zooplankton communities, such as size distribution, species composition and diversity (Declerck & De Meester, 2003; Lemmens, Declerck, Tuytens, Vanderstukken, & De Meester, 2017). Selective predation by fish can also have profound effects on population characteristics such as body size distribution, habitat use (Cousyn et al., 2001; De Meester, Weider, & Tollrian, 1995) and life history characteristics of species (Latta, Bakelar, Knapp, & Pfrender, 2007; Stoks, Govaert, Pauwels, Jansen, & De Meester, 2016). Adaptation to fish predation in zooplankton involves multiple life history (e.g. faster maturation at a smaller size, increased number of offspring, smaller offspring) and behavioral traits (e.g. diel vertical and horizontal migration) (Boersma, Spaak, & De Meester, 1998; Stoks et al., 2016). These traits are expected to have a substantial impact on zooplankton population dynamics by reducing mortality in the presence of fish, as well as by their costs in terms of food intake, such as in the case of predator avoidance by diel horizontal or vertical migration, or differential allocation of energy into number and size of offspring (Walsh & Post, 2011). Changes in behavioral traits, life history characteristics, and population dynamics of zooplankton in the presence of fish are expected to also influence the phytoplankton community by altering top-down control by zooplankton (Walsh, DeLong, Hanley, & Post, 2012). For example, a reduction in body size generally results in lower zooplankton grazing rates on phytoplankton (Gianuca, Pantel, & De Meester, 2016; Tessier, Leibold, & Tsao, 2000).

An increasing number of studies have shown that evolutionary responses to predation can impact predator-prey cycles and ecosystem characteristics. For example, the features of predator-prey cycles between rotifers and algae are profoundly altered by genetic variation in defense traits of the algae (Becks, Ellner, Jones, & Hairston, 2012; Fussmann, Ellner, & Hairston, 2003; Miller, Grand, Fondell, & Anthony, 2006; Yoshida, Jones, Ellner, Fussmann, & Hairston, 2003). Bassar et al. (2010) demonstrated that guppy populations adapted to different predation intensity change the features of small stream ecosystems by differentially lowering algal density and primary production, which results in altered nutrient cycles. In the water flea *Daphnia*, a set of studies quantifying eco-evolutionary feedbacks in a lake food chain involving alewife predation has amongst others reported that *Daphnia* populations adapted to different levels of fish predation differentially impact algal biomass and dynamics (Post & Palkovacs, 2009; Post, Palkovacs, Schielke, & Dodson, 2008). A laboratory experiment with *Daphnia* populations obtained from different lakes that differ in zooplanktivorous fish predation intensity demonstrated that life-history evolution in *Daphnia* resulted in divergence in the rate of population growth, which in turn altered consumer-resource dynamics and ecosystem functions (Walsh et al., 2012). Adult anadromous alewives migrate into lakes during spring for spawning and migrate back to the ocean each autumn. In some lakes, however, alewife are present year round because they are land-locked. *Daphnia* clones from lakes with anadromous alewives exhibited higher abundances and higher population growth, which resulted in consistently lower phytoplankton abundances compared to treatments with *Daphnia* from lakes with landlocked alewife populations or without alewife fish (Walsh et al., 2012).

While Walsh et al. (2012) documented a clear-cut impact of evolution on population densities in *Daphnia* and associated increases in top-down control of phytoplankton, in line with a priori expectations, the consequences of evolutionary change may not always be so straightforward. Given differential allocation into offspring and the impact of body size on grazing efficiency in zooplankton, the consequences of evolutionary change on population dynamics and ecosystem functions might depend on whether population dynamics are driven by resource limitation in juveniles or in adults (De Roos & Persson, 2013). In juvenile-driven cycles, juveniles are the strongest competitors and can prevent adults from reproducing (Nilsson, Persson, & Van Kooten, 2010). Reproduction then only occurs once a whole cohort of juveniles matures. In case of adult-driven cycles, adults are the strongest competitors and can prevent juveniles from maturing. The lack of new adults, while older ones die, eventually makes enough food available for some juveniles to mature (De Roos & Persson, 2013). *Daphnia* dynamics are still somewhat enigmatic in this respect, because the often observed high juvenile to adult biomass ratio suggests juvenile-driven dynamics, but individual level laboratory experiments have shown that adults are stronger competitors than juveniles (De Roos, McCauley, Nisbet, Gurney, & Murdoch, 1997; Nisbet, McCauley, Gurney, Murdoch, & Wood, 2004). Fish predation pressure often results in a change in allocation towards the production of more but smaller offspring (Boersma et al., 1998; Reznick, Butler, & Rodd, 2001; Roff, 1993) and this may change the competitive ability and starvation resistance of the *Daphnia* juveniles (Tessier, Henry, Goulden, & Durand, 1983). As a result, evolutionary change in response to an increase in predation pressure might change competitive interactions between adults and juveniles that drive population dynamics.

In earlier resurrection ecology studies, Cousyn et al. (2001) and Stoks et al. (2016) have reported rapid genetic adaptation of life history and behavioral traits in a natural population of the water flea *Daphnia magna* in response to changes in fish predation pressure that occurred over a time period of 16 years.

Given the substantial changes in 13 out of the 14 studied trait values that were reported in Stoks et al. (2016) combined with the well-documented high grazing pressure on algae that is exerted by large-bodied *Daphnia* such as *D. magna* (Carpenter, Cottingham, & Schindler, 1992; Gianuca et al., 2016; Lampert & Sommer, 2007; Verreydt et al., 2012), it is our hypothesis that these evolutionary changes likely influence population dynamics of the *Daphnia* themselves as well as algal dynamics and top-down control. We here took the opportunity to test the hypothesis of a feedback of evolution as it occurred in nature on a key ecosystem function in an outdoor mesocosm experiment in which we quantified *Daphnia* population densities and phytoplankton biomass over time in mesocosms inoculated with a representative set of clones of the three resurrected populations studied by Cousyn et al. (2001) and Stoks et al. (2016). These populations strongly differ in life history and behavioral traits (Stoks et al., 2016), and were here inoculated in mesocosms that did or did not contain fish. The presence and absence of fish provide very different selection pressures. For instance, *Daphnia* might adapt to the presence of visual predators such as fish by evolving a smaller body size (Stoks et al., 2016). In the absence of predation, however, *Daphnia* populations can reach a higher biomass, which increases food shortage, and thereby might select for larger *Daphnia* that produce larger-sized offspring with more reserves (Guisande & Gliwicz, 1992). Hence, we expected that in the treatment without fish the *Daphnia* population resurrected from the period prior to fish stocking would be able to attain the highest densities and exert the strongest top-down control on algae. In the presence of fish in the mesocosms, we expected that the population resurrected from the period with highest fish stocking would reach the highest densities because this population is adapted to coexist with fish and thus better protected from fish predation. As a result, we expected this population to exert the strongest control on algal biomass in the mesocosms with fish.

The main objective of our study was to test the feedback of evolution as it occurred in nature on an ecosystem function. Our study was therefore designed to test whether different populations established through a resurrection ecology study differed in population densities and top-down control of algae in a common gardening mesocosm experiment (Matthews et al., 2011). Our results do show pronounced differences among the populations, but the observed pattern was more complicated than our straightforward expectations. We therefore also engaged in an effort to elucidate the mechanisms underlying the observed differences between the populations that were resurrected from a layered egg bank of a single pond and document evolution as it occurred in a single population over a period of approximately 16 years.

Material and methods

Daphnia populations used in the experiment

The *Daphnia magna* clones used in the experiment were obtained from sediment cores from a relatively small (8.7 ha), shallow pond that was constructed in 1970 for the purpose of fish culture ("Oud-Heverlee Zuid", Belgium 50°50'22.16"N, 4°39'18.16"E). This pond has a well-documented fish stocking history over 30 years of its existence (see Cousyn et al., 2001). No fish stocking occurred in the period 1970-1972, while large numbers of planktivorous fish were stocked (>250 kg/ha) from 1976 to 1979. Thereafter, the stocking decreased, and completely stopped in 1993. We can therefore distinguish three main periods with regard to fish predation intensity in the history of the pond: a period corresponding to the first years (1970-1972) after the pond was dug when no fish were present (here called "Pre-fish period"), a period of high fish predation pressure (between 1976 and 1979; called "High-fish period"), and a period of relaxed fish predation pressure from 1988 -1990; called "Reduced-fish period; (see also Stoks et al., 2016). There was only a low level of genetic differentiation in neutral microsatellite markers between the three populations separated in time (Cousyn et al., 2001), supporting the view that they represent one single continuous population that showed strong adaptive evolution.

Ephippia of *Daphnia magna* clones were collected from three depth layers of a sediment core, corresponding to the Pre-fish, High-fish and Reduced-fish period (Cousyn et al., 2001). The sediment sampling and hatching was carried out as part of a previous resurrection ecology study (Stoks et al., 2016). In the laboratory, ephippia were exposed to optimal hatching stimuli (16h light/8 h dark; 20°C, fresh medium) and twelve clonal lineages from each fish stocking period were obtained and kept in the laboratory as clonal cohorts for several years before the experiment. During stock cultures, the clones were maintained at low food in order to keep them at low densities. Estimated population sizes (0.5 l jars) were less than 20 individuals; most individuals carried one or two eggs maximum, and average life span is estimated to be more than 3 months under those conditions (Luc De Meester personal observations). In this way, the turnover in individuals per year is very low (estimated to be less than 200 individuals per year) so that the probability of mutations impacting the genotypic trait values of individual clones is low, even over a period of ten years. The clones used in the present experiment were the same as used in Stoks et al. (2016), except for a contamination problem involving a few lineages (see supplementary information SI1).

In preparation for the experiment, we started up four independent, replicate cultures of all 36 clones individually (12 clones per time period x 3 time periods) under standardized conditions in a climate room (20 ± 1 °C with a 16L:8D photoperiod). Half of the culture medium (dechlorinated tap water) was renewed daily and the animals were fed fresh green algae (*Acutodesmus obliquus*, 1 x 10^5 cells ml^{-1}). Interference from maternal effects was minimized by growing the animals for two generations under those standard conditions prior to the mesocosm experiment. After the release of the second clutch of the second generation, we randomly selected 10 juveniles per clone and per replicate as the basis for the inoculum of the mesocosms. Per clone and replicate five individuals were assigned to the Predation treatment, the other five to the Control treatment. Those five individuals were grown together in a 500ml jar until release of the second clutch. In total, our set-up involved 3 time periods x 12 clones x 2 treatment groups x 4 replicates = 288 culture units. From the second clutch, we randomly selected 12 juveniles per clone, layer, replicate and treatment and combined them per layer to a population that was inoculated in a mesocosm. Each mesocosm thus received 144 individuals representing 12 genotypes of one population (Pre-fish, High-fish, or Reduced-fish), each represented by 12 individuals. Each replicate of a population (Pre-fish, High-fish or Reduced-fish) x treatment (presence or absence of predation) combination received an inoculum of animals that had been kept in separate culture for at least two generations. In this way, significant differences between mesocosms inoculated by different populations can be attributed to genetic differences among the populations rather than to maternal effects or effects of physiological acclimation. All animals were inoculated in the mesocosms when they were 24-48 hours old.

Mesocosms experiment

Twenty four cylindrical polyethylene 200 L mesocosms (three populations x two treatments x four replicates) were placed in an open grass field at the outdoor experimental area of the laboratory of Aquatic Ecology, Evolution and Conservation (ARENA) in Heverlee, Belgium. All mesocosms contained a fish cage made of 5 mm plastic mesh netting and representing 1/3 of the mesocosm volume, leaving a refuge of approximately 10 cm at the bottom and along the sides of the mesocosm. These refuges are similar to the ones used by zooplankton to avoid fish predation through horizontal and diel vertical migration. Each mesocosm was covered with mosquito netting (1.2 mm mesh size) to prevent mosquitos and other insects from entering into the mesocosms. On July 1 2014, the mesocosms were filled with 180 L of tap water, three liter of filtered (64 µm mesh size) water from a natural pond, and 10 ml of an *Acutodesmus obliquus* green algae suspension (1×10^8 cells ml^{-1}). The addition of pond water and the *Acutodesmus* inoculum was intended to stimulate the growth of phytoplankton. After twenty-one days, the mesocosms were randomly assigned to the Control (n=12) and Predation treatment (n=12), and within each of these two treatments to one of the three *Daphnia* population treatments (Pre-fish, High-fish and Reduced-fish). On that day (day 0 of the experiment), each mesocosm received 144 juvenile *Daphnia* representing independently cultured representatives of all clones from a given population (see above). Sixteen days after inoculating the mesocosms with *Daphnia* (i.e. day 16 of the experiment; slightly more than one parthenogenetic *Daphnia* generation at 20°C, ensuring that the inoculated individuals had reproduced) we added one three-spined stickleback (*Gasterosteus aculeatus*) of a standard body length of 5 cm to the cages of the Predation mesocosms.

Every six days, the fishes were taken out and redistributed using a randomization scheme to eliminate any possible biases that might arise because of differential activity among individual fishes.

Abiotic and biotic variables

We aimed for a regular increase in nutrient concentrations in the mesocosms to prevent nutrient limitation and promote the growth of phytoplankton, thereby challenging the capacity of the *Daphnia* population to achieve continued top-down control of the phytoplankton. In this way, we also buffered for the increase in nutrients imposed by excretion of the fish in the Predation treatment. To this end, water samples were taken every three days from each mesocosm, pooled per treatment (Predation and Control treatment), and immediately analyzed for total nitrogen (TN; two missing values on day 30 and 39) and total phosphorus (TP) using a HACH spectrometer. For TP, we aimed a weekly increase of 0.2mg/l starting from day 31. Based on the measured TP concentration, we calculated the amount of P that was needed to obtain an increase of 0.2mg TP/l per week in both the Predation and Control mesocosms. As the Predation mesocosms met the required increase of TP spontaneously (due to the excretion of P by the fish), we only added phosphorus (as KH_2PO_4) in mesocosms of the Control treatment. Every second time that we added P, we also added micronutrients (Na_2EDTA, $FeCl_3$, $CuSO_4$, $ZnSO_4$, $CoCl_2$, $MnCl_2$, $Na_2M_OO_4$ and H_3BO_3). In order to achieve a reasonable ratio between TP and TN concentrations, we added on two occasions nitrogen (as $NaNO_3$) in both the Predation and Control mesocosms. Because of the procedure to only add micronutrients every second time we added phosphorus and the spontaneous increase in TP in the mesocosms of the Predation treatment, we did not add micronutrients in mesocosms of the Predation treatment. This might be the cause for our observation that the Predation mesocosms experienced less pronounced algal blooms than the Control mesocosms (see further). Figure SI2 in supplementary information shows the changes in average total phosphorus and total nitrogen concentrations in the Predation and Control mesocosms as measured every three days along with the changes in temperature during the course of the experiment. The experiment lasted for 70 days.

From day 15 onwards, all mesocosms were intensively monitored until the end of the experiment. Water temperature was measured in each mesocosm every three days using a HACH multimeter. The concentration of *in vivo* chlorophyll *a* was used as a measure of phytoplankton biomass and was monitored daily (one missing value on day 59) with a handheld fluorometer (AquaFluor, Turner Designs, Sunnyvale, CA). The *Daphnia* population was sampled in each mesocosm every three days, except for the last sampling, which was delayed by one day (cf. day 70 instead of day 69). The *Daphnia* were sampled by taking a water sample (2 L) after gently mixing the water in the mesoscosm with a tube sampler. The two liter water sample was taken using a beaker and filtered over a 64 µm mesh size plankton gauze. Zooplankton samples were preserved in 4% formaldehyde. The number of adult and juvenile *Daphnia magna* individuals were determined in each sample by counting a minimum of 300 individuals from each sample using a stereomicroscope (Olympus ZS X 12). The counts were extrapolated to the total volume of the sample and transformed to abundances per liter (number of individuals/liter). *Daphnia* adults and juveniles were differentiated based on the length of the first abdominal process, which is clearly elongated in adult compared to immature females to be able to close the brood pouch (Benzie, 2005).

Data analysis

As a first test of differences among populations (categorical: Pre-fish, High-fish, and Reduced-fish) in *Daphnia* abundance and chlorophyll *a* concentration, we carried out a repeated measures linear mixed-effect model (pairing data according to date) using the 'nlme' and 'car' packages in R to compute approximate F-test statistics and p-values for fixed effects (R Development Core Team, 2016). For each variable, population was entered as a fixed effect and replicate populations were included as a random effect. We applied the restricted maximum-likelihood estimation method (REML). We analyzed the data of the Control and Predation treatment separately because of the difference in micronutrient addition during the experiment (see abiotic and biotic variables). Tukey Post-Hoc tests ('multcomp' package in R) were used to test for significant differences among specific populations in case of a significant main effect of population. We used (daily) chlorophyll *a* data and *Daphnia* abundance each three days from day 15 onwards. Chlorophyll *a* measurements before day 15 were part of the acclimation period and not used in the analyses (they are, however, plotted for clarification in Figure 1 and Figure SI3).

The repeated measures linear mixed-effect model we carried out is able to find some of the differences in population dynamics, but only when replicates behave in a synchronized and linear way. In table SI1 (see Supplementary information SI2) we show that for 15 out of the 18 population X treatment X variable combinations the dynamics are in fact nonlinear (i.e. theta > 0) and we also find a decay in forecast skill for long term forecasts, which is a characteristic of nonlinear systems. In the supplementary information, we therefore also provide a test for population differences based on simplex projections that do not assume linearity and synchrony, as additional support for differences in population dynamics. Simplex projections are an empirical dynamic modeling (EDM) technique (Deyle, Maher, Hernandez, Basu, & Sugihara, 2016; Deyle, May, Munch, & Sugihara, 2016; Sugihara, 1994; Sugihara et al., 2012; Sugihara & May, 1990, see supplementary information SI2). In the simplex projections based test for population differences, we compared the forecast skill of simplex projections using training and testing sets (replicate time-series) from the same or from different populations (see supplementary information SI2).

To explore the mechanisms underlying the differences in population densities and top-down control among populations, we examined the interactions between population densities of adult *Daphnia*, population densities of juvenile *Daphnia*, and phytoplankton biomass. Phytoplankton biomass, *Daphnia* adult abundance and *Daphnia* juvenile abundance together form a dynamic system in each mesocosm. They are (potentially) all affecting each other and these interactions can vary along a range of strengths depending on the state of the system. The interaction strength can, for instance, show us if adults are suppressing juveniles or vice–versa, and thereby provide a powerful way to distinguish adult-driven dynamics from juvenile-driven dynamics. Furthermore, the values of the interaction strengths between these three variables can differ among populations if genetic differences in life history or behavioral traits between these populations cause differences in the strengths and directions of interactions between juveniles, adults and phytoplankton biomass. To estimate the interaction strengths we used S-maps on the time series (Sugihara, 1994) as described in Deyle, May, et al. (2016). S-map is another empirical dynamic modeling (EDM) technique that has been used to detect non-linearity in dynamic systems (Sugihara & May, 1990) and make forecasts (Sugihara, 1994) of non-linear responses within time-series. The S-map method uses a locally weighted linear regression scheme, such that based on the state of the system, different regression coefficients are used for each forecast. These regression coefficients become estimates of interaction strength when making forecasts one time-step into the future, using a multivariate embedding (i.e. a set of variables used as predictors in the regression and to determine the state of the system), which contains different variables from that system. More precisely, these interaction strengths are dynamic forecasts of the effect one variable has on another variable one time step later (Deyle, May, et al., 2016). We produced S-maps based on normalized time-series data from each mesocosm. Libraries were created for each treatment and each population separately based on data from all four replicates combined. Combining replicate time-series was done following Hsieh, Anderson, and Sugihara (2007) and Clark et al. (2015). We only used chlorophyll *a* data from the days at which also the *Daphnia* densities were quantified, i.e., every third day (except for the last measurement, which was delayed by one day). We expressed time (t) in days and thus S-map forecasts were made for t+3. S-map coefficients were calculated to estimate the effect of each of the three variables (chlorophyll *a* concentration, adult *Daphnia* densities and juvenile *Daphnia* densities) on each other and on themselves. Each S-map used all three variables for the embedding (see supplementary information SI2). Before interpreting the S-map, we used convergent cross mapping (CCM) and associated null tests with surrogate time-series (see supplementary information SI2) to test whether the interactions are significant (Deyle, Maher, et al., 2016; Sugihara et al., 2012). CCM tests were done for each population and treatment separately.

Given that interactions between juveniles and adults and its effects on top-down control can be mediated by competition and thus be influenced by food levels, we tested for correlations between estimated interaction strengths and phytoplankton biomass using linear and quantile regression. Note that these tests show patterns in the model estimates of the interaction strengths rather than in the real interaction strengths. The forecast skills of the models were evaluated by the mean absolute error (MAE) and the correlation (ρ) between observations and model predictions. The degree to which patterns in model estimates reflect patterns in real interaction strengths can be derived from the skill of the model forecasts (supplementary information SI2, Table SI1).

All analyses and calculations were done in R v3.3.1 (R Development Core Team) using multiple functions from the R package rEDM developed by Ye, Clark, Deyele, Keyes, and Sugihara (2016) with additional information from Deyle, Maher et al. (2016) and Deyle, May et al. (2016). In all analyses, the data from the Control and Predation treatment were interpreted independently since both treatments received different concentrations of micro-nutrients throughout the duration of the experiment (only the Control mesocosms received micro-nutrients along with additions of phosphorus; the Predation mesocosms did not because TP increased spontaneously in these mesocosms; see abiotic and biotic variables).

Results

The overall dynamics were quite similar across mesocosms (Figure 1 and Figure SI3). During an initial phase (day 15 till approx. day 35), there was first a strong increase in the density of *Daphnia* adults and juveniles followed by a pronounced decrease. In between approximately day 40 to approximately day 55, densities of juveniles were very low and chlorophyll *a* levels tended to increase in many of the mesocosms. This increase in phytoplankton biomass was very strong in some mesocosms, whereas in others there were only moderate fluctuations. From approximately day 55 onwards, the number of juveniles in most mesocosms started to increase and chlorophyll *a* levels were suppressed.

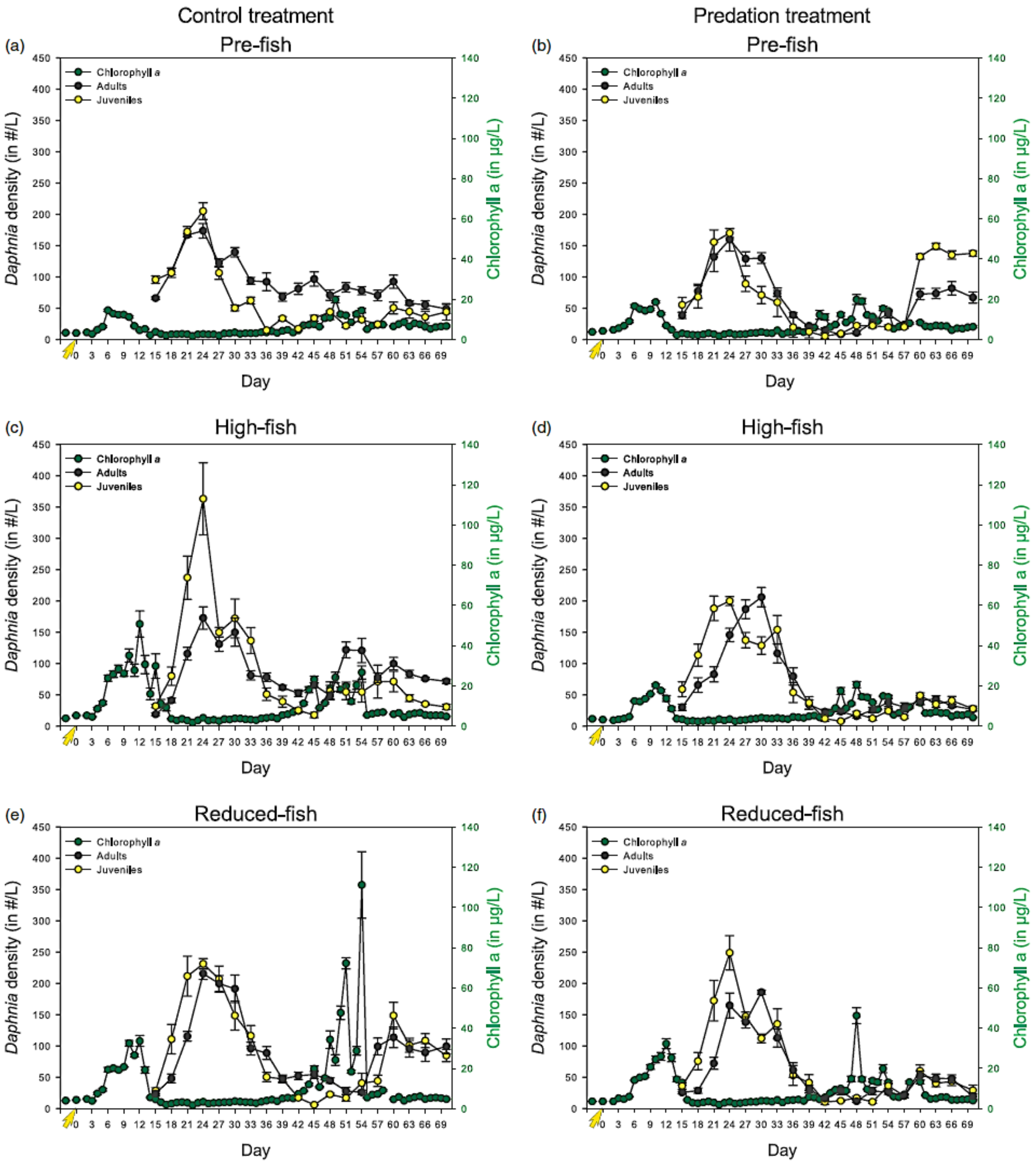


Figure 1. Average chlorophyll *a*, adult *Daphnia* and juvenile *Daphnia* abundance over the four replicates for each population (Pre-fish, High-fish and Reduced-fish) at each time point in the Predation and Control treatment. Error bars denote standard error. Yellow arrows denote the inoculation with juvenile *Daphnia* (0.8 individuals per liter).

Population differences

In the Control treatment, linear mixed-effect model revealed significant differences in chlorophyll *a* levels and juvenile abundances among *Daphnia* populations (Figure 1a, c & e and Figure 2, Table 1A).

The Reduced-fish population mesocosms had a significantly higher chlorophyll *a* concentration compared to the Pre-fish population mesocosms (posthoc Tukey test, Table 1A). The High-fish and Reduced-fish populations differed significantly in juvenile *Daphnia* abundances from the Pre-fish population (posthoc Tukey test, Table 1A). In the Predation treatment, the linear mixed-effect model showed no significant differences in chlorophyll *a* levels and *Daphnia* abundance among the three populations (Figure 2, Table 1B).

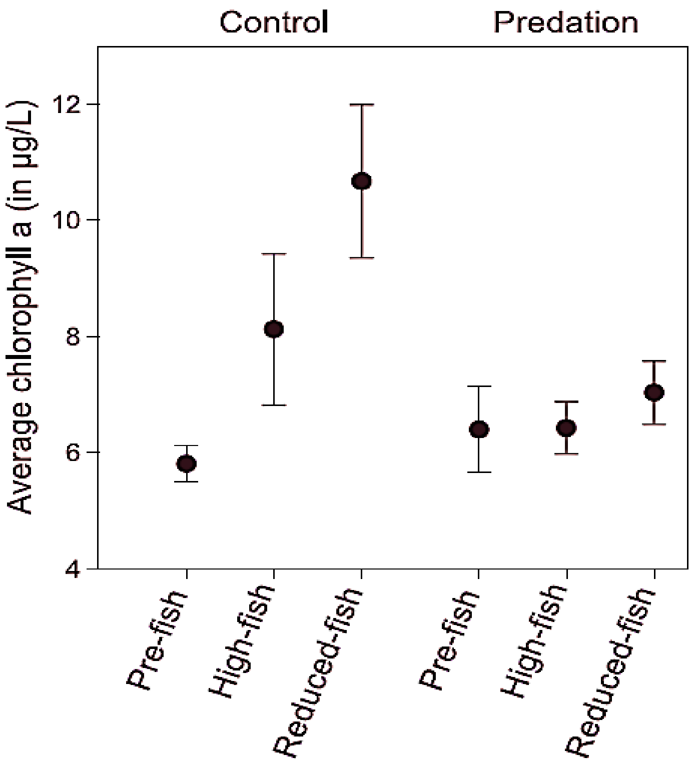


Figure 2. The average chlorophyll *a* concentration for the three populations (Pre-fish, High-fish and Reduced-fish) in the Predation and Control treatment. Error bars denote one standard error.

In the Control treatment, simplex projections of both the phytoplankton biomass and the juvenile *Daphnia* abundances in the Reduced-fish mesocosms were significantly better forecasted using other time-series from Reduced-fish mesocosms as library (i.e. training set) than when using one of the other two populations, i.e. Pre-fish or High-fish (Table 1A, supplementary information SI2, Figure SI8). Thus, Reduced-fish populations had dynamics in phytoplankton biomass and juvenile dynamics not present in the other two populations (also see supplementary information SI2). In accordance with the linear mixed-effect model, we found no significant differences among populations in adult *Daphnia* abundances in the Control treatment (Table 1A). In the Predation treatment, simplex projections revealed differences in phytoplankton biomass between the Reduced-fish population and the Pre-fish and High-fish populations (Table 1B, supplementary information SI2, Figure SI8). For Adult *Daphnia* abundances, simplex projections identified with statistical significance dynamics in the High-fish population not present in the Pre-fish population (Table 1B, supplement SI2, Figure SI8). For the juvenile dynamics, simplex projections identified dynamics in the Pre-fish population time-series not present in the High-fish and Reduced-fish populations (Table 1B, supplementary information SI2, Figure SI8).

Table 1A. Results on population differences in chlorophyll *a* concentration, adult *Daphnia* abundance and juvenile *Daphnia* abundance for the Pre-fish, High-fish and Reduced-fish populations in the (A) Control treatment and (B) Predation treatment using linear mixed-effect models structured with repeated measures and supplemented by Tukey posthoc tests and using simplex projections.

	Linear mixed-effect model									
	df	***F***	***p***	**Tukey post hoc test**			**Simplex projections**			
(A) Control treatment										
Chlorophyll *a* concentration					High-fish	Reduced-fish		Pre-fish	High-fish	Reduced-fish
Intercept	1	126.24	<.01	Pre-fish	X	✓ .001	Pre-fish		X	✓ (4)
Daphnia population	2	6.42	<.01	High-fish		X	High-fish	X		✓ (1)
Day	54	4.60	<.01				Reduced-fish	X	X	
Adult *Daphnia* abundance					High-fish	Reduced-fish		Pre-fish	High-fish	Reduced-fish
Intercept	1	364.63	<.01	Pre-fish	X	X	Pre-fish		X	X
Daphnia population	2	0.27	.76	High-fish		X	High-fish	X		X
Day	18	9.53	<.01				Reduced-fish	X	X	
Juvenile *Daphnia* abundance					High-fish	Reduced-fish		Pre-fish	High-fish	Reduced-fish
Intercept	1	284.05	<.01	Pre-fish	✓ .023	✓ .023	Pre-fish		X	✓ (4)
Daphnia population	2	4.54	.01	High-fish		X	High-fish	X		✓ (5)
Day	18	11.75	<.01				Reduced-fish	X	X	
(B) Predation treatment										
Chlorophyll *a* concentration					High-fish	Reduced-fish		Pre-fish	High-fish	Reduced-fish
Intercept	1	263.37	<.01	Pre-fish	X	X	Pre-fish		X	✓ (2)
Daphnia population	2	1.10	.33	High-fish		X	High-fish	X		✓ (5)
Day	54	10.47	<.01				Reduced-fish	X	X	
Adult *Daphnia* abundance					High-fish	Reduced-fish		Pre-fish	High-fish	Reduced-fish
Intercept	1	152.79	<.01	Pre-fish	X	X	Pre-fish		✓ (3)	X
Daphnia population	2	1.14	.32	High-fish		X	High-fish	X		X
Day	18	20.27	<.01				Reduced-fish	X	X	
Juvenile *Daphnia* abundance					High-fish	Reduced-fish		Pre-fish	High-fish	Reduced-fish
Intercept	1	307.16	<.01	Pre-fish	X	X	Pre-fish		✓ (4)	✓ (4)
Daphnia population	2	0.05	.95	High-fish		X	High-fish	✓ (1)		X
Day	18	12.21	<.01				Reduced-fish	✓ (1)	X	

Symbol “✓” indicates significance and symbol “X” indicates non-significance. The number between brackets for the report on the simplex projections indicates the number of forecast time step lengths, out of the 5 tested, in which the row population could significantly better predict itself than the column population could (see supplementary information SI2 for more details on the results). Significant p-values are shown in bold.

Interactions underlying the dynamics of juvenile and adult Daphnia

In the Control treatment, CCM tests showed significant effects of the density of adult *Daphnia* on the number of juvenile *Daphnia* in the High-fish and Reduced-fish populations, but not in the Pre-fish population (supplementary information SI2, Figure SI9). S-maps indicated this effect was on average negative (Figure 3a). The estimated strength of this interaction became smaller at high phytoplankton biomasses for the High-fish population (Figure 3a, 0.05 quantile regression: t =2.96869, p < 0.01), while we did not observed a significant relation between the estimated interaction strength and phytoplankton biomass for the Reduced-fish population. In the Predation treatment, CCM tests identified significant effects of the density of adult *Daphnia* on the number of juveniles in all three populations (supplementary information SI2, Figure SI9). In all three populations S-maps indicated this effect was on average negative and limited in strength at high phytoplankton biomasses (Figure 3b, 0.05 quantile regression, Pre-fish: t=3.78178, p < 0.001; High-fish: t=6.5038, p<0.001; Reduced-fish: t=3.53450, p<0.001).

In the Control treatment, CCM tests revealed that the population dynamics of *Daphnia* juveniles had a significant effect on the number of adults in all populations (supplementary information SI2, Figure SI9). S-maps indicated this effect was positive on average in all populations (Figure 3c). This effect was not associated with phytoplankton biomass in any of the populations (see Figure 3c). In the Predation treatment, CCM tests indicated juveniles had a significant effect on the number of *Daphnia* adults in all three populations (supplementary information SI2, Figure SI9). The S-map estimates of this interaction were on average positive in all three populations (Figure 3d). In the High-fish and Reduced-fish population there was little variation in the extent of this effect, while in the Pre-fish population it was highly variable (Figure 3d). There was no clear relation between the estimated effect of juvenile on adult *Daphnia* density and phytoplankton biomass (Figure 3d).

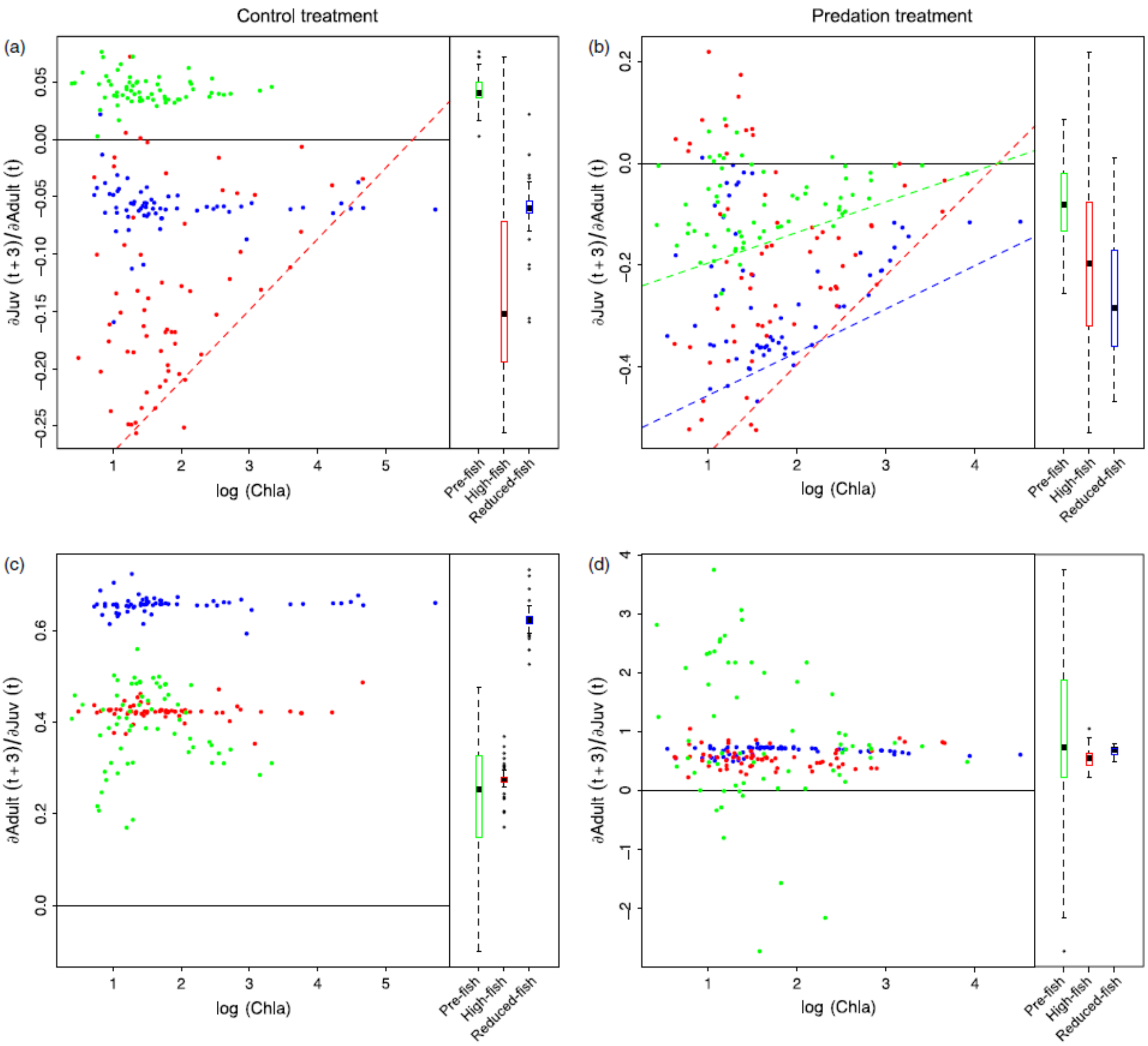


Figure 3. The effect of *Daphnia* adults on *Daphnia* juveniles [∂Juv(t+3)/∂Adult(t)] (a,b) and the effect of juveniles on adults [∂Juv(t+3)/∂Adult(t)] (c,d) as a function of phytoplankton biomass (log(Chla)) for each population separately (● Pre-fish, ● High-fish and ● Reduced-fish) in the absence (a,c) and presence (b,d) of predation. Simple linear regressions (dashed lines) show significant ($p < 0.005$, see text) 0.05 quantile regressions between estimated interaction strengths and the log(Chla) for each population. Boxplots show the distribution of estimated interaction strengths for the three populations. The bottom and top of the box show the lower and upper quartiles, the band in between them shows the median; whiskers show the minimum and maximum (excluding outliers), and circles show the outliers. Outliers are values more than 1.5 times the length of interquartile range larger than the upper quartile or smaller

than the lower quartile. The S-map estimated interaction strengths are in normalized units. The solid line shows the line of no effect.

The estimated effect of adult *Daphnia* density on adult *Daphnia* was positive in both the Predation and Control treatment (Figure SI4 a & b). In the Control treatment, this positive effect was considerably higher for the Pre-fish population than for the other two populations (Figure SI4 a). We did not observe this difference in the Predation treatment, but here the Pre-fish population showed larger variability in the estimated impact of adults on adults than the other two populations (Figure SI4 b). The effects of juvenile *Daphnia* on juveniles was estimated to be always positive in both Predation and Control treatment (Figure SI4 c & d).

In the Predation treatment, the CCM tests identified a significant effect of phytoplankton biomass on adults, which the S-maps estimated was negative in all populations (supplementary information SI2, Figure SI9, Figure SI5). CCM tests also identified a significant effect of phytoplankton biomass on juveniles in the Pre-fish and High-fish populations, which with S-maps was also estimated to be negative on average in both populations (supplementary information SI2, Figure SI9, Figure SI5).

Interactions underlying the dynamics of chlorophyll a

CCM tests revealed no significant (supplementary information SI2, Figure SI9) effect of the density of adult *Daphnia* on phytoplankton biomass in all three populations in the Control treatment (Figure 4a), whereas the effect of adults on phytoplankton biomass was significant for the Pre-fish and High-fish populations in the Predation treatment and estimated to be on average negative using S-maps (Figure 4b, Figure SI9, Figure 5). For both populations, this negative effect was stronger at higher phytoplankton biomass (Figure 4b).

In the Control treatment, the effect of juveniles on phytoplankton biomass was significant and had on average negative S-map estimates in the Pre-fish and Reduced-fish populations (Figure 4c, Figure SI9, and Figure 5). In the Predation treatment, the effect of juveniles on phytoplankton was significant in the Pre-fish and High-fish populations (Figure 4c-f, Figure SI9, and Figure 5). In all cases, simple linear regressions revealed that the estimated effect of juveniles on phytoplankton was stronger at high than at low phytoplankton concentrations (Table SI2) and at low rather than high ratios of *Daphnia* over phytoplankton biomass (Figure 4c-f, Table SI2). In all cases, the S-map estimated negative effect of *Daphnia* juveniles on phytoplankton biomass was (much) stronger than the estimated effect of *Daphnia* adults on phytoplankton (Figure 4). The negative effect of juveniles on phytoplankton biomass was large when the densities of adults and juveniles were low (Figure SI6 and Figure SI7).

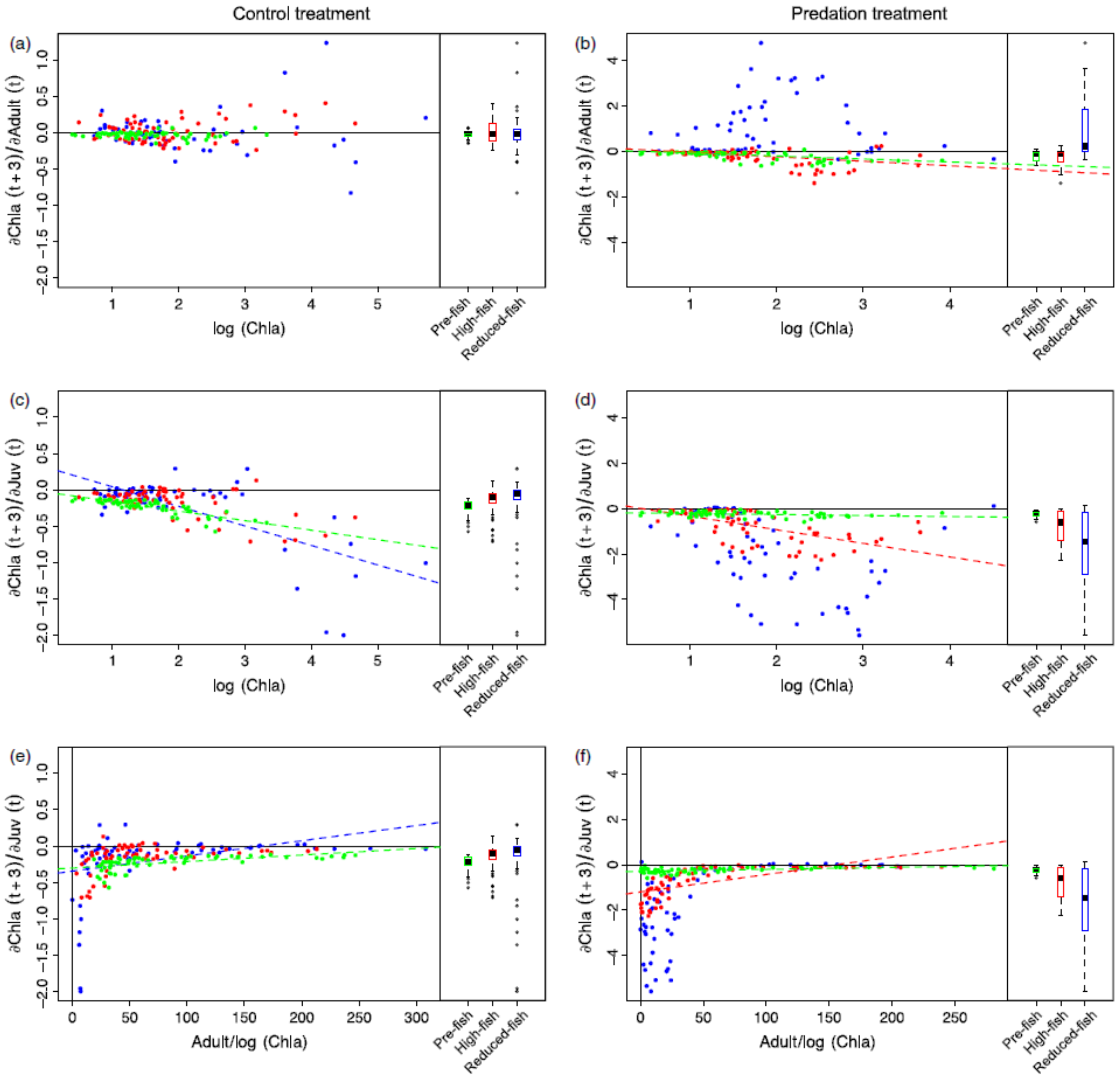


Figure 4. The effect of adults on phytoplankton [∂Chla(t+3)/∂Adult(t)] (a,b) and the effect of juveniles on phytoplankton [∂Chla(t+3)/∂Juv(t) (c,d) as a function of the phytoplankton biomass (log(Chla)) in each population in the absence (a,c) and presence of predation (b,d). (e,f) The effect of juveniles on phytoplankton [∂Chla(t+3)/∂Juv(t)] as a function of the ratio Adult : log(Chla) in the absence (e) and

presence of predation (f). The three populations are each time shown as: ● Pre-fish, ● High-fish and ● Reduced-fish. Simple linear regressions (dashed lines) between the S-map estimated interaction strengths and the log(Chla) in the Pre-fish (green), High-fish (red) and Reduced-fish (blue) populations all had significant slopes ($p<0.05$, see text). Boxplots show the distribution of estimated interaction strengths for the three populations. The bottom and top of the box show the lower and upper quartiles, the band in between them shows the median; whiskers show the minimum and maximum (excluding outliers), and circles show the outliers. Outliers are values more than 1.5 times the length of interquartile range larger than the upper quartile or smaller than the lower quartile. The S-map estimated interaction strengths are in normalized units. The solid line shows the line of no effect.

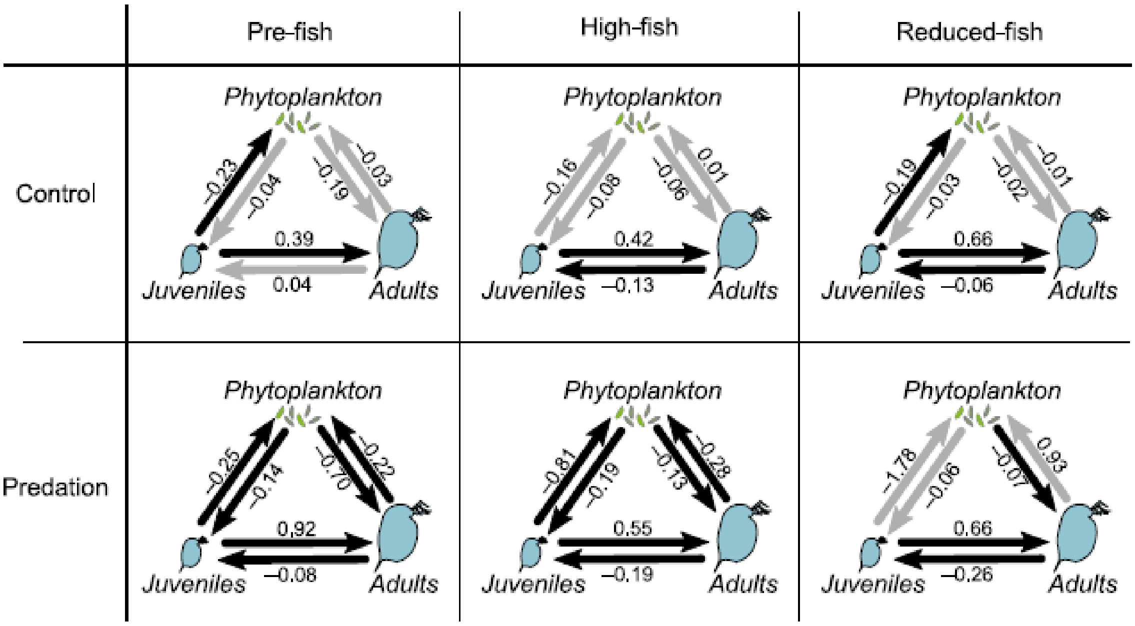


Figure 5. Interaction network for each population in both treatments (Predation and Control). Networks are based on cross map skills (ρ_{ccm}) and average S-map estimates of interaction strength. Black arrows show interactions for which the ρ_{ccm} was significantly larger than the surrogate time-series based null distributions of ρ_{ccm}. Numbers next to the arrows indicate the average interaction strengths as was estimated using S-maps.

Discussion

Population differences

At first glance, we observed a rather repeatable pattern in all mesocosms, which reflects observations in many other studies on *Daphnia* dynamics (Nelson, McCauley, & Wrona, 2005; Walsh et al., 2012), with a rapid population growth at the start of the experiment that apparently results in an overshooting of carrying capacity and is followed by a pronounced decline in population densities. During this initial phase, the *Daphnia* rapidly start to control phytoplankton growth, and chlorophyll *a* levels remain low in all mesocosms. At the end of this phase of decline in *Daphnia* densities, and in general when the number of juveniles becomes very low, there is in many mesocosms a quite pronounced increase in chlorophyll *a* biomass, reflecting that the top-down control by *Daphnia* is not effective anymore. After a period of increased phytoplankton biomass, the *Daphnia* densities start to slightly increase again, and the *Daphnia* again exert top-down control over the algae. As a result, the mesocosms in our 70 days experiment only showed a temporary increase in chlorophyll *a*, during the period between 40 and 55 days. The intensity of the resulting phytoplankton bloom differed strongly among mesocosms. *Daphnia* population (Pre-fish, High-fish, and Reduced-fish) and thus evolution of a single natural *Daphnia* population as it occurred in nature, impacted chlorophyll *a* levels in the Control treatment, where the Pre-fish *Daphnia* mesocosms exhibited lower chlorophyll *a* concentrations than the Reduced-fish *Daphnia* mesocosms (cf. results of both linear mixed-effect model and simplex projections; Figure 2 and Table 1A). Our experiment thus reveals differences in top-down control of algae associated with the evolutionary response of a single *Daphnia* population as quantified over a period of a few years (Pre-fish to High-fish: approximately 6 years; High-fish to Reduced-fish: approximately 10 years). Yet, our results did not support our initial predictions that top-down control in the absence of fish predation would be stronger in the Pre-fish population, while top-down control in the presence of fish predation would be stronger in the High-fish population. Instead, while top-down control in the Control treatment decreased from the Pre-fish to the Reduced-fish population, we found no significant differences between populations in the extent of phytoplankton blooms in the presence of fish predation.

Both the linear mixed-effect model and the simplex projections revealed differences in juvenile *Daphnia* dynamics between populations in the Control treatment, whereas in the Predation treatment only the simplex projections revealed differences in *Daphnia* dynamics between populations. Our results indicate that evolution in this natural *Daphnia* populations did not only result in a differential top-down control of phytoplankton but also in subtle differences in the dynamics of the *Daphnia* populations themselves.

Differences in dynamics often arise from differences in interactions between the state variables of the system (Chang, Ushio, & Hsieh, 2017; May, 1972; Mougi & Kondoh, 2012). In the following paragraphs, we discuss the differences in interactions between phytoplankton, *Daphnia* juveniles and *Daphnia* adults among populations and treatments that might explain the differences in top-down control of algae by the different *Daphnia* subpopulations.

Interactions underlying the dynamics of juvenile and adult Daphnia

We observed striking differences among populations in the interactions estimated between juveniles, adults and chlorophyll *a* in our time-series analyses (e.g. Figure 4). S-map estimates suggest that adult *Daphnia* in the Control treatment negatively affect juvenile abundances in the High-fish and Reduced-fish populations but not in the Pre-fish population (Figure 3a). Adult *Daphnia* are the strongest competitors (De Roos et al., 1997; McCauley, Nelson, & Nisbet, 2008), and our results suggest that they decrease survival of the juveniles more in the High-fish and Reduced-fish populations than in the Pre-fish population. This might reflect that populations adapted to fish predation pressure (here: High-fish and Reduced-fish) in general produce more but smaller juveniles (Boersma et al., 1998; Riessen, 1999; Walsh & Post, 2011). Stoks et al. (2016) characterized the three populations for their life history traits, and juveniles of the Pre-fish population genotypes are indeed slightly larger than those of the High-fish and Reduced-fish populations (see supplementary information SI3, Figure SI11a).

In the Predation mesocosms, the S-map estimates of interaction strength suggest that adult *Daphnia* have a negative impact on juveniles in all three populations. This is consistent with the fact that many studies have reported pronounced phenotypic plasticity in *Daphnia*, where animals exposed to fish kairomones often produce smaller offspring (Stibor & Lüning, 1994; Taylor & Gabriel, 1993). Admittedly, the data of Stoks et al. (2016) show divergent responses of neonate body length to the presence of fish kairomones in the different populations (Figure SI11a). Figure SI11 illustrates the relationship between average interaction strength of adults on juveniles (as estimated by S-maps based on the time-series in the different mesocosms) and three indices of juvenile quality: neonate size, 1/fecundity (assuming that the more juveniles a mother produces the less energy she can invest per individual juvenile), and size at maturity/fecundity (correcting for the fact that larger mothers might have more energy; see supplementary information SI3; all indices based on common garden life table data of Stoks et al. (2016). These scatter plots are suggestive of a link between interaction strength and differences among populations in life history traits, putatively investment in individual juveniles and its associated starvation resistance (Gorbi, Moroni, Sandra, & Rossi, 2011).

The estimated impact of juveniles on adults is generally positive in all mesocosms, supporting the view that juveniles do not exert a competitive control on adults (Figure 3c & d). The effect of adults on adults differs among populations. In the Control treatment, the Pre-fish population shows a more positive effect of adults on adults than the other populations (Figure SI4). The emerging picture on interactions between *Daphnia* is thus that (1) juveniles are competitively suppressed by adults, (2) the extent to which this happens differs among populations as they evolved through time, (3) juveniles do not competitively suppress adults but rather provide, through maturation, a source for new adults (De Roos et al., 1997; Gorbi et al., 2011; McCauley et al., 2008), and (4) the effect of adults on adults is impacted by evolution, as adults of the Pre-fish populations have a stronger positive association with their own densities three days later than adults of the other populations in the Control treatment (Figure 5).

Interactions underlying the dynamics of chlorophyll a

Our S-map estimates of the impact of *Daphnia* on phytoplankton indicate that the degree of top-down control of algae is not mainly linked to the density of adults but to the density of juveniles. This estimated impact of juveniles is stronger at low ratios of adult *Daphnia* over phytoplankton biomass (Figure 4e & f). The presence of juveniles (rather than adults) has a high estimated impact on the top-down control of algae when the densities of adults are low. While this link of dynamics in top-down control to juvenile rather than adult *Daphnia* might at first sight be surprising, it needs to be viewed against the observation that throughout nearly the whole experiment in most mesocosms the *Daphnia* populations kept the phytoplankton at low densities. Top-down control of phytoplankton by *Daphnia* is very strong during most of the experiment. As a result, the dynamics that are revealed by the S-maps rather refer to the mechanisms through which the *Daphnia* populations temporarily lost their capacity to control phytoplankton blooms. We observed a temporarily higher phytoplankton biomass during only a limited time frame (from approx. day 40 till day 55 in most mesocosms). The dominance of strong top-down control of algae during most of the experiment reflects the high grazing capacity of the large-bodied water flea *D. magna*, which is well-documented (Gianuca et al., 2016; Ye, Chang, García, Gong, & Hsieh, 2013). Given that grazing efficiency increases with body size (Brooks & Dodson, 1965; Mourelatos & Lacroix, 1990), this top-down control is largely a function of the densities of adults, not juveniles. Yet, the S-maps do not suggest this because efficient top-down control of the algae is the dominant, almost invariable state in our experiments, precisely because large-bodied *Daphnia* are such efficient grazers. Our analysis suggests a potential mechanism that leads to the occasional breakdown of this top-down control, leading to a (temporary) algae bloom. The capacity of the *Daphnia* population to dynamically increase its grazing capacity depends on the presence of juveniles, which can grow and mature to replace dying and senescing adults. Our analysis reveals that through severe competition, the adult *Daphnia* suppress survival of the juveniles, and this results in a gradual decline in the juveniles to adult ratio during the period following the initial peak population density (see Figure 1). If there are no juveniles available that can grow into adults, any increase in growth rates in phytoplankton is translated into higher chlorophyll *a* levels, as the *Daphnia* population cannot increase its grazing pressure. This situation is temporary, because the higher food availability will then result in the adults producing offspring, with the resulting reproduction (McCauley, Murdoch, & Nisbet, 1990) reinstating the capacity of the *Daphnia* population to increase its grazing impact. This is what we observe: a rapid increase in phytoplankton biomasses that is, however, temporary, and algal biomasses become low again after the re-appearance of juveniles in the populations.

Our analyses thus suggests a potential mechanism through which *D. magna* populations might (temporarily) lose the capacity to top-down control phytoplankton biomass. This mechanism follows from the fact that adults suppress juveniles, while the latter are crucial to the capacity of the population to show an immediate numerical (in terms of number of adults) response to increasing food levels and thus represent the flexibility of the population to maintain a strong top-down control on the algae under increasing nutrient loads. This proposed mechanism is directly related to the stage-structured view on populations developed by De Roos et al. (2008). It provides a link between the evolution of life history traits in response to changes in fish predation pressure in a natural population (Stoks et al., 2016) and changes in top-down control of algae, through a differential negative impact of adult *Daphnia* on juveniles.

Methodological considerations

Our experiment suffered from some methodological problems and limitation. The first is related to the fact that we adjusted phosphorus levels at regular intervals in the Control but not in the Predation mesocosms. We aimed for a regular increase in nutrient concentrations in the mesocosms to prevent nutrient limitation and promote the growth of phytoplankton, thereby challenging the capacity of *Daphnia* population to achieve continued top-down control of the phytoplankton. There was, however, no need to increase phosphorus levels in the Predation mesocosms as the presence of fish resulted in a spontaneous gradual enrichment. Every second time that we added P, we also added micronutrients. As a result of this procedure, micronutrients were added in the Control but not in the Predation mesocosms. This likely resulted in the higher phytoplankton biomasses in the Control compared to the Predation mesocosms. These higher phytoplankton biomasses in the absence compared to the presence of fish predation are opposite to expectations built on a rich literature on the impact of fish on algal blooms (Brönmark & Hansson, 2005; Scheffer, 1998). This difference in nutrient concentrations prohibits a direct comparison of phytoplankton concentrations of Predation and Control treatment, but does not interfere with comparisons of the dynamics of populations within treatments, and thus does not impact our interpretation of the results.

We note that, irrespective of the differences in nutrient concentrations, the low chlorophyll *a* levels in the mesocosms of the Predation treatment might also reflect the fact that the cages in which the fish were kept provided relatively good refuges for zooplankton. Even though the cages were quite large (1/3 of the volume of the mesocosms), they provided for a refuge of approximately 10-15 cm along the walls and bottom of the container. The efficiency of the refuge might have been enhanced by the fact that *D. magna,* when food stressed, engages in a browsing behavior, where they graze algae along hard surfaces (Horton, Rowan, Webster, & Peters, 1979). In doing so, they automatically were in a predator-safe zone.

A second methodological problem is that our experiment suffered from contamination during inoculation of the mesocosms. More specifically, our High-fish populations contained one clone from the Reduced-fish population and one clone from the Pre-fish population. Our Reduced-fish population contained one clone from the High-fish population. The contaminants did not dominate the populations (see supplementary information, Figure SI1) and thus likely did not impact our results on differences in chlorophyll *a* concentrations and on S-map estimated interactions between adults and juveniles and between these two life stages and chlorophyll *a*. If anything, this contamination made our observations on among-population differences conservative.

We related our results to the life history data of Stoks et al. (2016, see also SI3). There are, however, some limitations associated with establishing this link. First, the number of data points was low (cf.

three populations X two predation treatment conditions). Second, the life table data collected by Stoks et al. (2016) were assessed under optimal conditions of high food and low population densities, whereas the populations in our mesocosm experiment were exposed to widely varying population densities and food concentrations. These differences in context make it less straightforward to expect associations between the data in our mesocosm experiment (e.g. interaction strength) and the life history characteristics of the different populations. Yet, the tendencies revealed by the scatterplots linking interaction strengths of adults on juveniles with energy invested in newborns are suggestive.

Conclusion

Summarizing, our mesocosm experiment using resurrected *Daphnia* populations revealed a clear feedback of evolutionary trait change in a natural *D. magna* population that was exposed to changing levels of fish predation pressure on population dynamics and an ecosystem function, top-down control of phytoplankton. Such a feedback of genetic differences in antipredator traits on top-down control of algae was also observed by Walsh et al. (2012) in their study on *D. ambigua* populations from lakes with different levels of predation by alewife. Our results indicate that the differences in top-down control that we observed in our experiment were a consequence of an evolutionary change resulting in the production of smaller juveniles, resulting in a stronger suppression of juveniles by adults in the evolved populations in the Control treatment. Our results suggest that the interactions between adults and juveniles can strongly impact the dynamics of populations and their top-down effect on algae, and can be modulated by rapid evolution.

The introduction of predators can cause complex dynamics with feedback loops that can cause alternative stable states (Scheffer, Carpenter, Foley, Folke, & Walker, 2001). In the context of shallow lakes, fish can eat large zooplankton, preventing them from suppressing the algae, which can lead to a regime shift to the turbid state (Scheffer, Hosper, Meijer, Moss, & Jeppesen, 1993). Our results, however, suggest that predators can also influence top-down control of algae in more subtle ways, through the evolutionary responses they elicit when their densities are not so high as to entirely wipe out large zooplankton from the system. Our experimental results suggest that adaptive evolution in response to the presence of fish can facilitate a breakdown of top-down control of algae through changes in demographic interactions. More specifically, the production of smaller offspring can lead to a stronger effect of adults on juveniles, which can lead to strong changes in population dynamics and consequences at the level of communities and ecosystems (De Roos & Persson, 2013). Top-down control is a crucial ecosystem function in standing waters, and key to the ecosystem services ponds and lakes deliver to society (Moss, 2013; Scheffer, 1998). Algae blooms, and especially blooms to toxic cyanobacteria, strongly reduce the ecosystem services of ponds (e.g. aesthetic value, swimming water, production of drinking water, watering cattle) and might even cause health problems (Brooks et al., 2016).

Our study provides an example of an evolutionary response that fundamentally alters the processes structuring population dynamics and as a consequence also impacts ecosystem features. Our analysis is particularly strong because we could show these dynamics in a comparison of the behavior of resurrected populations derived from different time periods in the history of a single, natural population. Studies on ecosystem feedbacks of rapid evolution as it occurred in nature are a powerful new application of resurrection ecology.

References

Bassar, R. D., Marshall, M. C., Lopez-Sepulcre, A., Zandona, E., Auer, S. K., Travis, J., . . . Reznick, D. N. (2010). Local adaptation in Trinidadian guppies alters ecosystem processes. *Proceedings of the National Academy of Sciences of the United States of America, 107*(8), 3616-3621.

Becks, L., Ellner, S. P., Jones, L. E., & Hairston, N. G. (2012). The functional genomics of an eco-evolutionary feedback loop: linking gene expression, trait evolution, and community dynamics. *Ecology Letters, 15*(5), 492-501.

Benzie, J. A. H. (2005). Cladocera: The Genus Daphnia (including Daphniopsis). Leiden: Backhuys Publishers.

Boersma, M., Spaak, P., & De Meester, L. (1998). Predator-mediated plasticity in morphology, life history, and behavior of Daphnia: the uncoupling of responses. *The American Naturalist, 152*(2), 237-248.

Brönmark, C., & Hansson, L. A. (2005). The Biology of Lakes and Ponds: OUP Oxford.

Brooks, B. W., Lazorchak, J. M., Howard, M. D. A., Johnson, M. V., Morton, S. L., Perkins, D. A. K., . . . Steevens, J. A. (2016). Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems? *Environmental toxicology and chemistry, 35*(1), 6-13.

Brooks, J. L., & Dodson, S. I. (1965). Predation, body size, and composition of plankton. *science, 150*(3692), 28-35.

Carpenter, S. R., Cole, J. J., Hodgson, J. R., Kitchell, J. F., Pace, M. L., Bade, D., . . . Schindler, D. E. (2001). Trophic cascades, nutrients, and lake productivity: whole-lake experiments. *Ecological monographs, 71*(2), 163-186.

Carpenter, S. R., Cottingham, K. L., & Schindler, D. E. (1992). Biotic feedbacks in lake phosphorus cycles. *Trends in Ecology & Evolution, 7*(10), 332-336.

Chang, C. W., Ushio, M., & Hsieh, C. H. (2017). Empirical dynamic modeling for beginners. *Ecological Research*.

Clark, A. T., Ye, H., Isbell, F., Deyle, E. R., Cowles, J., Tilman, G. D., & Sugihara, G. (2015). Spatial convergent cross mapping to detect causal relationships from short time series. *Ecology, 96*(5), 1174-1181.

Cousyn, C., De Meester, L., Colbourne, J. K., Brendonck, L., Verschuren, D., & Volckaert, F. (2001). Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. *Proc Natl Acad Sci U S A, 98*(11), 6256-6260.

Crutsinger, G. M., Collins, M. D., Fordyce, J. A., Gompert, Z., Nice, C. C., & Sanders, N. J. (2006). Plant genotypic diversity predicts community structure and governs an ecosystem process. *science, 313*(5789), 966-968.

De Meester, L., Weider, L. J., & Tollrian, R. (1995). Alternative antipredator defenses and genetic-polymorphism in a pelagic predator-prey system. *Nature, 378*(6556), 483-485.

De Roos, A. M., McCauley, E., Nisbet, R. M., Gurney, W. S. C. , & Murdoch, W. W. (1997). What individual life histories can (and cannot) tell about population dynamics. *Aquatic ecology, 31*(1), 37-45.

De Roos, A. M., & Persson, L. (2013). Population and community ecology of ontogenetic development: Princeton University Press.

De Roos, A. M., Schellekens, T., Van Kooten, T., & Persson, L. (2008). Stage-specific predator species help each other to persist while competing for a single prey. *Proceedings of the national Academy of Sciences, 105*(37), 13930-13935.

Declerck, S., & De Meester, L. (2003). Impact of fish predation on coexisting Daphnia taxa: a partial test of the temporal hybrid superiority hypothesis *Aquatic Biodiversity* (pp. 83-94): Springer.

Deyle, E. R, Maher, M. C., Hernandez, R. D., Basu, S., & Sugihara, G. (2016). Global environmental drivers of influenza. *Proceedings of the National Academy of Sciences*, 201607747.

Deyle, E. R., May, R. M., Munch, S. B, & Sugihara, G. (2016). Tracking and forecasting ecosystem interactions in real time. *Proceedings of the Royal Society B: Biological Sciences, 283*(1822).

Deyle, E. R., & Sugihara, G. (2011). Generalized theorems for nonlinear state space reconstruction. *PloS one, 6*(3), e18295.

Ellner, S. P., Geber, M. A., & Hairston, N. G. (2011). Does rapid evolution matter? Measuring the rate of contemporary evolution and its impacts on ecological dynamics. *Ecology Letters, 14*(6), 603-614.

Fussmann, G. F., Ellner, S. P., & Hairston, N. G. (2003). Evolution as a critical component of plankton dynamics. *Proceedings. Biological sciences / The Royal Society, 270*(1519), 1015-1022.

Fussmann, G. F., Loreau, M., & Abrams, P. A. (2007). Eco-evolutionary dynamics of communities and ecosystems. *Functional Ecology, 21*(3), 465-477.

Gianuca, A. T., Pantel, J. H., & De Meester, L. (2016). Disentangling the effect of body size and phylogenetic distances on zooplankton top-down control of algae. *Proceedings. Biological sciences / The Royal Society, 283*(1828).

Gorbi, G., Moroni, F., Sandra, S., & Rossi, V. (2011). Anticipatory maternal effects in two different clones of Daphnia magna in response to food shortage. *Journal of Limnology, 70*(2), 222-230.

Guisande, C., & Gliwicz, Z. M. (1992). Egg size and clutch size in two Daphnia species grown at different food levels. *Journal of Plankton Research, 14*(7), 997-1007.

Hairston, N. G., Ellner, S. P., Geber, M. A., Yoshida, T., & Fox, J. A. (2005). Rapid evolution and the convergence of ecological and evolutionary time. *Ecology Letters, 8*(10), 1114-1127.

Hendry, A. P. (2016). Eco-evolutionary dynamics: Princeton University Press.

Horton, P. A., Rowan, M., Webster, K. E., & Peters, R. H. (1979). Browsing and grazing by cladoceran filter feeders. *Canadian Journal of Zoology, 57*(1), 206-212.

Hsieh, C. H., Anderson, C., & Sugihara, G. (2007). Extending nonlinear analysis to short ecological time series. *The American Naturalist, 171*(1), 71-80.

Jansen, B., Geldof, S., De Meester, L., & Orsini, L. (2011). Isolation and characterization of microsatellite markers in the waterflea Daphnia magna. *Molecular Ecology Resources, 11*, 418-421.

Jeppesen, E., Jensen, J. P., Jensen, C., Faafeng, B., Hessen, D. O., Søndergaard, M., . . . Christoffersen, K. (2003). The impact of nutrient state and lake depth on top-down control in the pelagic zone of lakes: a study of 466 lakes from the temperate zone to the arctic. *Ecosystems, 6*(4), 313-325.

Johnson, M. T., Vellend, M., & Stinchcombe, J. R. (2009). Evolution in plant populations as a driver of ecological changes in arthropod communities. *Philos Trans R Soc Lond B Biol Sci, 364*(1523), 1593-1605.

Lampert, W., & Sommer, U. (2007). Limnoecology: the ecology of lakes and streams: Oxford university press.

Latta, L. C., Bakelar, J. W., Knapp, R. A., & Pfrender, M. E. (2007). Rapid evolution in response to introduced predators II: the contribution of adaptive plasticity. *BMC evolutionary biology, 7*, 21.

Lemmens, P., Declerck, S., Tuytens, K., Vanderstukken, M., & De Meester, L. (2017). Bottom-up effects on biomass versus top-down effects on identity: A multiple-lake fish community manipulation experiment. *Ecosystems*, 1-12.

Matthews, B., Narwani, A., Hausch, S., Nonaka, E., Peter, H., Yamamichi, M., . . . Turner, C. B. (2011). Toward an integration of evolutionary biology and ecosystem science. *Ecology Letters, 14*(7), 690-701.

May, R. M. (1972). Will a large complex system be stable? *Nature, 238*(5364), 413-414.

McCauley, E., Murdoch, W. W., & Nisbet, R. M. (1990). Growth, reproduction, and mortality of Daphnia pulex Leydig: life at low food. *Functional Ecology*, 505-514.

McCauley, E., Nelson, W. A., & Nisbet, R. M. (2008). Small-amplitude cycles emerge from stage-structured interactions in Daphnia–algal systems. *Nature, 455*(7217), 1240-1243.

Mergeay, J, Aguilera, X., Declerck, S., Petrusek, A., Huyse, T., & De Meester, L. (2008). The genetic legacy of polyploid Bolivian Daphnia: the tropical Andes as a source for the North and South American D. pulicaria complex. *Mol Ecol, 17*(7), 1789-1800.

Miller, D. A., Grand, J. B., Fondell, T. F., & Anthony, M. (2006). Predator functional response and prey survival: direct and indirect interactions affecting a marked prey population. *The Journal of animal ecology, 75*(1), 101-110.

Moss, B. R. (2013). Ecology of Fresh Waters: A View for the Twenty-First Century: John Wiley & Sons.

Mougi, A., & Kondoh, M. (2012). Diversity of interaction types and ecological community stability. *science, 337*(6092), 349-351.

Mourelatos, S., & Lacroix, G. (1990). In situ filtering rates of Cladocera: Effect of body length, temperature, and food concentration. *Limnology and Oceanography, 35*(5), 1101-1111.

Nelson, W. A., McCauley, E., & Wrona, F. J. (2005). Stage-structured cycles promote genetic diversity in a predator-prey system of Daphnia and algae. *Nature, 433*(7024), 413.

Nilsson, K. A., Persson, L., & Van Kooten, T. (2010). Complete compensation in Daphnia fecundity and stage-specific biomass in response to size-independent mortality. *Journal of animal ecology, 79*(4), 871-878.

Nisbet, R. M., McCauley, E., Gurney, W. S. C., Murdoch, W. W., & Wood, S. N. (2004). Formulating and testing a partially specified dynamic energy budget model. *Ecology, 85*(11), 3132-3139.

Orsini, L., Spanier, K. I, & De Meester, L. (2012). Genomic signature of natural and anthropogenic stress in wild populations of the waterflea Daphnia magna: validation in space, time and experimental evolution. *Mol Ecol, 21*(9), 2160-2175.

Pantel, J. H., Duvivier, C., & De Meester, L. (2015). Rapid local adaptation mediates zooplankton community assembly in experimental mesocosms. *Ecology Letters, 18*(10), 992-1000.

Pauwels, L., Stoks, R., Decaestecker, E., & De Meester, L. (2007). Evolution of heat shock protein expression in a natural population of Daphnia magna. *The American Naturalist, 170*(5), 800-805.

Post, D. M., & Palkovacs, E. P. (2009). Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play. *Philos Trans R Soc Lond B Biol Sci, 364*(1523), 1629-1640.

Post, D. M., Palkovacs, E. P., Schielke, E. G., & Dodson, S. I. (2008). Intraspecific variation in a predator affects community structure and cascading trophic interactions. *Ecology, 89*(7), 2019-2032.

R Development Core Team. (2016). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Reznick, D. N., Butler, M. J., & Rodd, H. (2001). Life-history evolution in guppies. The comparative ecology of high-and low-predation environments. *The American naturalist, 157*(2), 126-140.

Riessen, H. P. (1999). Predator-induced life history shifts in Daphnia: a synthesis of studies using meta-analysis. *Canadian Journal of Fisheries and Aquatic Sciences, 56*(12), 2487-2494.

Roff, D. (1993). Evolution of life histories: theory and analysis: Springer Science & Business Media.

Scheffer, M. (1998). Ecology of shallow lakes. London, UK: Chapman and Hall.

Scheffer, M., Carpenter, S. R., Foley, J. A., Folke, C., & Walker, B. (2001). Catastrophic shifts in ecosystems. *Nature, 413*(6856), 591.

Scheffer, M., Hosper, S. H, Meijer, M. L, Moss, B., & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution, 8*(8), 275-279.

Schoener, T. W. (2011). The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *science, 331*(6016), 426-429.

Stibor, H, & Lüning, J. (1994). Predator-induced phenotypic variation in the pattern of growth and reproduction in Daphnia hyalina (Crustacea: Cladocera). *Functional Ecology*, 97-101.

Stoks, R., Govaert, L., Pauwels, K, Jansen, B., & De Meester, L. (2016). Resurrecting complexity: the interplay of plasticity and rapid evolution in the multiple trait response to strong changes in predation pressure in the water flea Daphnia magna. *Ecology Letters, 19*(2), 180-190.

Sugihara, G. (1994). Nonlinear forecasting for the classification of natural time series. *Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences, 348*(1688), 477-495.

Sugihara, G., & May, R. M. (1990). Nonlinear forecasting as a way of distinguishing chaos from measurement error in time series. *Nature, 344*(6268), 734-741.

Sugihara, G., May, R., Ye, H., Hsieh, C., Deyle, E., Fogarty, M., & Munch, S. (2012). Detecting causality in complex ecosystems. *Science, 338*(6106), 496-500.

Takens, F. (1981). Detecting strange attractors in turbulence *Dynamical systems and turbulence, Warwick 1980* (pp. 366-381): Springer.

Taylor, B. E., & Gabriel, W. (1993). Optimal adult growth of Daphnia in a seasonal environment. *Functional Ecology*, 513-521.

Terhorst, C. P., Lennon, J. T., & Lau, J. A. (2014). The relative importance of rapid evolution for plant-microbe interactions depends on ecological context. *Proceedings. Biological sciences / The Royal Society, 281*(1785), 20140028.

Tessier, A. J., Henry, L. L., Goulden, C. E, & Durand, M. W. (1983). Starvation in Daphnia: energy reserves and reproductive allocation. *Limnology and Oceanography, 28*(4), 667-676.

Tessier, A. J., Leibold, M. A., & Tsao, J. (2000). A fundamental trade-off in resource exploitation by Daphnia and consequences to plankton communities. *Ecology, 81*(3), 826-841.

Urban, M. C., Leibold, M. A., Amarasekare, P., De Meester, L., Gomulkiewicz, R., Hochberg, M. E., . . . Wade, M. J. (2008). The evolutionary ecology of metacommunities. *Trends in ecology & evolution, 23*(6), 311-317.

Verreydt, D., De Meester, L., Decaestecker, E., Villena, M. J., Van Der Gucht, K., Vannormelingen, P., . . . Declerck, S. (2012). Dispersal-mediated trophic interactions can generate apparent patterns of dispersal limitation in aquatic metacommunities. *Ecology Letters, 15*(3), 218-226.

Walsh, M. R., DeLong, J. P., Hanley, T. C., & Post, D. M. (2012). A cascade of evolutionary change alters consumer-resource dynamics and ecosystem function. *Proceedings. Biological sciences / The Royal Society, 279*(1741), 3184-3192.

Walsh, M. R., & Post, D. M. (2011). Interpopulation variation in a fish predator drives evolutionary divergence in prey in lakes. *Proceedings. Biological sciences / The Royal Society, 278*(1718), 2628-2637.

Whitham, T. G., Bailey, J. K., Schweitzer, J. A., Shuster, S. M., Bangert, R. K., LeRoy, C. J., . . . Potts, B. M. (2006). A framework for community and ecosystem genetics: from genes to ecosystems. *Nature Reviews Genetics, 7*(7), 510-523.

Ye, H., Clark, A., Deyele, E., Keyes, O. , & Sugihara, G. (2016). rEDM: Applications of Emeperical Dynamic Modelling from Time Series (Version 0.5.4). Retrieved from https://CRAN.R-project.org/package=rEDM

Ye, L., Chang, C. Y., García, C. C., Gong, G. C., & Hsieh, C. H. (2013). Increasing zooplankton size diversity enhances the strength of top-down control on phytoplankton through diet niche partitioning. *Journal of animal ecology, 82*(5), 1052-1061.

Yoshida, T., Jones, L. E., Ellner, S. P., Fussmann, G. F., & Hairston, N. G. (2003). Rapid evolution drives ecological dynamics in a predator-prey system. *Nature, 424*(6946), 303-306.

Supplementary information to chapter 4

SI1. *Genotype screening, clonal identities and contamination*

Genotype screening

The clonal identity of a total of 600 individuals, on average 25 randomly isolated individuals per mesocosm sampled at the end of the experiment (day 70), was determined by means of microsatellite analyses following Jansen, Geldof, De Meester, and Orsini (2011) and Orsini, Spanier, and De Meester (2012). Genomic DNA was extracted from random *Daphnia magna* individuals isolated from the mesocosms using the Proteinase K digestion method, as described by Mergeay et al. (2008). Live *Daphni*a were homogenized in 100 µl proteinase K-buffer (16mM $[NH_4]_2SO_4$, 67 mM Tris-HCl, pH 8.8, 0.01% Tween-20, 10% DTT and 0.5mM proteinase K). Following overnight incubation at 56°C, a 10 minute denaturation of samples was carried out at 96°C. Qualitative PCR (T1 PCR machine; Biometra, Germany) was conducted with the QIAGEN multiplex PCR kit (QIAGEN, Netherlands). Nine microsatellite markers, structured in one multiplex (multiplex MO1, as in Jansen et al. (2011) and Orsini et al. (2012); EST4276 was added as an additional marker) were used to identify clonal lineages.

PCR cycling conditions included an initial denaturation step at 95°C for 15 minutes, 30 cycles of 94°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 45 seconds, and a final elongation step at 60°C for 30 minutes. Microsatellite alleles were scored using an ABI PRISM 3031 automated sequencer (Applied Biosystems) and analyzed with the Gene Mapper software (applied Biosystems) using LIZZ500 as standard size. Based on reference samples (composed of animals from stock cultures and maternal individuals that gave rise to the animals that were used to stock mesocosms), all genotyped individuals were classified and named according to their layer of origin (B, M, T) and specific clone identification code (as in Pauwels, Stoks, Decaestecker, & De Meester, 2007; Stoks, Govaert, Pauwels, Jansen, & De Meester, 2016). The multilocus-genotypes were also compared to earlier genotyping efforts on the same clones (Orsini et al., 2012).

Clonal identities and contamination

The 36 clones used in the present experiment were the same as used in Stoks et al. (2016), however, we detected that some contamination had occurred when we screened the clones for their genotype using 9 microsatellite markers (see above). For the pre-fish population our multi-locus genotype identification suggests that one clone (B7) was accidently replaced by another clone from the same population (B9). For this population, the contamination only occurred within the population and resulted in 11 instead of 12 clones being used in the experiment (Figure SI1 a & b). For the high-fish population, two clones (M10 and M12) were accidently replaced by another clone from the same population (M11), one clone (M3) was accidently replaced by a clone with the same multilocus genotype as clone B6 of the pre-fish population, and one clone (M2) was accidently replaced by a clone with the same multilocus genotype as clone T12 from the reduced-fish population. So for this population, 8 clones were inoculated in addition to two clones that was derived from another population (Figure SI1 c & d). In the reduced-fish population, multi-locus genotype identification suggests that one clone (T3) was accidently replaced by a clone from the high-fish population (M7). For this population, 11 clones were used in the experiment in addition to one clone from another population (Figure SI1 e & f). As we screened the lineages for their genotypes after inoculating the mesocosms, we could not prevent the contamination. Although the contamination was unfortunate, only the among-population contaminations (one clone in the reduced-fish population and two clones in the high-fish population) can potentially interfere with the interpretation of our results, which are based on comparisons among populations. Moreover, to the extent that the interpretation of our results depends on the detection of among-population differences, the contamination does not induce false positives, but rather results in a conservative assessment of the impact of evolutionary change. The average detected relative abundance of the pre-fish clone in the high-fish population treatment at the end of the experiment was 0% in both Control and Predation treatments (Figure SI1 c & d), which translates into at most very low abundances of this clone. The average observed relative abundance of the reduced-fish population clone in the high-fish population treatment at the end of the experiment was 1% in the Control mesocosms and 26% in the Predation mesocosms (Figure SI1 c & d). The average observed relative abundance of the high-fish population clone in the reduced-fish population treatment at the end of the experiment was 38.1 % in the Control mesocosms and 28.7% in the Predation mesocosms (Figure SI1 e & f).

SI2. *Data analysis*

Empirical dynamic modeling

Empirical dynamic modeling (EDM), uses time-series to reconstruct the attractor manifold (see further) and allows for the exploration of the mechanisms underlying the dynamics of the system (Deyle, May, Munch, & Sugihara, 2016; Sugihara, 1994; Sugihara et al., 2012; Sugihara & May, 1990). Simplex projections are a forecasting technique from the EDM framework (Sugihara & May, 1990). The forecast skill of simplex projections using one group of time-series as a library (i.e. learning set) to make forecasts for data points in another time-series can be used to assess the similarity in the attractor manifold of those time-series. The S-map method is a technique from the EDM framework that can be used to estimate interaction strengths. S-maps do so by recovering the Jacobians (i.e. partial derivatives) at each time-point (Deyle, May, et al., 2016; Sugihara, 1994). Another technique from the EDM framework is convergent cross mapping (CCM) (Sugihara et al., 2012). CCMs can be used to identify causal links in the system. A brief explanation of EDM and the techniques that we used are given below, together with details of our implementation of them. For more in-depth explanations and further examples, we refer the reader to the rEDM user guide Ye, Clark, Deyele, Keyes, and Sugihara (2016) and empirical dynamic modeling for beginners (Chang, Ushio, & Hsieh, 2017).

Dynamic systems are often described as a set of multiple equations, in which each equation describes how the dynamics of a certain variable depends on itself and other variables. When different states of a system are very similar, the state of the system will over the short run evolve very similarly. Representing all the relevant variables of a system as a set of Cartesian coordinates in state space and the observations in the time-series of these variables as coordinates visited by that system, results in a collection of trajectories forming a geometric object called an attractor manifold. The attractor manifold is the product of the specific rules and equations that describe the interactions between variables of the system, and thus is an empirical description of the dynamics of the system. An animation explaining the reconstruction of the attractor manifold by its variables is given in Sugihara et al. (2012; https://youtu.be/8DikuwwPWsY). As similar states evolve similarly over the short run, so do nearby states in state space. Thus, when time-series of the relevant variables are available, short-term forecasts for a given state can be made based on the predicted short-term future of nearby states in state space. However, when time-series for some variables in the system are not available, trajectories cross and nearby states will not go in exactly the same direction. In reality there might be countless variables influencing every system, but often the majority of the changes over time in a certain state variable are caused by only a few other variables. Hence, relatively skillful forecasts can be made based on these few relevant variables. It is, however, not always feasible to obtain measurements of or know all relevant variables of the system. Takens (1981) addressed this problem by using the fact that in a dynamic system, time-series of a variable that is influenced by other variables also contain information on these variables. An everyday used example of this principle is our ability to estimate the future location of moving objects by using consecutive snapshots of these objects, locations, without directly observing the momentum of the object and the forces (e.g. gravity) acting on it. Thus, instead of representing the state of the system as a vector (i.e. a multidimensional point) in state space with as coordinates the relevant state variables, one can use time-lagged observations (i.e. snapshots) of one variable as the coordinates. This is called a time-lagged embedding. There are a minimum number of time-lagged observations needed to capture all the necessary information and thereby prevent trajectories from crossing in the time-lagged embedding (i.e. for the embedding to be diffeomorphic). Before Takens' theorem, it was not clear if the number of time-lags needed would be too high for any practical usage. Takens (1981) found a connection between the number of required lags, i.e. the embedding dimension E, and the number of state variables, i.e. the number of dimensions D. He demonstrated that a time-lagged embedding using just $E = 2 * D + 1$ lags is the maximum needed to obtain a diffeomorphism of the original attractor manifold of a dynamic system (i.e. to prevent lines crossing in the embedding). Thus, if the relevant variables of a system are the two variables X and Y, no more than five lags are needed in the embedding (i.e. {Y(t), Y(t-1), Y(t-2), Y(t-3), Y(t-4)}). This means one can obtain a shadow version (i.e. a time-lagged embedding) of the original attractor manifold by using only a few lags of one variable. Although this shadow manifold is a globally distorted (e.g. stretched or bent) version of the original manifold, this distortion is a smooth invertible change in coordinates. A short animation by Sugihara et al. (2012) explaining Takens' theorem can be found here: https://youtu.be/QQwtrWBwxQg. The same points in time that are close on the original attractor manifold are also close on the shadow manifold. Thus, as the state of the system changes over time and visits different neighborhoods on the attractor manifold, it will pass by neighborhoods on the manifold that it has visited before and the time points in history close to each other on the original attractor are also close on the shadow manifold. In (univariate) simplex projections, this fact is used to make forecasts using only one variable (Sugihara & May, 1990). As differences in dynamics between different populations can be more pronounced in some variables (i.e. dimensions in state space) than others, we decided to test for significant differences between populations independently for different variables. For this test we were thus able to use univariate simplex projections.

An interesting consequence from Takens' theorem is that when a variable *X* influences another variable *Y*, then *X* will leave its mark on the dynamics of *Y*. Time-points close on the time-lagged embedding of *X* will also be close on the time-lagged embedding of *Y*. Thus, if nearby points on the shadow manifold of *Y* are also close on the shadow manifold of *X*, than *X* likely influences variable *Y*. This is the basis for convergent cross mapping (Sugihara et al., 2012). An animation explaining this principle by Sugihara et al. (2012) can be found here: https://youtu.be/NrFdIz-D2yM.

Simplex projections

We used univariate simplex projections to compare the similarity in dynamics of individual variables between populations. In simplex projections, for each state in the time-lagged embedding of the prediction set (i.e. for each target state $\underline{x}(t^*)$), the *E+1* closest points on the shadow manifold of *X*, which is reconstructed using only the library set, are taken and a weight is calculated based on their Euclidian distance from *x(t*)*. To make forecasts with a specified time step, t_p, into the future, the states that the *E+1* nearest states have t_p into the future are multiplied with their respective weights and the average of these products is used as the forecast(Sugihara & May, 1990). If the time-series used in the library can be used to make skillful forecasts of the time-series in the prediction set, then the dynamics underlying the time-series in the prediction set must be similar to those in the time-series used in the library. We expressed forecast skill in MAE (mean absolute error). For each population, to determine whether the dynamics of replicates of the same population are more similar than between different populations, we used a one sided Mann-Whitney test comparing the skill of within population forecasts to forecasts from other populations. We made forecasts for all possible combinations of (replicate) time-series as library and prediction sets, with 3 time-series as library predicting one other time-series (i.e. excluding combinations where the same time-series occurred in both library and prediction set). This leads to four MAEs for each set of within population forecasts and 16 for the between population forecasts. For simplex projections the number of lags used in the reconstructed state space has to be specified (i.e. the embedding dimension *E*). We here each time tried all embedding dimensions below 7 and used the *E* that resulted in the highest value.

Another parameter that has to be set in simplex predictions is the forecast time step t_p. The standard choice for this is to use the smallest step (i.e. 3 days for *Daphnia* time-series and 1 day for phytoplankton). However, when dynamics are relatively slow, small time steps might not be sufficiently challenging to distinguish in forecast skill. In contrast, as is characteristic to non-linear systems, large forecast time steps result in lower predictability and forecast skill decreases in all models. Therefore, in testing the difference between populations, we tested different forecast time-steps (3, 6, 9, 12 and 15 days). Although within population simplex projections might not be significantly more skillful (i.e. lower MAE) with very small time-steps or too large time-steps, if they significantly differ from another population, than at least within a certain range of forecast time steps, they would do significantly better when using libraries from the same population than when using libraries from a different population. In Simplex projections, unlike the other EDM methods we applied, each time-series is analyzed separately. Hence, we were able to make use of the finer resolution of the phytoplankton time-series and use a time lag of one day between the time lags in the embedding. The P-values of these tests against the forecast time-step used are shown in figure SI8 and the number of significant p-values (<0.05) among the tests using the five different time steps, are shown in Table SI1 in the main text.

Cross mapping

For each time point t_p, the *E+1* closest points on the shadow manifold of *Y* are taken and a weight is calculated based on their distance from t_p. Multiplying the *E+1* nearest states with their respective weights and averaging them gives the value of *Y* at t_p. The same *E+1* time points, but from the time-series of *X* are then multiplied with these weights to make a prediction. Note that these do not have to be the *E+1* closest points on *X* as well, as long as they are close enough, the prediction will be reasonably skillful. The Pearson correlation coefficient between the predicted values of *X* based on the manifold of *Y* at each time point and the true value of *X* at those time points is the prediction skill of the cross map (ρ_{ccm}). In the presence of a causal link between the considered variables, the prediction skill will increase until they converge to a certain ρ_{ccm} when more time-points are used to make the shadow manifold of the library variable (in our case *Y*), (Sugihara et al., 2012).

Given that each selection of days from the time-series to use in the library will result in a different cross map skill (ρ_{ccm}), the days of the time-series used at each library size is drawn multiple times (in our case 100 times) resulting in a distribution of cross map skills (ρ_{ccm}). In all our analysis, we combined time-series data of the four replicates of each population within each treatment, but in such a way that no one vector (i.e. set of time lagged observations) contained data points from different replicates (Clark et al., 2015; Hsieh, Anderson, & Sugihara, 2007). To avoid problems with overfitting, we first performed CCMs predicting the state of *X* three days before based, on the shadow manifold of *Y* (see Deyle, Maher, Hernandez, Basu, & Sugihara, 2016; Deyle, May, et al., 2016) and then used the embedding dimension resulting in the highest ρ_{ccm} from this as embedding dimension for the actual CCMs. In the actual CCMs, the shadow manifold of Y was used to predict the state of X on the same day (see Figure SI10). False signs of positive cross maps were further eliminated by testing against randomly generated surrogate time-series that were used as a null distribution (Deyle, Maher, et al., 2016; Deyle, May, et al., 2016). We generated 500 surrogate time-series in which the order of the days was randomly permutated. If the cross map skill is significantly greater in the original time-series than in the surrogate time-series based null distribution, the properties that were incorporated in the surrogate time-series are not enough to explain the size of the cross map skill. Importantly, we used the same permutation of days for each of the 4 replicates in generating null distributions to also consider potential false signs of causal influences resulting from synchrony of variables with external forces that acted upon the four replicates simultaneously. We determined the embedding dimensions for both the surrogate and the regular time-series similarly to the regular CCMs (i.e. based on the ρ_{ccm} in 3 day backward predictions). When the original time-series performed better than 95% of the surrogates using ρ_{ccm} as a criterion, we considered them to be significant (i.e. $p<0.05$). Results of this analysis are given in Figure SI9. All cases with significant surrogate tests (Figure SI9) showed convergence in the CCM plots (Figure SI10). An overview of significant interactions between variables is given in Figure 5 in the main text.

S-maps

The closer one zooms into a small neighborhood on the attractor manifold, the more linear the manifold becomes. The S-map method is a locally weighted linear regression scheme (Sugihara, 1994). It approximates the best local linear model at each measured state by giving more weight to states on the attractor manifold that are more nearby that state. Similar to a multivariate linear regression, S-maps average out noise by using all data points, rather than just a few neighboring points in state space. In contrast to multivariate linear regression, S-maps allow points more closely located on the manifold to the target point $\underline{x}(t)$ to be given a higher weight in the forecast, thereby accounting for potential state-dependent-differences in interaction strengths over time, which is typical for non-linear dynamic systems. S-maps contain one variable, theta (θ), which sets the degree of non-linearity by determining to what extent points more nearby on the attractor manifold are given more weight than distant ones. A theta of zero leads to equal weights for all points and basically results in a simple multivariate linear regression. As each target point $\underline{x}(t)$ is positioned differently on the manifold with respect to the other points in the dataset, a separate weighted linear regression is made for each location $\underline{x}(t^*)$ on the manifold. The weight given to observation *i* when making the local linear approximation of $\underline{x}(t^*)$ is given by $w_i = e^{\frac{-\theta\|\underline{x}(t_i)-\underline{x}(t^*)\|}{\bar{d}}}$, where $\left\|\underline{x}(t_i) - \underline{x}(t^*)\right\|$ is the Euclidian distance between the two vectors and $\bar{d} = \frac{1}{n}\sum_{i=1}^{n}\left\|\underline{x}(t_i) - \underline{x}(t^*)\right\|$. With the separate local weightings around each target point, separate linear regressions can be made by solving the SVD (singular value decomposition) for $\boldsymbol{C}$ in the linear equation $\boldsymbol{B} = \boldsymbol{A} \cdot \boldsymbol{C}$, where **A** is the $n \times E$ dimensional matrix (*E* is the embedding dimension or number of variables used to reconstruct the attractor manifold) of states $\underline{x}_j(t_i)$ weighted based on the proximity to the target states $\underline{x}(t^*)$ given by $A_{ij} = w_i x_j(t_i)$, ***B*** is the *n*-element vector of future values of the target variables x_k, also weighted based on the proximity to the target states, given by $B_i = w_i x_k(t_i + 1)$, and ***C*** is the *E*-element vector of Jacobian elements at the target point $\underline{x}(t^*)$ for the target variable x_k.

S-maps can be used on both univariate embeddings of one variable or using a multivariate embedding (Deyle & Sugihara, 2011). When using a multivariate embedding, the S-map coefficients, i.e. the regression coefficients of each locally weighted linear regression (which are equivalent to the partial derivatives on the manifold or the Jacobian) give the strengths and signs of the interactions between variables (Deyle, May, et al., 2016).

In our study, all the forecasts were done 3 days into the future, so that direct comparisons with our observed data could be carried out, and using leave-one-out cross validation. We made separate library sets for each of the 3 populations in each treatment (6 library sets in total) and always used the same multivariate embedding: {Chla(t), Adults(t), Juv(t)}, irrespective of which of the three target variables (x_k) (Chla, Juveniles or Adults) was used. We performed S-maps with the following theta values: 0, 10^{-}

^{4}, $3*10^{-4}$, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 15 and 20. The theta resulting in the best forecast skill ρ was used for each population, treatment and forecasted variable. The forecast skill ρ was found to be always significantly (<0.0001) better than zero using Fisher's z-transformation (Table SI2). The resulting S-map estimates of interaction strengths (i.e. the elements of ***C***) are plotted against time in Figure SI6 and SI7.

The effect of X on the future (3 days later) of Y is given by $\frac{\partial X(t+3)}{\partial Y(t)}$. The interaction strengths are calculated separately at each time point in the time-series. At each time point, based on the location of the state in state space, a locally weighted linear regression is performed. For Adult *Daphnia*, for instance, a linear regression would look like this:

$$Adult(t+3) = \beta_0 + \beta_1 Chla(t) + \beta_2 Adult(t) + \beta_3 Juv(t) + \epsilon_t.$$

But in the non-linear forecasts by the S-maps, for each forecast different parameters are estimated because of the local weighting, and the β_1, β_2 and β_3 become the elements of the Jacobian matrix:

$$\beta_1 = \frac{\partial Adult(t+3)}{\partial Chla(t)}, \beta_2 = \frac{\partial Adult(t+3)}{\partial Adult(t)}, \beta_3 = \frac{\partial Adult(t+3)}{\partial Juv(t)}.$$

Non-linear systems, as analyzed by S-maps, can thus be interpreted as linear systems, but with changing parameters depending on the position in state space of the system. By interpreting interactions this way, the straightforward intuition we have with linear systems can be extended to explore non-linear systems, the difference being that the interaction strengths change over time depending on the states. So we see a cloud of interaction strengths in Figure 3, Figure 4, Figure SI4 and Figure SI5 that shows how interaction strengths vary with state. By plotting the interaction strengths estimated at each time point against measured observations at the same time points of an important variable (e.g. phytoplankton in Figure 3, Figure 4a-d, Figure SI4 and Figure SI5) we are able to see how the interaction strengths from the locally weighted linear regressions predicted by the model depend on this variable. To test for the significance of relations between estimated interaction strengths and variables, we performed several linear regressions (Figure 4). The statistics of these linear regressions are shown in Table SI2.

SI3. *Linking interaction strengths to genotypic trait values*

Figure SI11 contains scatter plots showing the association between average interaction strengths of adults on juveniles against three measures of juvenile 'quality' based on the life history data provided by Stoks et al. (2016): (A) average neonate size (body length); (B) inverse average number of offspring of the first two clutches (1/average fecundity); (C) average size at maturity divided by average fecundity. All data points represent averages of all clones of a given population and in either control or fish kairomone exposure conditions as studied in the common garden experiment carried out by Stoks et al. (2016).

All three variables capture some aspects of juvenile energy content, but should be considered loose approximations rather than precise estimates. We plot neonate body size because of its link to body mass. We plot average number of offspring assuming that investment per individual offspring would become lower as their numbers increase. This assumes an equal amount of available energy of all mothers. The third index (average size at maturity divided by average fecundity) tries to take variation in energy content of mothers into consideration by scaling the number of offspring to the size of the mother.

All three scatterplots are suggestive for a relationship between the interaction strengths of adults and juvenile 'quality', even though none of the relationships is significant. The lack of significance might reflect: (i) the limited number of data points, (ii) the approximate nature of our measures of energy content, (iii) the fact that the life history data are limited to size of neonates, size of adults, and number of offspring, whereas the mesocosm data integrate information on different age classes within the categories of juveniles and adults, and (iv) the impact of density effects on body sizes (all life history data in Stoks et al. (2016) were collected under optimal food conditions). Given that Stoks et al. (2016) quantified among population differences in genotypic trait values under highly standardized conditions, while the mesocosm experiment quantified dynamics of these same populations under highly fluctuating population densities and food conditions, we consider the observed relationships (with correlation coefficients reaching 0.59) to be suggestive of a mechanistic link between the life histories of the populations and the observed dynamics of *Daphnia* and chlorophyll a in the mesocosms.

Table **SI1**. Degree of non-linearity (theta) resulting in optimal S-map forecast skill (i.e. highest ρ) and probability that the ρ is greater than zero using Fisher's z-transformation.

Treatment	Population	Forecasted variable:								
		Chlorophyll a			Adults			Juveniles		
		theta	ρ	probability (Fisher)	theta	ρ	probability (Fisher)	theta	ρ	probability (Fisher)
Control	Pre-fish	1	0.65	1.89E-10	0.75	0.59	2.38E-08	0.1	0.68	1.21E-11
	High-fish	1.5	0.57	1.18E-07	0	0.52	1.42E-06	0.5	0.55	2.63E-07
	Reduced-fish	2	0.69	3.29E-12	0	0.79	2.60E-18	0	0.70	1.06E-12
Predation	Pre-fish	1	0.59	2.87E-08	6	0.79	2.75E-18	1	0.68	1.32E-11
	High-fish	2	0.68	1.23E-11	3	0.84	4.45E-23	1.5	0.77	1.52E-16
	Reduced-fish	4	0.53	1.27E-06	0.5	0.74	9.27E-15	0.75	0.68	1.06E-11

Table **SI2**. Results of the simple linear regressions in Figure 4. The S-map estimates of the effect of *Daphnia* on phytoplankton were regressed on chlorophyll *a* concentration (log(chla)) and (only for juveniles) the ratio of Adult *Daphnia* : chlorophyll *a* (Adult/log(chla)) for pre-fish, high-fish and reduced-fish in the Control and Predation treatments. Linear regressions were only performed for all population x treatment combinations when the interactions were significant ($p<0.05$) in the CCM test.

Treatment	Regression	Pre-fish				High-fish				Reduced-fish			
		R^2	R^2adj	$F_{1,62}$	p	R^2	R^2adj	$F_{1,62}$	p	R^2	R^2adj	$F_{1,62}$	p
Control	∂Chla/∂Adult on log(Chla)	ρCCM is not significant				ρCCM is not significant				ρCCM is not significant			
	∂Chla/∂Juv on log(Chla)	0.68	0.6776	133.40	< 0.001	ρCCM is not significant				0.49	0.4804	59.25	0.001
	∂Chla/∂Juv on Adult/log(chla)	0.33	0.3239	31.19	< 0.001	ρCCM is not significant				0.12	0.1038	8.298	< 0.01
Predation	∂Chla/∂Adult on log(Chla)	0.45	0.4411	50.73	0.001	0.25	0.2421	21.13	< 0.001	ρCCM is not significant			
	∂Chla/∂Juv on log(Chla)	0.09	0.078	6.30	< 0.05	0.39	0.3767	39.08	< 0.001	ρCCM is not significant			
	∂Chla/∂Juv on Adult/log(chla)	0.22	0.2084	17.58	< 0.001	0.47	0.4621	55.12	< 0.001	ρCCM is not significant			

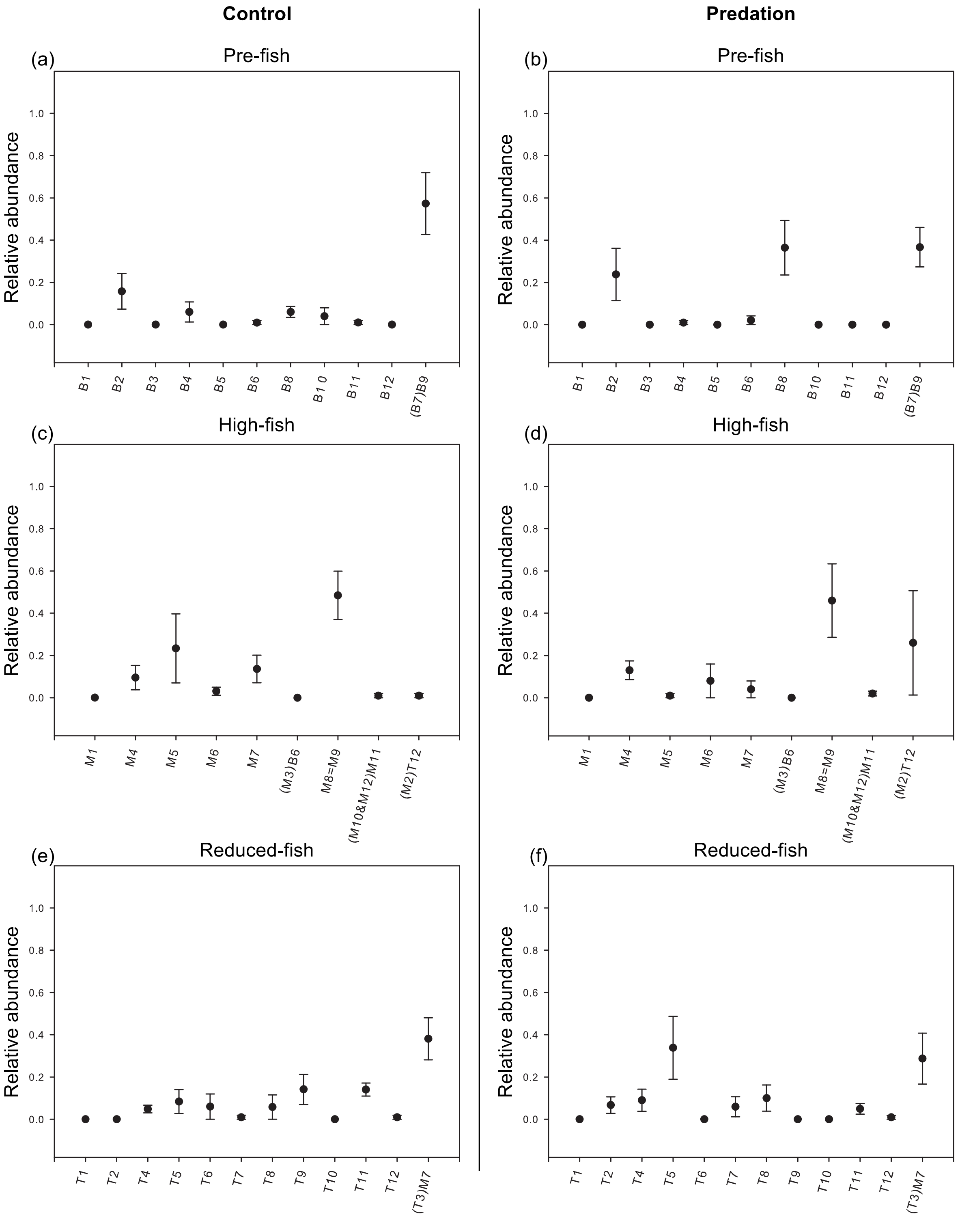


Figure SI1. The average relative abundance of individual clones in each population in the Control and Predation treatment at the end of the experiment. Clones codes that start with a letter “T” belong to the reduced-fish population (“Top”), codes starting with “M” indicate clones that belong to the high-fish population (“Middle”) and “B” indicates clones from the pre-fish population (“Bottom”). In the pre-fish population clone B7 was accidently replaced by clone B9 from the same population. In the high-fish population clones M8 and M9 are the same multi-locus genotype (cannot be discriminated with the marker set used), clones M10 and M12 were accidently replaced by clone M11 from the same population, clone M3 was accidently replaced by a clone with the same multilocus genotype as B6 of the pre-fish population and clone M2 was accidently replaced by a clone with the same multilocus genotype as clone T12 from the reduced-fish population. In the reduced-fish population clone T3 was replaced by clone M7 from the high-fish population. Error bars indicate standard error.

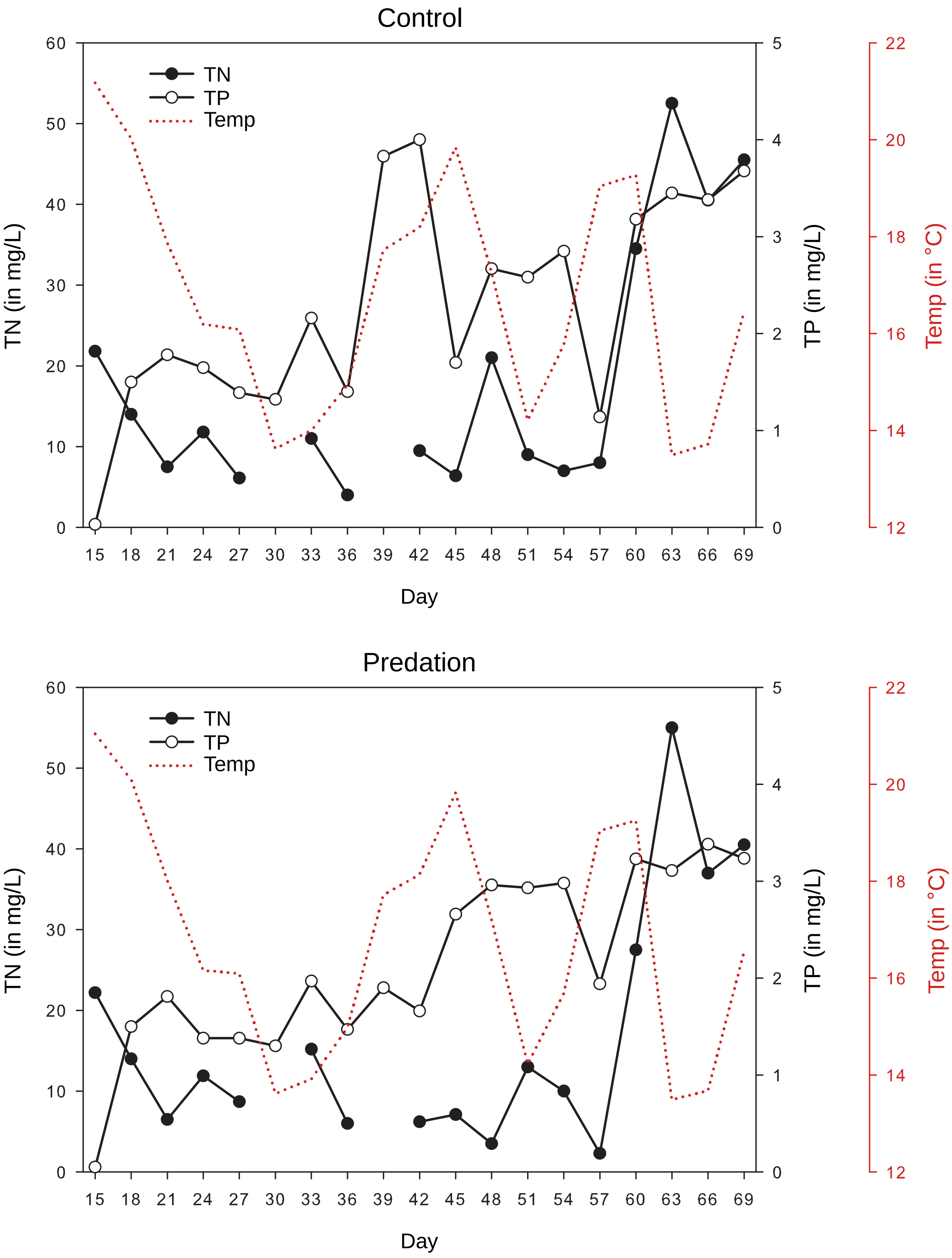


Figure SI2. Total nitrogen concentration (TN), total phosphorus concentration (TP) and water temperature (Temp) for each time point over the duration of the experiment based on pooled samples of the Control (upper panel) and Predation (lower panel) treatment mesocosms separately .

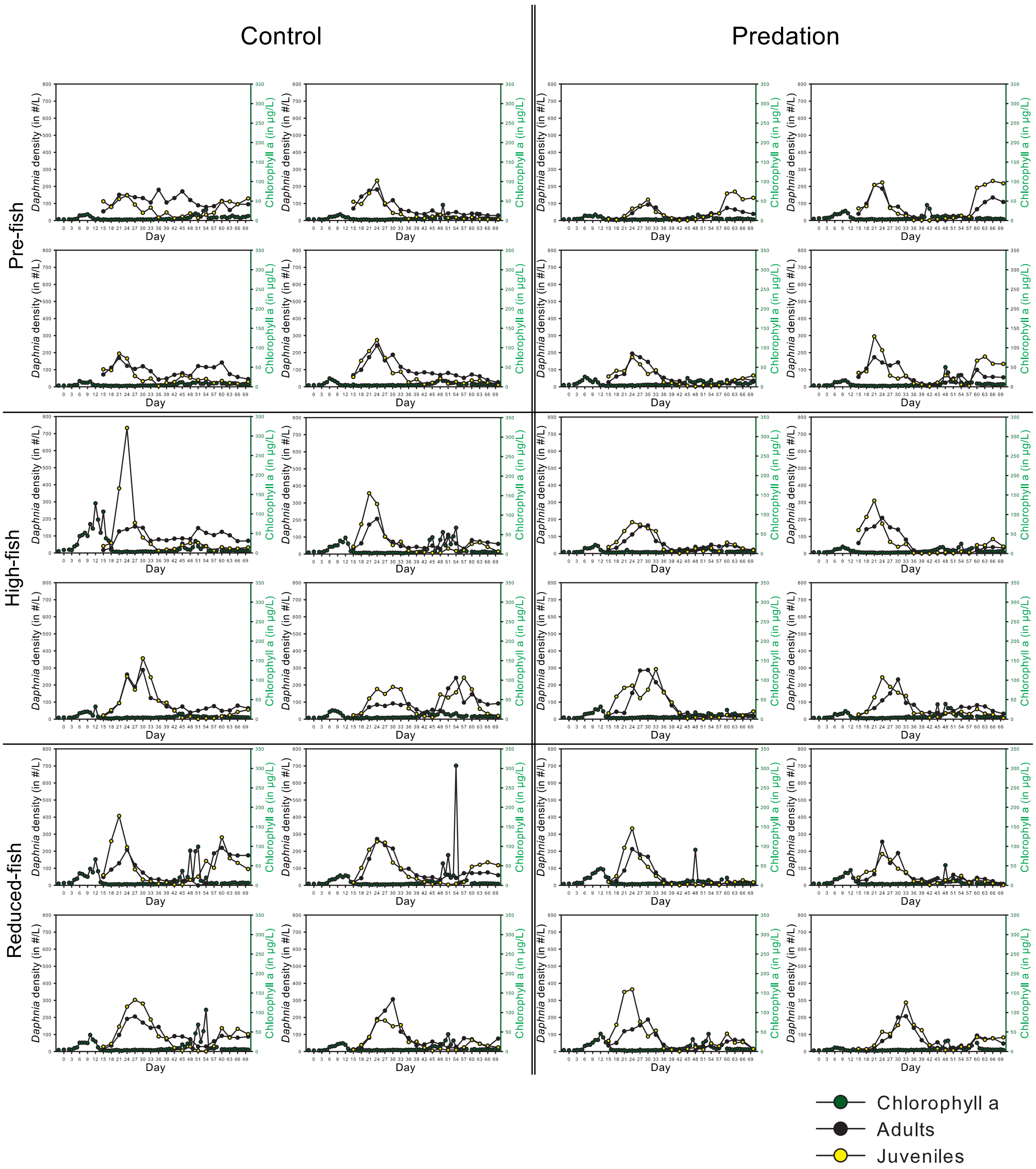


Figure SI3. The density of *Daphnia* adults, *Daphnia* juveniles, and chlorophyll *a* concentration at each time point for each replicate mesocosms of each population separately in the Control and Predation treatments.

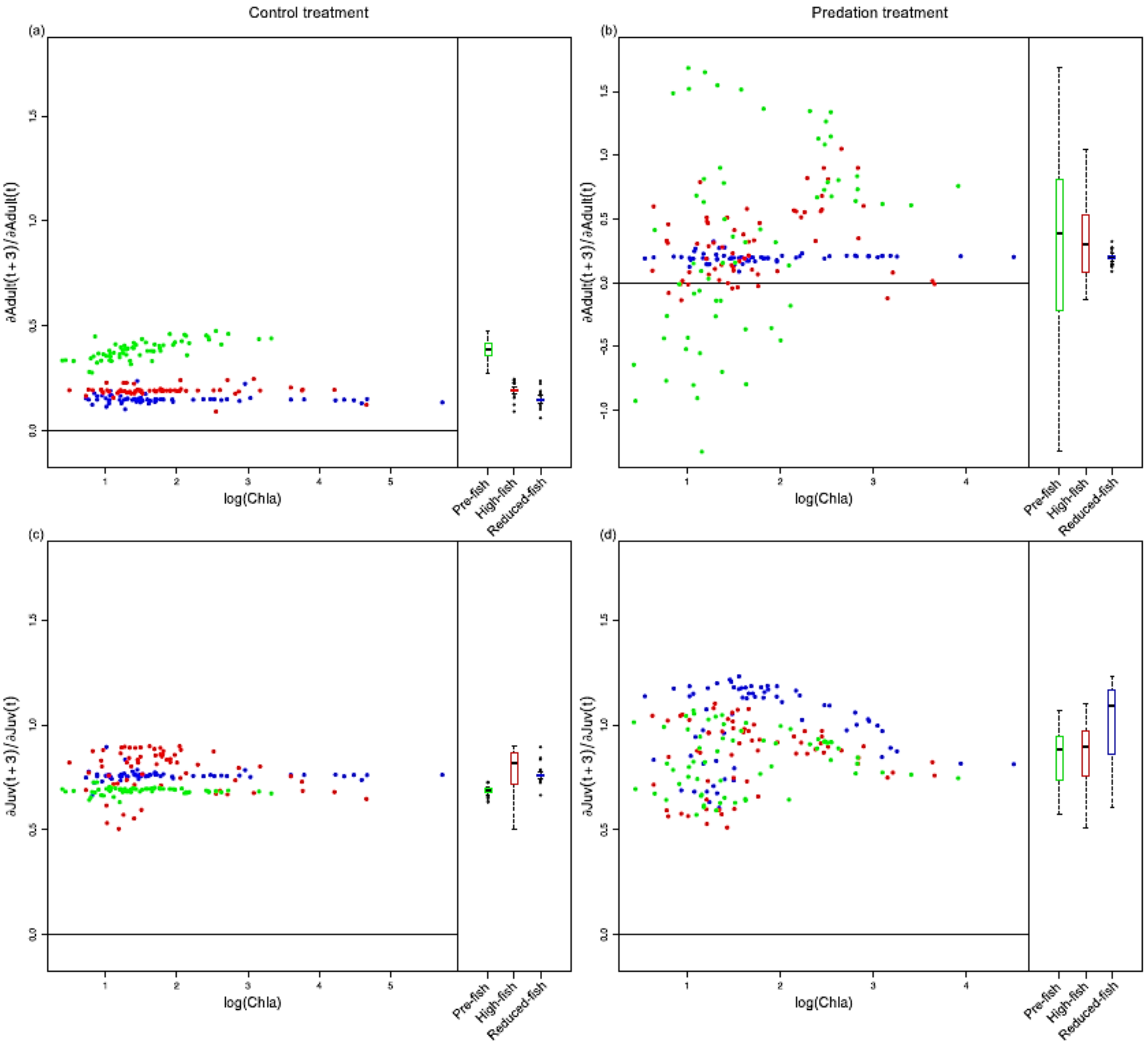


Figure SI4. The effect of adults on adults [∂Adult(t+3)/∂Adult(t)] (a,b) and the effect of juveniles on juveniles [∂Juv(t+3)/∂Juv(t)] (c,d) as a function of the phytoplankton biomass (log(Chla) for the three populations: ● pre-fish, ● high-fish and ● reduced-fish, in the Control (a,c) and Predation (b,d) treatments. The effect of adults and juveniles on their own future densities is a mixture of negative effects from resource competition with themselves and positive effects from survival. Boxplots show the distribution of estimated interaction strengths for the three populations. The bottom and top of the box show the lower and upper quartiles, the band in between them shows the median, whiskers show the minimum and maximum (excluding outliers) and circles show the outliers. Outliers are values more than 1.5 times the length of interquartile range greater than the upper quartile or smaller than the lower quartile. The S-map estimated interaction strengths are in normalized units. The solid line shows the line of no effect.

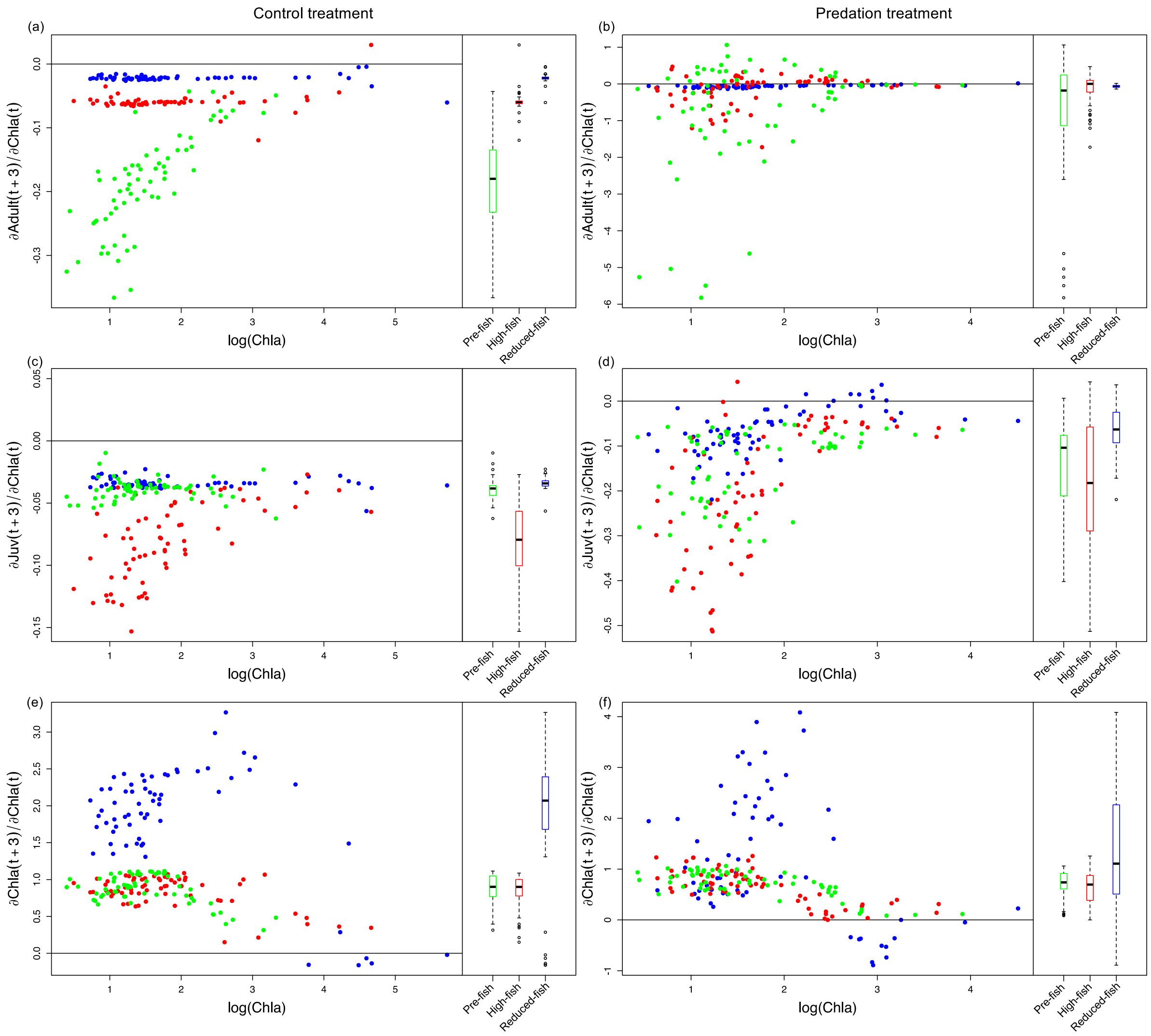


Figure SI5. The effect of phytoplankton on *Daphnia* adults [∂Adults(t+3)/∂ Chla(t)] (a,b) on *Daphnia* juveniles [∂Juv(t+3)/∂ Chla(t)] (c,d), and on phytoplankton itself [∂Chla(t+3)/∂ Chla(t)] (e,f) as a function of the phytoplankton biomass (log(Chla)) in the three populations: ● pre-fish, ● high-fish and ● reduced-fish, in the Control (a,c,e) and Predation (b,d,f) treatments. Boxplots show the distribution of estimated interaction strengths for the three populations. The bottom and top of the box show the lower and upper quartiles, the band in between them shows the median, whiskers show the minimum and maximum (excluding outliers) and circles show the outliers. Outliers are values more than 1.5 times the length of interquartile range greater than the upper quartile or smaller than the lower quartile. The S-map estimated interaction strengths are in normalized units. The solid line shows the line of no effect.

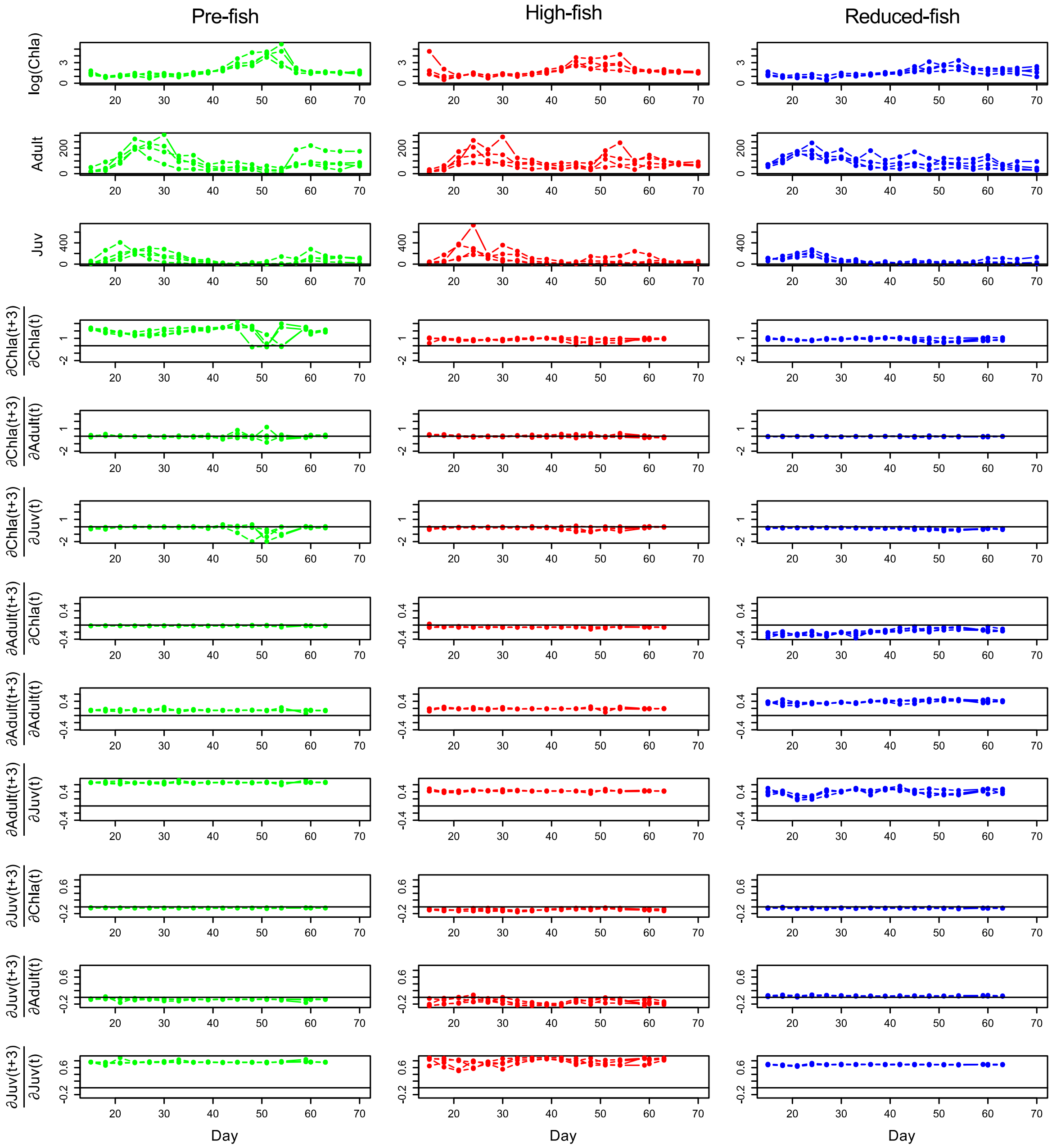


Figure SI6. Dynamic interactions in the Control treatment. The top three rows show the time-series of the three variables (phytoplankton given by log(Chla) (in log(µg/L)), adult densities (in #/L) and juvenile densities (in #/L)); the other rows show all the different S-map estimated interaction strengths as a function of the day. Row 4-6 show effects on phytoplankton, row 7-9 show effects on adults and row 10-12 show effects on juveniles. The first column contains the ● pre-fish population, the second the ● high-fish population and the third the ● reduced-fish population. The S-map estimated interaction strengths are in normalized units. The solid line shows the line of no effect.

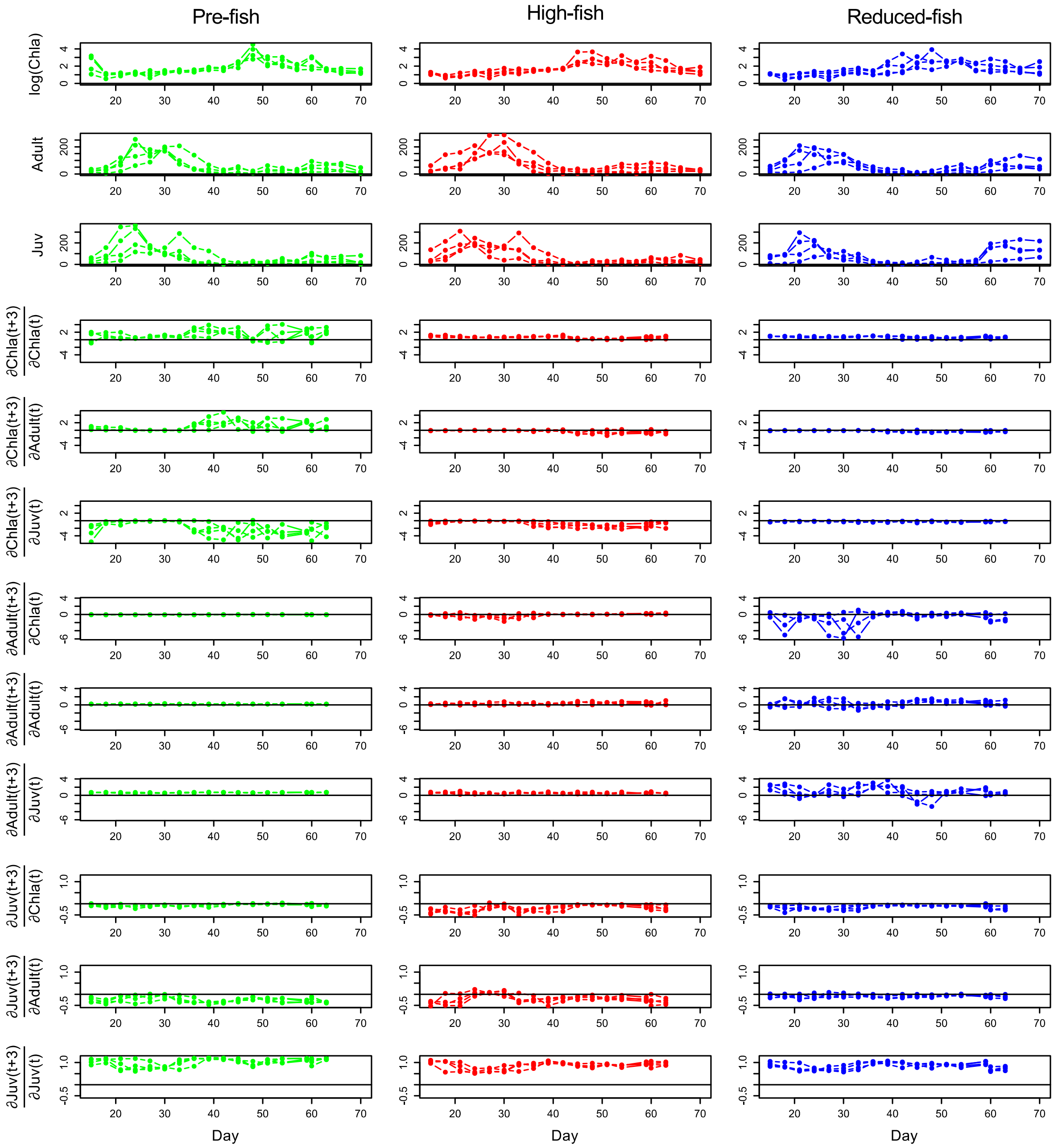


Figure SI7. Dynamic interactions in the Predation treatment. The top three rows show the time-series of the three variables (phytoplankton given by log(Chla) (in log(µg/L)), adult densities (in #/L) and juvenile densities (in #/L)); the other rows show all the different S-map estimated interaction strengths as a function of the day. Row 4-6 show effects on phytoplankton, row 7-9 show effects on adults and row 10-12 show effects on juveniles. The first column contains the ● pre-fish population, the second the ●

high-fish population and the third the • reduced-fish population. The S-map estimated interaction strengths are in normalized units. The solid line shows the line of no effect.

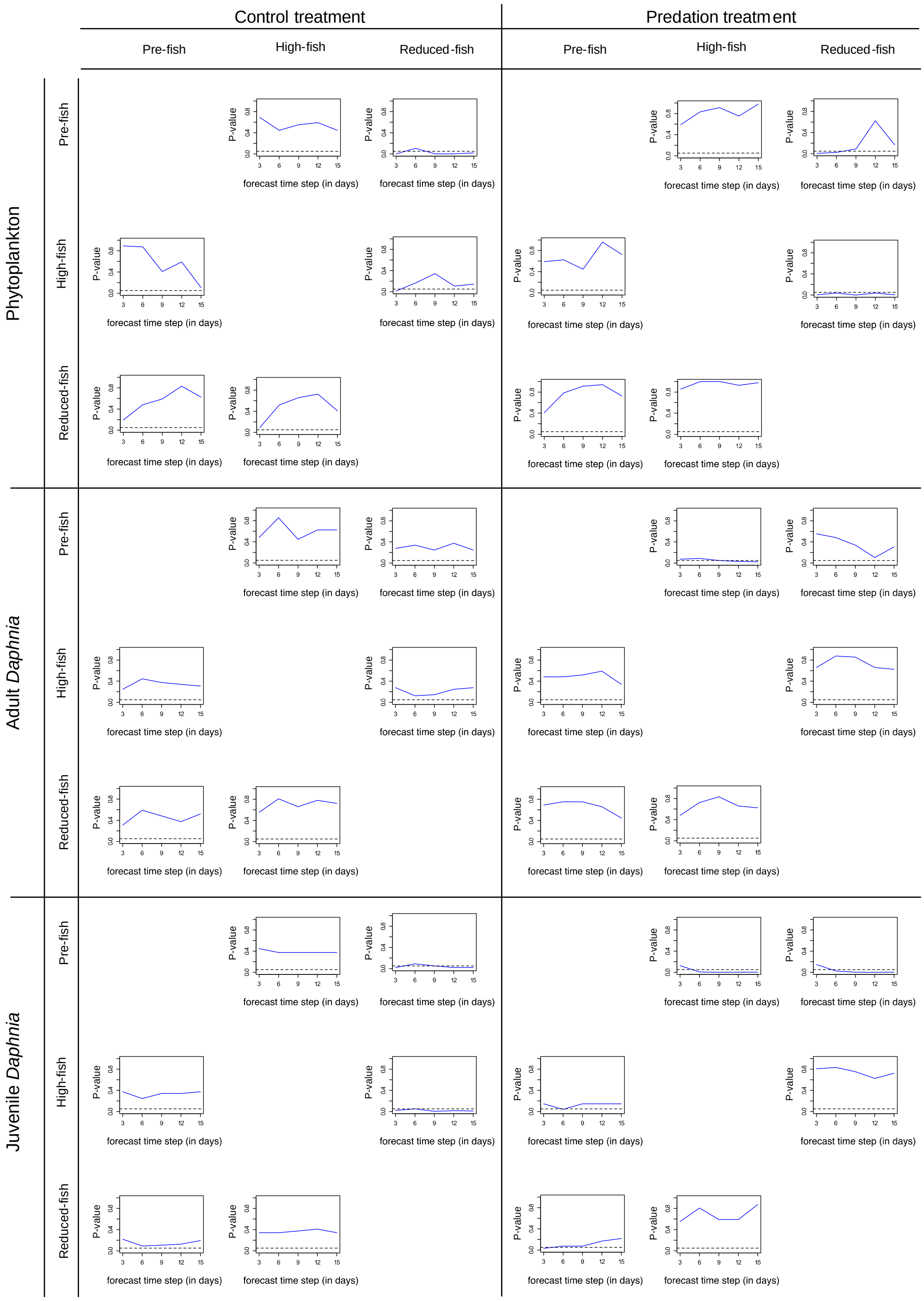


Figure SI8. Simplex projection tests for population differences. One sided Mann-Whitney tests were used to determine if forecasts between replicate mesocosms of the same population are more skillful than predictions from a replicate mesocosms from another population. This is done with different forecast time step lengths (i.e. 3, 6, 9, 12, 15 days). Below the dashed line, the within population forecasts are significantly ($p<0.05$) more skillful (i.e. lower MAE) than the between population forecasts.

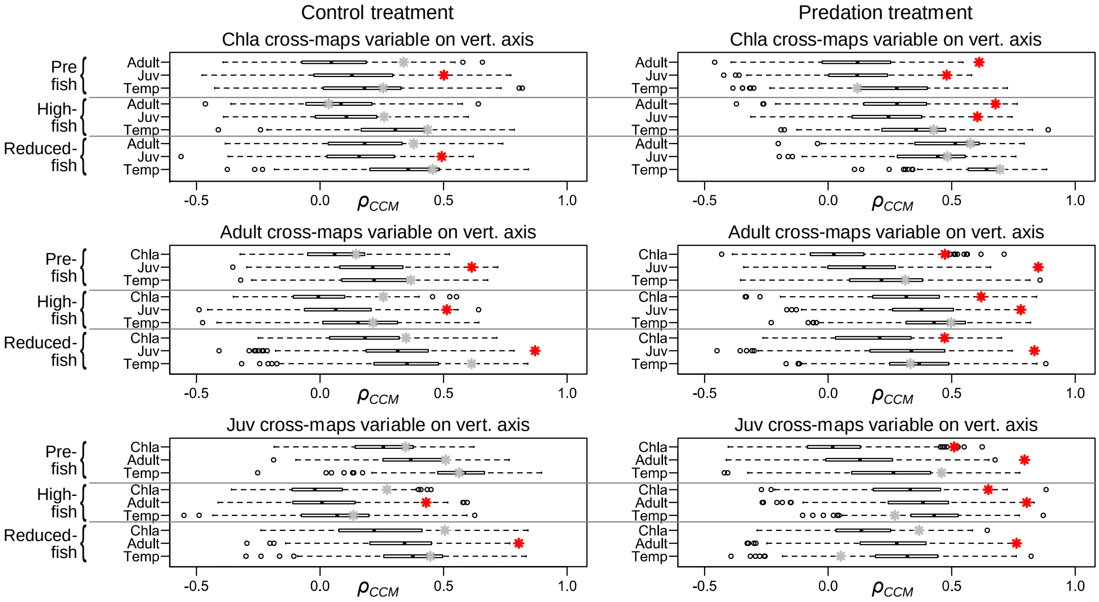


Figure SI9. Boxplots showing the null distributions of cross map skills (ρ_{ccm}) in the surrogate time-series for chlorophyll *a* concentration, the density of *Daphnia* adults and the density of *Daphnia* juveniles, for each population and both treatments separately. Cross map skills (ρ_{ccm}) in the original time-series are indicated with grey asterisks. Red asterisks indicate cross map skills (ρ_{ccm}) that were significantly larger than the surrogate time-series. The bottom and top of the box show the lower and upper quartiles, the band in between them shows the median, whiskers show the minimum and maximum (excluding outliers) and circles show the outliers. Outliers are values more than 1.5 times the length of interquartile range greater than the upper quartile or smaller than the lower quartile.

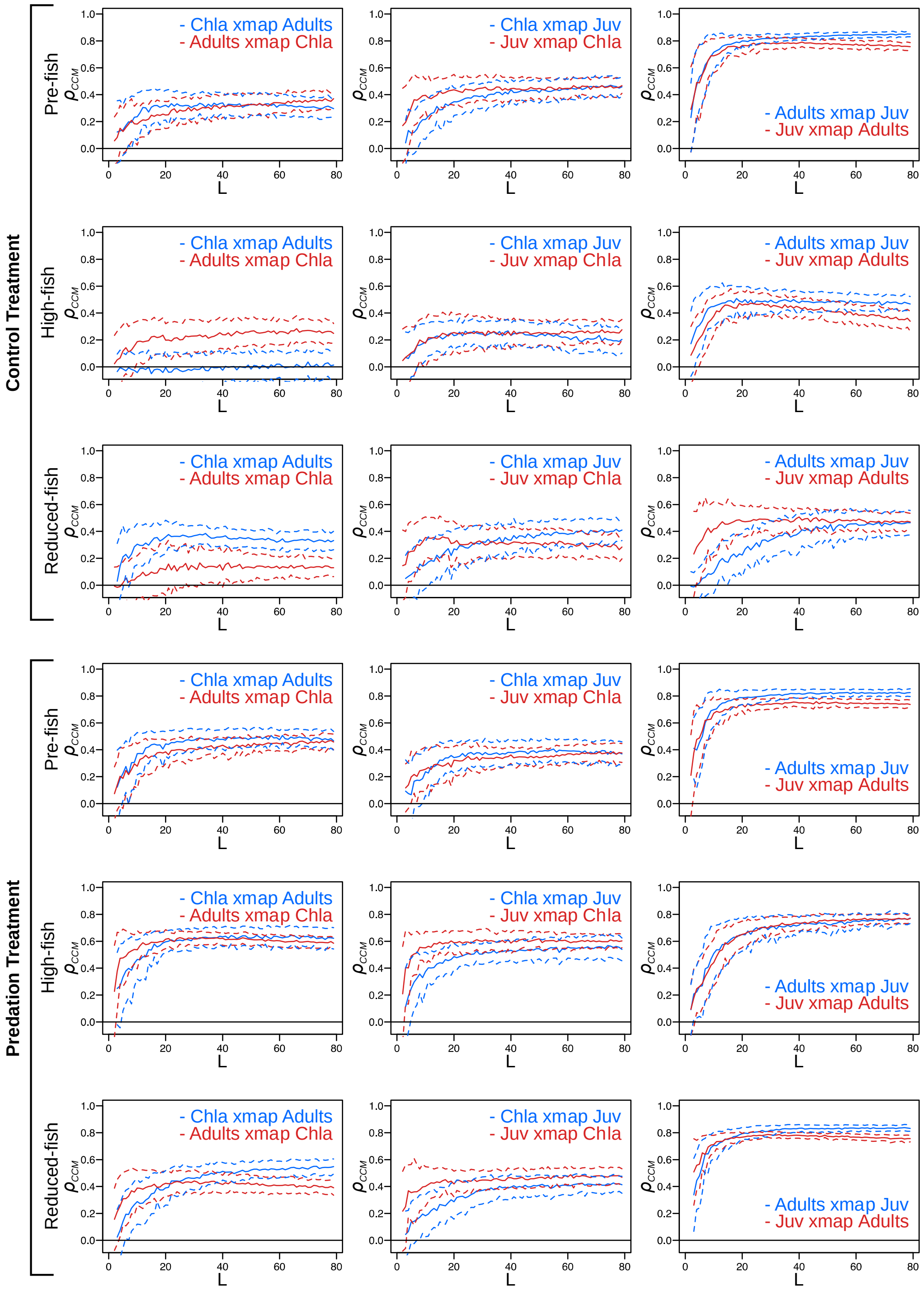


Figure SI10. Convergent cross-maps for each population in both treatments. Each plot shows the cross map between two variables in both directions against the length of the library set used to make the cross map. At each library length 100 random samples were used to make cross maps. The solid line shows the average cross map skill (ρ_{ccm}) and the dashed line shows the standard deviation. If variable X influences variable Y, the skill of the cross map from Y to X should initially increase and then converge to an upper limit, as the length of the library set used is increased.

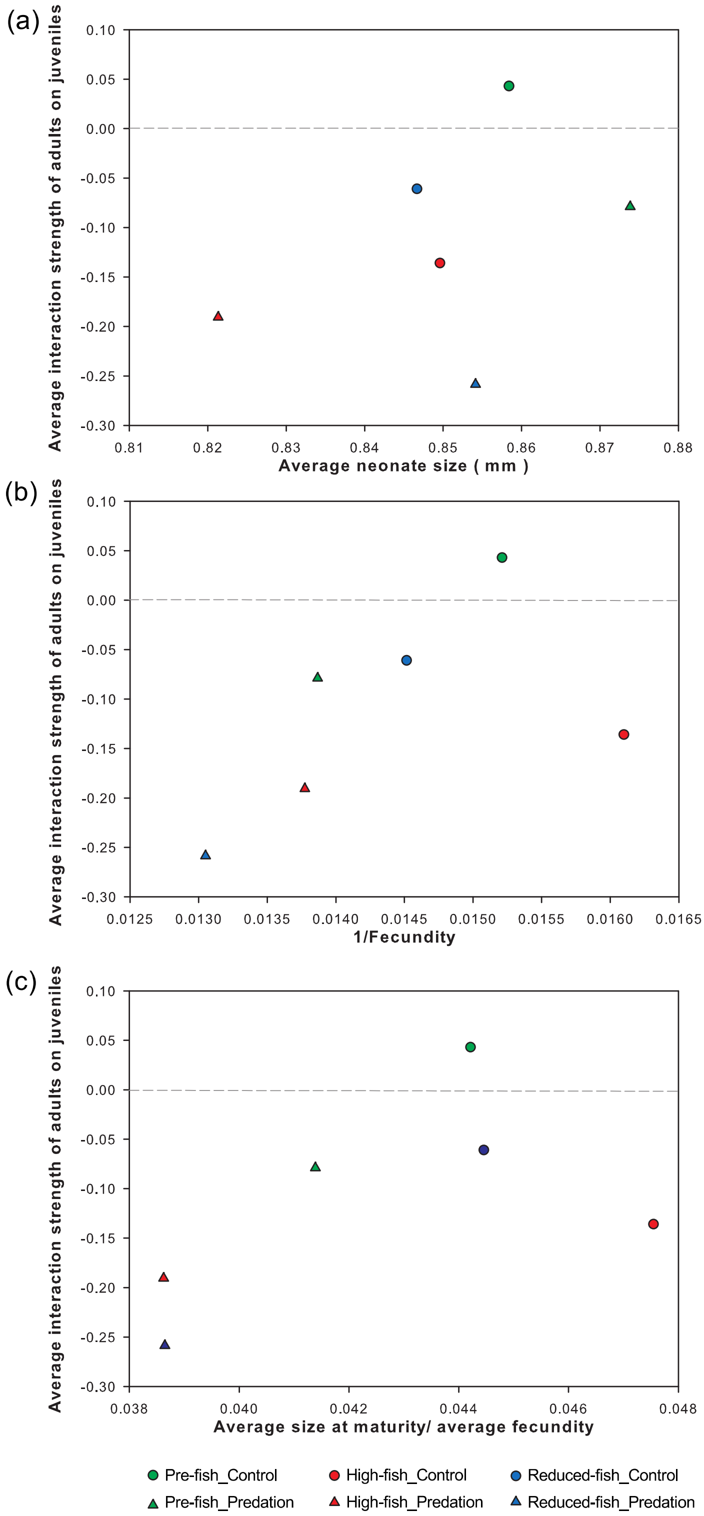


Figure SI11. Scatter plots showing the association between average interaction strengths of adults on juveniles against three measures of juvenile 'quality' based on life history data (Stoks et al., 2016): (a) average neonate size (body length); (b) inverse average number of offspring of the first two clutches (1/average fecundity); (c) average size at maturity divided by average fecundity.

References

Chang, C. W., Ushio, M., & Hsieh, C. H. (2017). Empirical dynamic modeling for beginners. *Ecological Research*.

Clark, A. T., Ye, H., Isbell, F., Deyle, E. R., Cowles, J., Tilman, G. D., & Sugihara, G. (2015). Spatial convergent cross mapping to detect causal relationships from short time series. *Ecology, 96*(5), 1174-1181.

Deyle, E. R, Maher, M. C., Hernandez, R. D., Basu, S., & Sugihara, G. (2016). Global environmental drivers of influenza. *Proceedings of the National Academy of Sciences*, 201607747.

Deyle, E. R., May, R. M., Munch, S. B, & Sugihara, G. (2016). Tracking and forecasting ecosystem interactions in real time. *Proceedings of the Royal Society B: Biological Sciences, 283*(1822).

Deyle, E. R., & Sugihara, G. (2011). Generalized theorems for nonlinear state space reconstruction. *PLoS One, 6*(3), e18295.

Hsieh, C. H., Anderson, C., & Sugihara, G. (2007). Extending nonlinear analysis to short ecological time series. *The American naturalist, 171*(1), 71-80.

Jansen, B., Geldof, S., De Meester, L., & Orsini, L. (2011). Isolation and characterization of microsatellite markers in the waterflea Daphnia magna. *Molecular Ecology Resources, 11*, 418-421.

Mergeay, J, Aguilera, X., Declerck, S., Petrusek, A., Huyse, T., & De Meester, L. (2008). The genetic legacy of polyploid Bolivian Daphnia: the tropical Andes as a source for the North and South American D. pulicaria complex. *Molecular Ecology, 17*(7), 1789-1800.

Orsini, L., Spanier, K. I, & De Meester, L. (2012). Genomic signature of natural and anthropogenic stress in wild populations of the waterflea Daphnia magna: validation in space, time and experimental evolution. *Molecular Ecology, 21*(9), 2160-2175.

Pauwels, L., Stoks, R., Decaestecker, E., & De Meester, L. (2007). Evolution of heat shock protein expression in a natural population of Daphnia magna. *The American naturalist, 170*(5), 800-805.

Stoks, R., Govaert, L., Pauwels, K, Jansen, B., & De Meester, L. (2016). Resurrecting complexity: the interplay of plasticity and rapid evolution in the multiple trait response to strong changes in predation pressure in the water flea Daphnia magna. *Ecology Letters, 19*(2), 180-190.

Sugihara, G. (1994). Nonlinear forecasting for the classification of natural time series. *Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences, 348*(1688), 477-495.

Sugihara, G., & May, R. M. (1990). Nonlinear forecasting as a way of distinguishing chaos from measurement error in time series. *Nature, 344*(6268), 734-741.

Sugihara, G., May, R., Ye, H., Hsieh, C., Deyle, E., Fogarty, M., & Munch, S. (2012). Detecting causality in complex ecosystems. *Science, 338*(6106), 496-500.

Takens, F. (1981). Detecting strange attractors in turbulence *Dynamical systems and turbulence, Warwick 1980* (pp. 366-381): Springer.

Ye, H., Clark, A., Deyele, E., Keyes, O. , & Sugihara, G. (2016). rEDM: Applications of Emeperical Dynamic Modelling from Time Series (Version 0.5.4). Retrieved from https://CRAN.R-project.org/package=rEDM

General discussion

Ecosystem stability, resilience and regime shifts in a set of interconnected shallow lakes

Ecosystem stability and resilience has become a central theme in ecology and environmental biology (Tilman, Reich, & Knops, 2006; Donohue et al., 2013; Donohue et al., 2016) in the context of human-induced change and the Anthropocene (Hannah et al., 1994; Vitousek et al., 1997; Rockström et al., 2009; Pereira, Navarro, & Martins, 2012). There is special interest in regime shifts (Scheffer et al., 2001; Carpenter et al., 2011; Dakos et al., 2011). This is for several reasons. From a practical and management point of view, regime shifts are important as they can lead to massive shifts in ecosystem state when environmental stress is just marginally increased, and once a regime shift has occurred it takes major efforts to reverse the state shift (Scheffer et al., 2001; Carpenter, Kinne, & Wieser, 2003). Thus in terms of policy and management, prevention and mitigation are important in systems that exhibit regime shifts. From a conceptual point of view, regime shifts attract interest because they are intrinsically linked to resilience (Holling, 1973; May, 1977; Scheffer, 1998). It is precisely because regime states are stabilized by negative feedback loops that they exhibit a regime shift when the system is pushed across a threshold that leads to a breakdown of the buffering mechanisms.

Shallow lakes have since more than two decades been studied as exhibiting alternative stable states and regime shifts (Scheffer et al., 1993) and are a prime and often cited example of such regime shifts in the literature (Scheffer et al., 2001; Søndergaard et al., 2007; Kéfi et al., 2013). The mechanisms underlying stabilization of the turbid and clear-water state in shallow lakes are well-documented (Van Donk et al., 1990; Scheffer et al., 1993; Jeppesen et al., 1997; Kéfi, Holmgren, & Scheffer, 2016), and the existence of alternative stable states and their link to food web structure have been used in policy and lake restoration (Van Donk et al., 1990; Jeppesen et al., 2007; Hobbs et al., 2012). Yet, the ubiquity of regime shifts in shallow lakes has been contested in recent literature. Schröder et al. (2005) argued that not all variation in ecosystem characteristics in shallow lakes should be pushed in the framework of alternative stable states , and several studies emphasized that gradients in ecosystem state of shallow lakes often seem rather smooth and gradual (Jeppesen et al., 2000; McGowan et al., 2005; Bayley et al., 2007; Zimmer et al., 2009; Hanson et al., 2012).

It was a key aim of my PhD study to provide a better understanding of how shallow lakes behave in terms of their “state” (clear-water versus turbid) in time and space, focusing on a system of interconnected ponds (Vijvergebied Midden-Limburg) that is characterized by the presence of both clear-water as well as turbid systems at any point in time and is characterized by a shared water source. The latter provides added value, as the differences in turbidity and chlorophyll *a* concentration are in this system likely a consequence of differences in ecological functioning of the systems and their biota rather than being a consequence of major differences in water quality. As a matter of fact, these systems from the start are to some extent an illustration of the fact that both the turbid and clear-water states can coexist in the same region, in ponds with the same water source and thus likely exposed to similar levels of external phosphorus loading. When one does a survey of these systems, they show strong ecological differences and range from clear-water systems with abundant submerged vegetation to very turbid systems exhibiting intensive algal blooms (Lemmens et al., 2018). But the existence of both turbid and clear-water systems does not imply that any pond can shift from one alternative stable state to another one at any moment. Such surveys when not repeated in time do not inform us on the degree to which each system is stable through time. Conversely, not all shifts that one would observe in these systems would necessarily reflect regime shifts, nor does the absence of regime shifts preclude that the mechanisms typically linked to alternative stable states and regime shifts operate to stabilize the systems in their current state. My aim was to shed light into the stability of lake state and the occurrence of regime shifts in this system by carrying out repeated surveys of ecosystem state and some of its presumed drivers through time, and by quantifying ecosystem features at different temporal resolutions. In Chapter 2 I focused on year-to-year variation, whereas in Chapter 1 I looked at a weekly resolution that also captures seasonality. Finally, in Chapter 3 I could use data from chlorophyll *a* loggers that quantified variation in phytoplankton biomass every fifteen minutes.

The results of the analyses put forward in this PhD provide support both for the importance of the framework of alternative stable states as well as for the perspective that not all variation in lake characteristics is linked to regime shifts. Our data suggest that key mechanisms that are linked to the framework of alternative stable states, such as the structure of the food web and the importance of macrophytes, indeed are of key importance in explaining variation in the studied set of ecologically disparate but interconnected shallow lakes. In addition, our analyses provide evidence for the occurrence of regime shifts, as we do observe changes in lake state (turbid versus clear-water) in the absence of strong environmental gradients or strong changes in environmental conditions. Our data also show, however, that part of the variation in lake state observed in our study system can likely be explained by strong differences in food web structure among systems linked to management, or by seasonal changes likely linked to reproduction cycles of fish. A more explicit analysis of early warning signals on high density data on chlorophyll *a* in four study systems provided evidence of a critical slowing down preceding only one of the multiple strong changes in state that were observed in our data. This result at this stage needs to be interpreted with caution, however, as the field of early warning signals is in full development and needs to be evaluated for its potential to unambiguously identify regime shifts. In the following paragraphs, I critically reflect on the results presented in the various chapters of this PhD.

Temporal scales: parallels, differences, and limitations

My three studies focusing at different temporal scales also differed in the extent that I could gather data on environmental conditions and food web structure. In the year-to-year analysis (Chapter 2), I could incorporate data on abiotic and biotic variables that were quantified in a highly standardized way (De Bie et al., 2012; Lemmens et al., 2013; Lemmens et al., 2015), and I could assess repeatability of the observed relationships between ecosystem state and environmental gradients across years. In my study of seasonal patterns involving weekly samples of 25 systems (Chapter 1), I could only incorporate measures of turbidity, chlorophyll *a* concentration and macrophyte cover at a weekly basis, and I supplemented these data with three snapshot surveys of zooplankton community composition and body size distribution each year. For the highest temporal resolution study (Chapter 3), the data-loggers only provided data on chlorophyll *a* and phycocyanin concentration. These differences in coverage of environmental variables and potential drivers of ecosystem state shifts limit comparisons across temporal scales. Yet, I here explore parallels across studies in terms of (1) drivers of relationships and (2) repeatability of pond identity effects on chlorophyll *a* across studies.

Drivers of ecosystem state

In Chapter 2 I showed that pond management is a key explanatory variable of local environmental conditions and zooplankton community composition. Environmental differences that are linked to zooplankton community composition are themselves explained by pond management, which are in essence food web manipulations. In Chapter 1 I showed that variation in chlorophyll *a* concentration among ponds can be linked to zooplankton community structure and average body size in spring, and to macrophyte cover in summer. These results are in line with earlier studies showing that food web structure and the trophic cascade are important in determining structure and function of shallow lakes, both directly and through their impact on the establishment and growth of macrophytes (cf. Scheffer et al., 1993). Macrophytes reduce, through competition for light and nutrients, the growth of phytoplankton and are a key driver of differences in chlorophyll *a* concentration in ponds with similar nutrient loading (Jeppesen et al., 1997; Scheffer, 1998).

Large zooplankton are key grazers of phytoplankton and promote establishment of macrophytes by reducing competition from algae, but are themselves dependent on sufficiently low predation rates by planktivorous fish and refuges offered by macrophytes (Timms & Moss, 1984; Carpenter & Kitchell, 1996; Jeppesen et al., 1997). These are the feedback loops between top-down control and macrophyte growth that stabilize the clear-water state. Interestingly, in Chapter 1 I showed that macrophyte cover in summer has a higher explanatory power for phytoplankton abundance in spring than macrophyte cover in spring. Conversely, zooplankton body size is especially important in spring and not in summer. The latter is explained by the fact that a well-established macrophyte vegetation reduces the growth of zooplankton because of a lack of food (Blindow et al., 2000). Zooplankton grazing in spring can through top-down control of algae allow macrophyte vegetation to establish (Norlin, Bayley, & Ross, 2005). So my results nicely fit in the body of knowledge on the importance of top-down control in shallow lakes and how fish, zooplankton and macrophytes jointly determine variation in phytoplankton biomass given a certain nutrient load. The relationships are, however, not always straightforward and simple. For instance, given the patterns and mechanisms outlined above, one might expect a significant relationship between zooplankton body size in spring (expected to promote establishment and growth of macrophytes) and macrophyte cover in summer, but I did not observed such a relationship (Pearson correlation: R = 0.37, p = 0.08). This suggests that there are multiple mechanisms that can operate jointly to determine phytoplankton biomass.

There is considerable variation in environmental conditions among the ponds in terms of nutrient concentrations, turbidity, phytoplankton and zooplankton biomass, and macrophyte cover, even though all ponds are interconnected and share a similar water source. This is in line with earlier studies on the same (Lemmens et al., 2013; Lemmens et al., 2015) and other systems (Cottenie et al., 2003), and suggests that the observed differences in lake characteristics are the consequence of differences in biotic interactions, illustrating the importance of food web structure and top-down control in lakes and ponds (Carpenter, Kitchell, & Hodgson, 1985; Carpenter et al., 1996). This is corroborated by my results that the main axes of variation in environmental conditions among the lakes are linked to differences in pond management (Chapter 2). In the generally meso- to eutrophic systems studied here, the whole spectrum of very turbid (Snell's depth 5 cm) to very clear (Snell's depth 64 cm; bottom view) ponds can be obtained. Given that these two extremes occur in a setting that is fed by the same water source and involves interconnected systems is suggestive of the existence of alternative stable state, and this observation is reinforced by the observation that the mechanisms underlying this variation correspond to the key factors highlighted in the theory of alternative stable states in shallow lakes (Scheffer et al., 1993).

Repeatability of pond identify effect on chlorophyll a concentration

Across Chapters 1-3, my study integrates data over a period of five years (2013 till 2017). Here I explore the repeatability in behavior of ponds across studies. Figure 1A shows the correlation between average chlorophyll *a* levels across Chapter 1 and 2 when the data are paired by pond identity. This graph suggests a high impact of pond identity on average chlorophyll *a* concentrations in the study system. In many cases this might be related to the fact that also pond management remained constant across years. There are some cases, however, in which pond management changed across years. Figure 1B shows for all years studied the average value of chlorophyll *a* level in summer (August) for ponds grouped according to the five pond management types. This plot illustrates indeed that there are differences in average chlorophyll *a* among pond management types, with the same pattern reflected across studies.

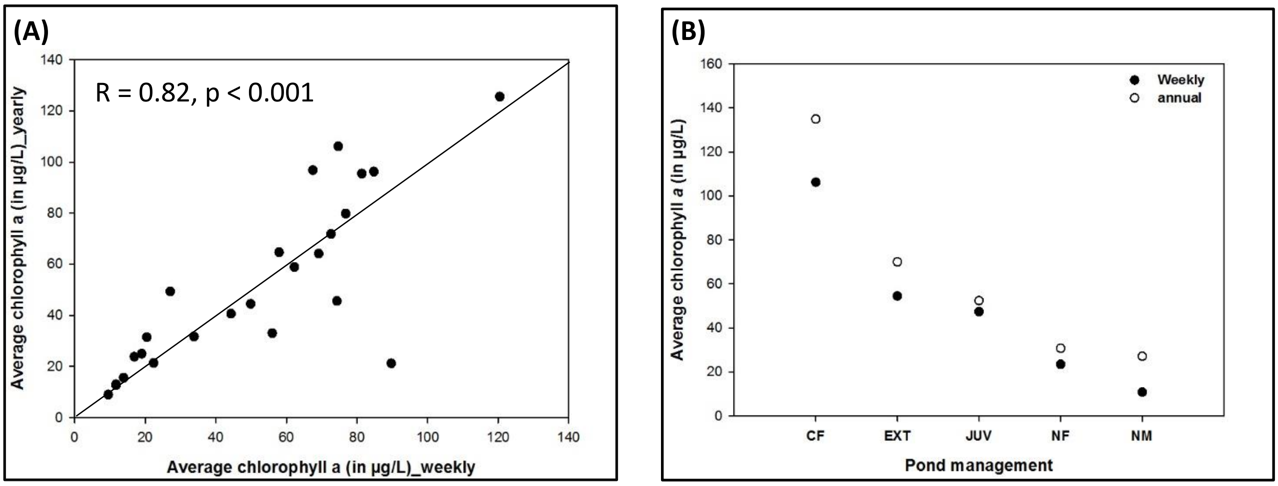


Figure 1. Pearson correlation between average chlorophyll *a* levels across the study described in Chapter 1 (weekly samples) and Chapter 2 (yearly samples) (A), and average value of chlorophyll *a* level in summer for the five categories of ponds as determined by pond management type (CF: carp farming; EXT: extensive fish farming; JUV: juvenile fish farming; NF: no fish farming; and NM: no management). The line in panel A indicates the 1:1 isocline.

Alternative stable states and regime shifts

In the previous paragraphs I suggested that the patterns we observe are consistent with the existence of alternative stable states and the mechanisms stabilizing them, and I showed that differences among ponds in average chlorophyll *a* level tend to be repeatable across years and studies. But how stable are the systems, and how common are regime shifts? To explore that, I can use three criteria, which I briefly highlight in the following paragraphs.

First, as I did in Chapter 1, one can check whether variation in ecosystem state as observed across lakes reflects fixed differences among lakes that are in essence stable. In these systems, even though the state may be stabilized by the feedbacks that underlie alternative stable states and may be linked to food web manipulations linked to pond management (see previous paragraph), we have no evidence for regime shifts. In Chapter 1 I showed that this is the case for 8 of the study systems. In another 8 systems, there seems to be a repeatable seasonal shift in turbidity. Given its seasonal and repeatable pattern, such a shift may reflect a major environmental change such as juvenile fish entering the pelagic. This cannot be categorized as a true regime shift, as it does not reflect a change in state linked to small changes in external environmental drivers. Again, if fish predation is involved, the mechanism underlying the seasonal change in turbidity is strongly related to the mechanisms that stabilize alternative states. However, here the driver for state change is an internal shift in food web structure that is a repeatable, seasonal phenomenon, not a small change in external forcing. In a remaining 9 of the 25 studied lakes, I report in Chapter 1 a change in pattern across years, and this might reflect regime shifts.

As a second approach, I used the early warning indicator outlined by Dakos et al. (2012; see also Scheffer et al., 2009; Drake & Griffen, 2010; Carpenter et al., 2014). For these indicator one needs extensive time series data, which I only had for the four lakes in which we monitored changes in chlorophyll *a* level using data-loggers. While these detailed data showed that several of the studied lakes showed strong fluctuations in chlorophyll *a* level through time, in many cases these changes appeared quite gradual and were not associated with changes in early warning indicator for regime shifts, such as an increase in temporal autocorrelation (Chapter 3; Dakos et al., 2012). This suggests that the systems studied are highly variable in chlorophyll *a* levels through time and that regime shifts do occur, but that they do not occur often and that many rapid and pronounced changes in turbidity through time may potentially not represent regime shifts. More specifically, the data on four lakes and two years only revealed one case of a regime shift if we use the criterion that it should be preceded by a strong increase in temporal autocorrelation. We need to interpret this in a prudent way, however, as the field of early warning indicators is in full development, and there is a risk that the criteria need to be fine-tuned (Dakos et al., 2012; Wang et al., 2012; Spears et al., 2017). If my observations are true, one also needs to be prudent in the context of applications linked to environmental safety, as my data suggest that it may perhaps not be enough to have early warning indicators for regime shifts to develop a policy or management that precludes changes in ecosystem state. Indeed, many of the pronounced changes in ecosystem state I observed in Chapter 3 were perhaps not regime shifts in the strict sense (or could at least not be identified as such using early warning indicators as a criterion), but still their changes were profound and might strongly impact biodiversity and ecosystem services.

As a third criterion, I also checked whether the change in chlorophyll *a* in relation to nutrient loading showed a linear pattern. I carried out this analysis for the data of Chapter 1 and Chapter 2 combined. Overall, the pattern reveals no significant correlation between chlorophyll *a* and total phosphorus (Chapter 1: $R = 0.32$, $p = 0.12$; Chapter 2: $R = 0.34$, $p = 0.09$; Figure 2A) and a pattern of greater variation in chlorophyll *a* levels with increasing nutrient loading (Figure 2A). We did, however, observe a significant positive correlation between average chlorophyll *a* and total nitrogen (Chapter 1: $R = 0.60$, $p = 0.001$; Chapter 2: $R = 0.57$, $p = 0.003$; Figure 2B). A similar pattern was observed for water transparency (with phosphorus: Figure 2C; Chapter 1: $R = -0.24$, $p = 0.24$; Chapter 2: $R = -0.24$, $p = 0.25$; with nitrogen: Figure 2D; Chapter 1: $R = -0.68$, $p < 0.001$; Chapter 2: $R = -0.61$, $p = 0.001$). We thus observe that for this set of interconnected lakes there is a stronger link between chlorophyll *a* and turbidity with nitrogen concentration than with phosphorus concentration. This is in line with observations of Lemmens et al. (2018). Our data show that a large range of chlorophyll *a* concentrations can be obtained in a single system of interconnected ponds. Overall, the relationship between chlorophyll *a* and nitrogen concentration shows no abrupt transitions but rather is consistent with a gradual transition. While such a gradual transition may suggest that no real alternative stable states or regime shifts are involved, we need to interpret this pattern with caution, as it is possible that an abrupt transition within systems results in a gradual pattern across lakes as the patterns are smoothened by variation in other environmental conditions among lakes.

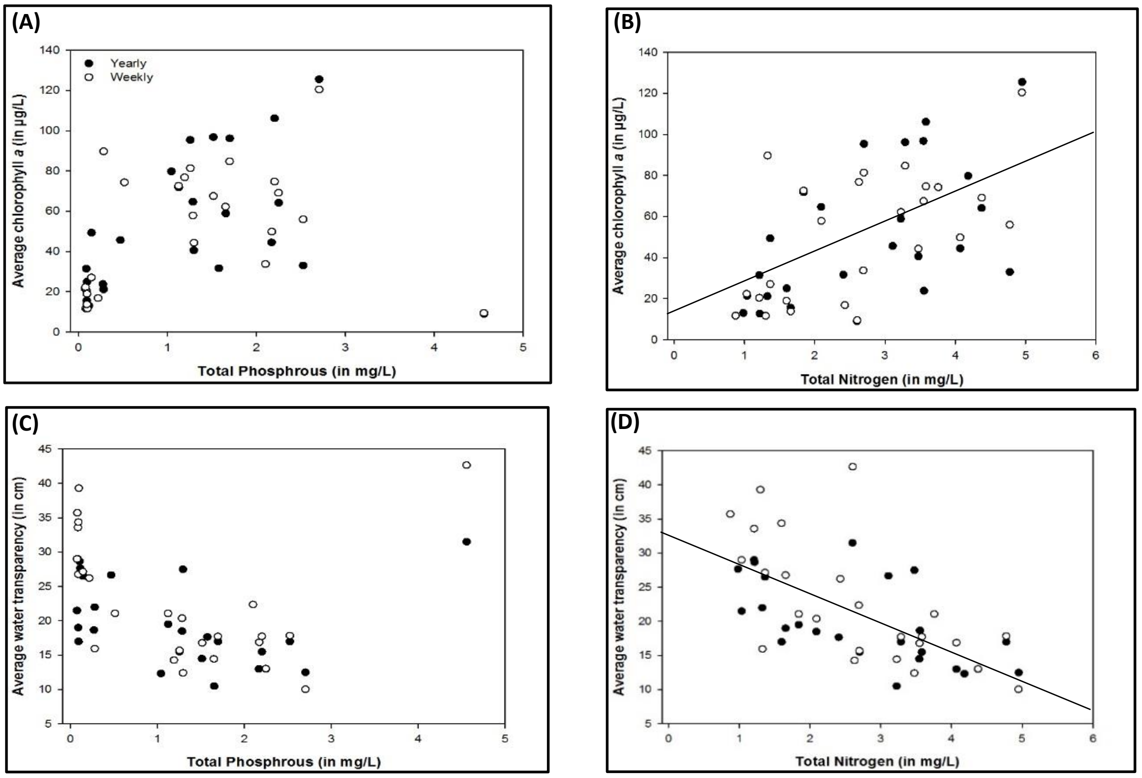


Figure 2. Scatter plot of observed chlorophyll *a* level during summer (A and B), and water transparency (Snell's depth) (B and D) against average total phosphorus concentration (A and C) and nitrogen concentration (C and D) for all studied ponds in Chapter 1 (weekly samples) and Chapter 2 (yearly samples).

Overall, my conclusion is that (1) the interconnected ponds of Vijvergebied Midden-Limburg vary considerably in chlorophyll *a* concentration among systems and across time, even though they share the same water source; (2) this variation is linked to pond management and food web structure, and my data suggest that chlorophyll *a* levels in ponds are driven by zooplankton body size in spring, macrophyte cover in summer, and nitrogen loading; (3) this suggests that the mechanisms known to stabilize the clear-water and turbid state in shallow lakes are also operating in the study system; (4) while variation in chlorophyll *a* and turbidity is striking, a subset of systems is either turbid or clear-water during the whole study period and another set of systems shows a systematic change in turbidity with season; while these systems behave in line with mechanisms known to be linked to regimes shifts, my data do not provide evidence that regime shifts occurred in these systems; (5) my data suggest that a few systems did show regime shifts during the study period, but the number of such events was rather low even though variation in chlorophyll *a* levels was very high. Summarizing, my data support the vast body of literature on alternative stable states and the mechanisms stabilizing them, but also indicate that a spatial survey documenting the occurrence of lakes in both the turbid and clear-water state should not be viewed as evidence of the regular occurrence of regime shifts. Rather, my data show that regime shifts can occur, but within a context of a wide range of dynamics, from highly stable systems to systems showing regular changes, gradual changes and true regime shifts.

Repeatability of variability across temporal resolutions

Several of the study sites of the present study showed strong variation in turbidity and chlorophyll *a* concentrations through time. I reported this in the different chapters, which raises two questions: (1) are the levels of variation of the different lakes significantly correlated across temporal resolutions? and (2) are the levels of variation in absolute terms similar across temporal resolutions? Figure 3 shows the correlations of coefficients of variation in chlorophyll *a* levels across studies. There is indeed a significant correlation, which suggests that weekly data can be used to predict variation at larger time scales. Strikingly, the coefficients of variation are lower for the weekly samples than for the year-to-year samples. This suggests that year-to-year variation results in larger variation in chlorophyll *a* levels than seasonal variation. We should not here that our weekly samples were only taken during spring, summer and early autumn, so did not include samples of the winter season. Yet it is striking that across spring and summer variation in chlorophyll *a* levels tend to be less than across years. Given the positive correlation among the coefficients of variation across weeks and years when paired according to pond, however, variation in chlorophyll *a* levels within the growing season provides information on how strong the ponds respond to year-to-year variation in environmental conditions.

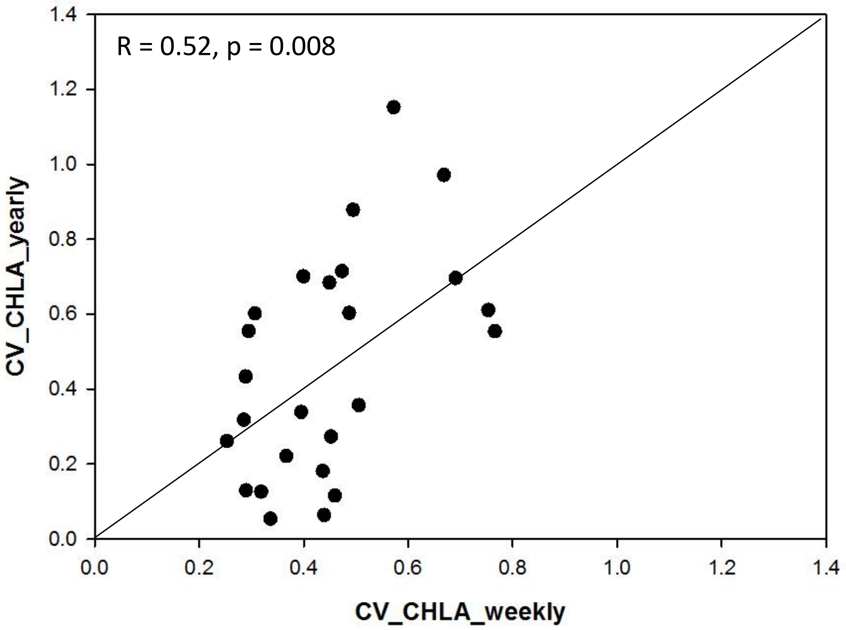


Figure 3. Pearson correlation coefficient of variation in chlorophyll *a* levels across two studies. The line indicates the 1:1 isocline.

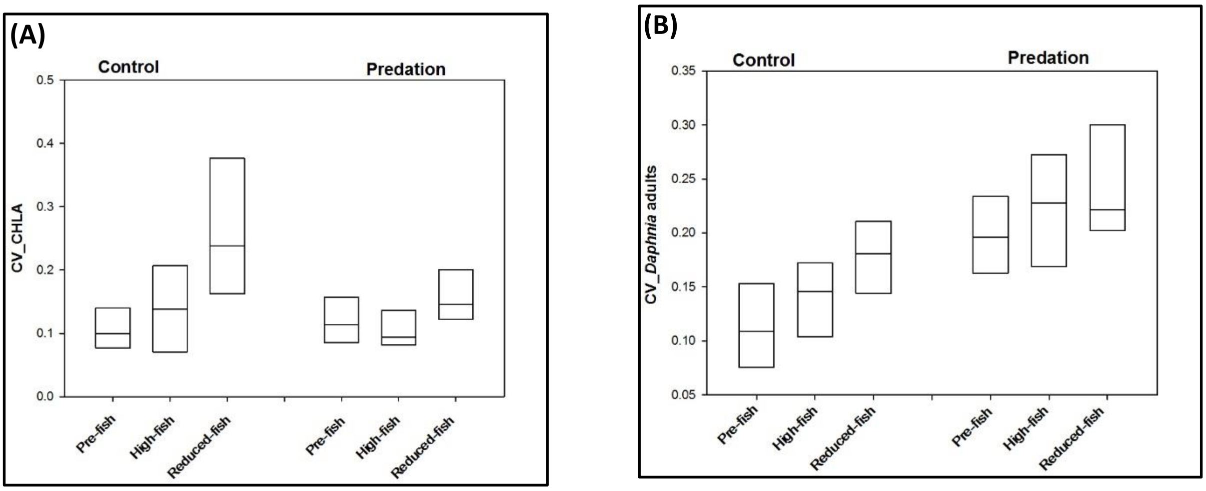
Cyanobacteria blooms

So far I focused on phytoplankton blooms in general, as I used chlorophyll *a* as a measure of phytoplankton biomass. Cyanobacteria blooms are from a management point of view very important as they are often toxic (Anderson, Glibert, & Burkholder, 2002; Brooks et al., 2016). In Chapter 3 I quantified phycocyanin concentration as *a* proxy for cyanobacteria biomass. For this variable too, I observed both pronounced differences among ponds as well as among years. In addition, within-year variation within a single pond was also very high. In both years, ponds M12 and K9 had the highest average concentrations of phycocyanin, but their relative position in the ranking differed among years. Pond V16 in both years had the lowest average phycocyanin concentration.

Eco-evolutionary dynamics and ecosystem stability

In Chapter 4, I carried out a mesocosm experiment to quantify to what extent evolution as it happened in a natural system (Oud-Heverlee Zuid Pond, Belgium) in response to pronounced changes in fish predation pressure and involving multiple life history traits (Stoks et al., 2016) as well as phototactic behavior (Cousyn et al., 2001; Stoks et al., 2016) influenced top-down control of algae. I showed that evolution has a significant effect on the degree to which top-down control of algae by *Daphnia* temporarily collapses, and I show that these differences are related to life history changes linked to predator avoidance. More specifically, investment in smaller juveniles as an anti-predator strategy results in a stronger negative impact of adults on juveniles through competition, and this results in a strong algal bloom (Chapter 4). In the mesocosm experiment carried out in Chapter 4, the *D. magna* populations were most of the time exerting strong top-down control on the algae, such that phytoplankton biomasses were low. In one specific time period (day 45 – day 55), however, this top-down control collapsed and the algae started to bloom. This bloom was subsequently suppressed when the *Daphnia* populations had produced new juveniles such that through their growth grazing pressure could be increased again. Here I explore two additional ideas: (1) does evolution impact stability of both phytoplankton biomass and *Daphnia* densities? and (2) do we have evidence for the fact that the sudden and temporary algal blooms in the mesocosms involved regime shifts?

For the first question, I calculated coefficients of variation of both phytoplankton biomass (chlorophyll *a*) and abundances of adult and juvenile *Daphnia* for the six treatments (three populations and presence/absence of fish during the entire experiment). Figure 4 shows the boxplots of coefficients of variation in chlorophyll *a* and abundances of adult and juvenile *Daphnia* for the three *Daphnia magna* populations in the control and predation treatments. A one-way ANOVA reveals no significant impact of evolution (population) on coefficients of variation in both treatments (Table 1).



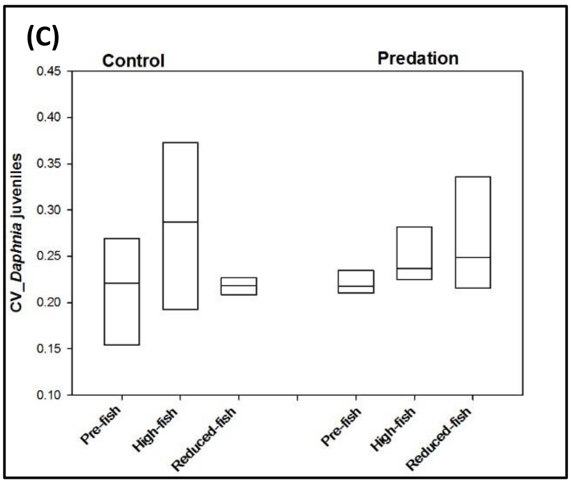


Figure 4. Boxplots of coefficients of variation in (A) chlorophyll *a* and (B) abundances of adult and (C) juvenile *Daphnia* for the Pre-fish, High-fish, and Reduced-fish *Daphnia magna* populations in the control and predation treatments in the mesocosm experiment reported upon in Chapter 4.

However, there was a (marginally non-significant, with p=0.059, Table 1A) tendency for an effect of population on variability in chlorophyll *a* levels in the control treatment, with mesocosms inoculated with the pre-fish population exhibiting less variation compared to those inoculated with the high-fish and reduced-fish populations. If this tendency would be corroborated in a larger experiment, this would indicate that in the absence of fish, evolution of *Daphnia* in response to an increase in fish predation reduces ecological stability.

Table 1. Results of a one-way ANOVAs testing for the effect of evolution (population) on the coefficients of variation (CV) in chlorophyll *a* and the abundances of adult and juvenile *Daphnia* in the mesocosm experiment carried out in Chapter 4 for the control (A) and predation treatment (B) separately.

(A) Control treatment	**df**	**Sum of Squares**	**Mean Square**	**F**	**p**
Chlorophyll *a* concentration					
Daphnia population	2	0.052	0.026	3.939	0.059
Adult *Daphnia* abundance					
Daphnia population	2	0.009	0.004	3.093	0.095
Juvenile *Daphnia* abundance					
Daphnia population	2	0.012	0.006	1.443	0.286
(B) Predation treatment					
Chlorophyll *a* concentration					
Daphnia population	2	0.006	0.003	2.067	0.182
Adult *Daphnia* abundance					
Daphnia population	2	0.004	0.002	0.766	0.493
Juvenile *Daphnia* abundance					
Daphnia population	2	0.004	0.002	1.163	0.355

For the second question, I applied the early warning indicator tools presented by Dakos et al. (2012) on the mesocosm data. The number of data points in the time series is rather low, so the results should be interpreted with caution. The analysis does not provide strong indications for regime shifts. Rather the shift in algal biomass seems to gradually build up. While I need to interpret the data with caution as the power of our analysis was low, I can tentatively conclude that this mesocosm experiment does not provide evidence for evolution impacting the occurrence of regime shifts (Figure 5).

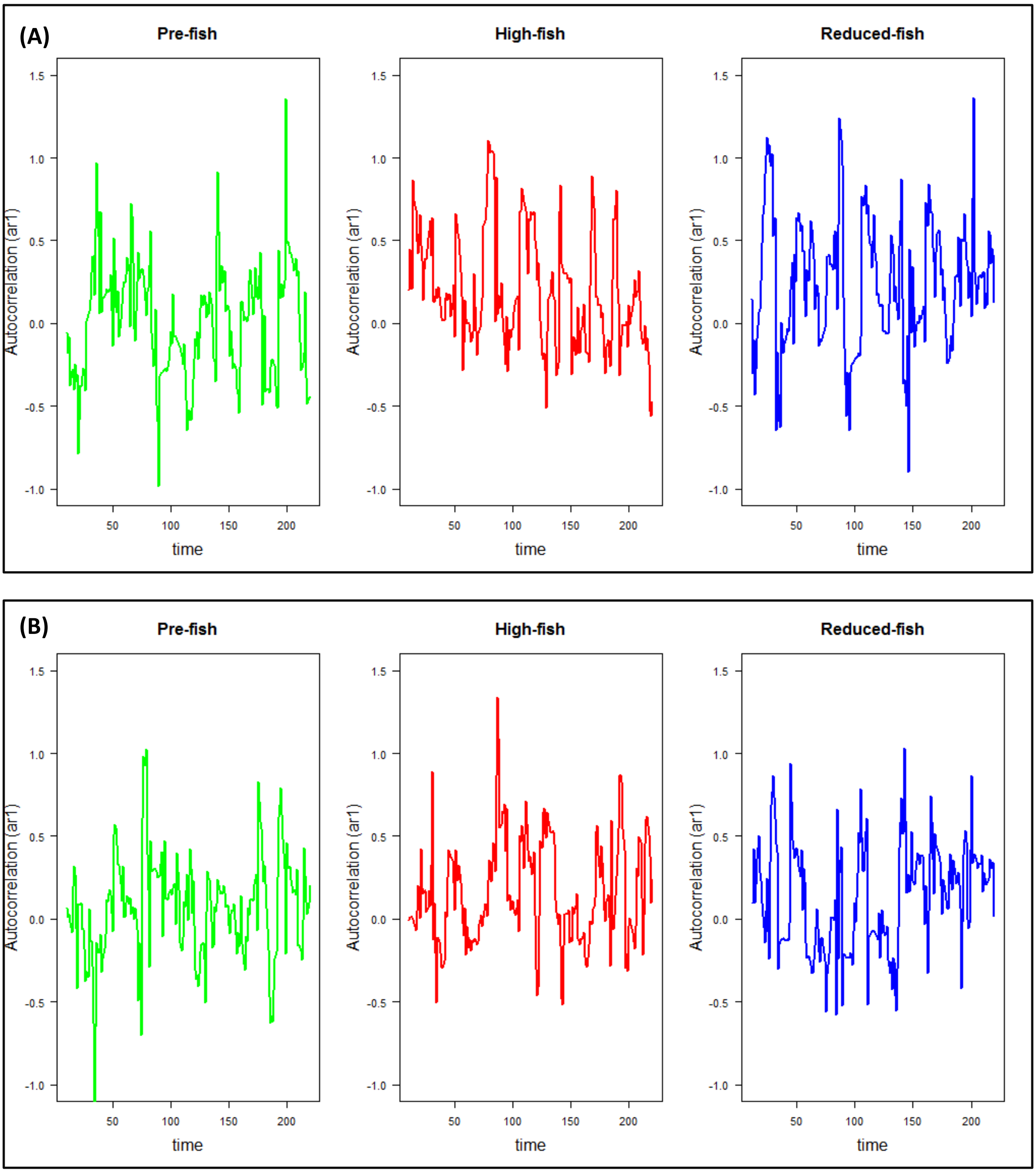


Figure 5. Plots showing the autocorrelation in chlorophyll *a* time series data in the mesocosm experiment documented up on in Chapter four for the Pre-fish, High-fish, and Reduced-fish *Daphnia magna* populations, in the control (A) and predation treatments (B). A rolling time window of 5% of the length of the time series was used.

Perspectives and future work

I suggest three possible directions for future research, which can be divided into two major parts: one linked to ecosystem stability, regime shifts and early warning signals (1-2) and one linked to eco-evolutionary dynamics on ecosystem functioning in natural complexity (3). I briefly outline them below.

First, there is a need to clear out the conceptual and semantic confusion about regime shifts, alternative stable states and early warning signals. In order to be able to assess to what extent alternative stable states and regime shifts are common in natural systems, it is important to collect long-term data for multiple systems. Regrettably, only a relatively small number of lakes are regularly monitored, and historical data on ecosystem dynamics are lacking for most lakes. Although it is not possible to go back in time and collect information using traditional field-based methods, remote sense tools such as Landsat images have been acquired and archived on a regular basis since the early 1970s, enabling a reconstruction of historical water quality of lakes. There are technical challenges such as deriving the right indices for assessing underwater vegetation development using satellite images and coping with interference from vegetation surrounding lakes, and these challenges are especially important in small, shallow systems. Yet, if these technical challenges can be dealt with, then one could apply remote sensing on the entire set of ponds of the Vijvergebied Midden-Limburg, which would provide unprecedented power to cover both temporal (decades) and spatial (>1000 lakes of Vijvergebied Midden-Limburg) scales. High resolution assessments on multiple systems over long time periods have the potential to vastly improve our understanding on ecosystem stability, regime shifts and alternative stable states, as well as on the capacity of early warning signals to predict regime shifts.

Second, I think more work is needed on testing the performance of various early warning signals (EWSs). Most studies have derived the early warning indicators from theory and have then linked regime shifts with the occurrence of indicators such as critical slowing down. There is a need for a more integrated perspective that quantifies the performance of early warning indicators taking false positives and false negatives into account. Up to now, critical transitions in modeled time series, experimental time series and whole lake experiments have been shown to be preceded by, for instance, critical slowing down in one or another parameter. This does not provide sufficient confidence and precision to make the reverse link, i.e. using indicators to determine whether a change in state was a regime shift. Until now, an assessment of the generality and detection power of early warning indicators for regime shifts using monitoring data on natural ecosystems is lacking.

Third, there is a need for more studies quantifying the importance of eco-evolutionary dynamics at the ecosystem level in systems exhibiting natural complexity. Because ecological and evolutionary dynamics processes are strongly intertwined and can occur at the same time scales, evolutionary trait changes can mediate changes in population dynamics, community composition, and ecosystem functions. So far however, only few studies report feedbacks of evolution at the ecosystem level, and only a handful of studies do this in in nature in a well-defined time frame. Given the link between top-down control of algae by *Daphnia* with the occurrence of regime shifts and with management, it is likely that trait evolution in *Daphnia* has the capacity to alter ecosystem state and regime shifts, and quantifying the contribution of evolution to regime shifts is an important challenge for the future.

Conclusions

We quantified stability in ecosystem state at different temporal scales in a set of shallow lakes, a system that has been foundational to the development of regime shift theory in ecology. The emerging conclusion of our analysis is that internal food web structure and biotic interactions can indeed strongly influence chlorophyll *a* dynamics in the studied meso- to eutrophic systems, and that even within one set of interconnected lakes variation in chlorophyll *a* levels can be very high. Clear-water and turbid systems do coexist in space and can occur within a single lake over time, and shifts are often erratic and pronounced. All these observations point to the importance of the concept of alternative stable states in shallow lakes (Scheffer, 1998) as a powerful framework for ecological studies in shallow lakes.

Yet, not all differences among lakes or changes from the turbid to the clear-water state within lakes reflect regime shifts, as some of the shifts might be gradual or might be in response to pronounced changes in environmental conditions. This thesis hopefully represents a contribution to a more open and subtle debate, where it is on the one hand recognized that regime shifts may not be ubiquitous and may not occur in all lakes, but that, at the other hand, the occurrence of alternative stable states does imply the risk of sudden regime shifts. The concept of regime shift remains important in terms of its policy implications related to both mitigation as well as to restoration. With respect to mitigation, the theory of regime shifts teaches us to be prudent in systems that show high resilience, such as shallow lakes. With respect to restoration, the theory provides us clear guidelines on how to act: on the one hand one needs to take hysteresis into account and restoration efforts will need to be powerful, but on the other hand our insights into the mechanisms underlying the stabilization of states also gives us handles to pull to achieve the reverse shift, as is illustrated by successful biomanipulation efforts. I identified three important lines for future research: developing remote sensing to test for the degree to which regime shifts are common, improving early warning indicators so that they can be used in a quantitative way to make a link to regime shifts, and better quantifying the degree to which eco-evolutionary dynamics impact the occurrence of regime shifts.

References

Anderson, D. M., Glibert, P. M., & Burkholder, J. M. (2002). Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries, 25*(4), 704-726.

Bayley, S. E., Creed, I. F., Sass, G. Z., & Wong, A. S. (2007). Frequent regime shifts in trophic states in shallow lakes on the boreal plain: Alternative" unstable" states? *Limnology and Oceanography, 52*(5), 2002-2012.

Blindow, I., Hargeby, A., Wagner, B. M. A., & Andersson, G. (2000). How important is the crustacean plankton for the maintenance of water clarity in shallow lakes with abundant submerged vegetation? *Freshwater Biology, 44*(2), 185-197. doi: 10.1046/j.1365-2427.2000.00552.x

Brooks, B. W., Lazorchak, J. M., Howard, M. D. A., Johnson, M. V. V., Morton, S. L., Perkins, D. A. K., . . . Steevens, J. A. (2016). Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems? *Environmental Toxicology and Chemistry, 35*(1), 6-13.

Carpenter, S. R., Brock, W. A., Cole, J. J., & Pace, M. L. (2014). A new approach for rapid detection of nearby thresholds in ecosystem time series. *Oikos, 123*(3), 290-297.

Carpenter, S. R., Cole, J. J., Pace, M. L., Batt, R., Brock, W. A., Cline, T., . . . Seekell, D. A. (2011). Early warnings of regime shifts: A whole-ecosystem experiment. *science, 332*(6033), 1079-1082.

Carpenter, S. R., Kinne, O., & Wieser, W. (2003). *Regime shifts in lake ecosystems: Pattern and variation* (Vol. 15): International Ecology Institute Oldendorf/Luhe.

Carpenter, S. R., & Kitchell, J. F. (1996). *The trophic cascade in lakes*: Cambridge University Press.

Carpenter, S. R., Kitchell, J. F., & Hodgson, J. R. (1985). Cascading trophic interactions and lake productivity. *Bioscience, 35*(10), 634-639.

Cottenie, K., Michels, E., Nuytten, N., & De Meester, L. (2003). Zooplankton metacommunity structure: Regional vs. Local processes in highly interconnected ponds. *Ecology, 84*(4), 991-1000.

Cousyn, C., De Meester, L., Colbourne, J. K., Brendonck, L., Verschuren, D., & Volckaert, F. (2001). Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. *Proc Natl Acad Sci U S A, 98*(11), 6256-6260. doi: 10.1073/pnas.111606798

Dakos, V., Carpenter, S. R., Brock, W. A., Ellison, A. M., Guttal, V., Ives, A. R., . . . van Nes, E. H. (2012). Methods for detecting early warnings of critical transitions in time series illustrated using simulated ecological data. *PloS one, 7*(7), e41010.

Dakos, V., Kéfi, S., Rietkerk, M., Van Nes, E. H., & Scheffer, M. (2011). Slowing down in spatially patterned ecosystems at the brink of collapse. *The American Naturalist, 177*(6), E153-E166.

De Bie, T, De Meester, L, Brendonck, L, Martens, K, Goddeeris, B, Ercken, D, . . . Gucht, K. (2012). Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. *Ecology Letters, 15*(7), 740-747.

Donohue, I., Hillebrand, H., Montoya, J. M., Petchey, O. L., Pimm, S. L., Fowler, M. S., . . . McClean, D. (2016). Navigating the complexity of ecological stability. *Ecology Letters, 19*(9), 1172-1185.

Donohue, I., Petchey, O. L., Montoya, J. M., Jackson, A. L., McNally, L., Viana, M., . . . Emmerson, M. C. (2013). On the dimensionality of ecological stability. *Ecology Letters, 16*(4), 421-429.

Drake, J. M., & Griffen, B. D. (2010). Early warning signals of extinction in deteriorating environments. *Nature, 467*(7314), 456.

Hannah, L., Lohse, D., Hutchinson, C., Carr, J. L., & Lankerani, A. (1994). A preliminary inventory of human disturbance of world ecosystems. *Ambio*, 246-250.

Hanson, M. A., Herwig, B. R., Zimmer, K. D., Fieberg, J., Vaughn, S. R., Wright, R. G., & Younk, J. A. (2012). Comparing effects of lake-and watershed-scale influences on communities of aquatic invertebrates in shallow lakes. *PloS one, 7*(9), e44644.

Hobbs, W. O., Hobbs, J. M. R., LaFrançois, T., Zimmer, K. D., Theissen, K. M., Edlund, M. B., . . . Carlson, T. J. (2012). A 200-year perspective on alternative stable state theory and lake management from a biomanipulated shallow lake. *Ecological Applications, 22*(5), 1483-1496.

Holling, C. S. (1973). Resilience and stability of ecological systems. *Annual Review of Ecology and Systematics, 4*(1), 1-23.

Jeppesen, E., Jensen, J. P., Søndergaard, M., Lauridsen, T., Pedersen, L. J., & Jensen, L. (1997). Top-down control in freshwater lakes: The role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia, 342*, 151-164.

Jeppesen, E., Meerhoff, M., Jacobsen, B. A., Hansen, R. S., Søndergaard, M., Jensen, J. P., . . . Branco, C. W. C. (2007). Restoration of shallow lakes by nutrient control and biomanipulation—the successful strategy varies with lake size and climate. *Hydrobiologia, 581*(1), 269-285.

Jeppesen, E., Peder, J. J., SØndergaard, M., Lauridsen, T., & Landkildehus, F. (2000). Trophic structure, species richness and biodiversity in danish lakes: Changes along a phosphorus gradient. *Freshwater Biology, 45*(2), 201-218.

Kéfi, S., Dakos, V., Scheffer, M., Van Nes, E. H., & Rietkerk, M. (2013). Early warning signals also precede non-catastrophic transitions. *Oikos, 122*(5), 641-648.

Kéfi, S., Holmgren, M, & Scheffer, M. (2016). When can positive interactions cause alternative stable states in ecosystems? *Functional Ecology, 30*(1), 88-97.

Lemmens, P., Declerck, S. A. J., Tuytens, K., Vanderstukken, M., & De Meester, L. (2018). Bottom-up effects on biomass versus top-down effects on identity: A multiple-lake fish community manipulation experiment. *Ecosystems, 21*(1), 166-177. doi: 10.1007/s10021-017-0144-x

Lemmens, P., Mergeay, J., De Bie, T., Van Wichelen, J., De Meester, L., & Declerck, S. A. (2013). How to maximally support local and regional biodiversity in applied conservation? Insights from pond management. *PLoS One, 8*(8), e72538. doi: 10.1371/journal.pone.0072538

Lemmens, P., Mergeay, J., Van Wichelen, J., De Meester, L., & Declerck, S. A. J. (2015). The impact of conservation management on the community composition of multiple organism groups in eutrophic interconnected man-made ponds. *PloS one, 10*(9), e0139371.

May, R. M. (1977). Thresholds and breakpoints in ecosystems with a multiplicity of stable states. *Nature, 269*(5628), 471-477.

McGowan, S., Leavitt, P. R., Hall, R. I., Anderson, N. J., Jeppesen, E., & Odgaard, B. V. (2005). Controls of algal abundance and community composition during ecosystem state change. *Ecology, 86*(8), 2200-2211.

Norlin, J., Bayley, S. E., & Ross, L. (2005). Submerged macrophytes, zooplankton and the predominance of low-over high-chlorophyll states in western boreal, shallow-water wetlands. *Freshwater Biology, 50*(5), 868-881.

Pereira, H. M., Navarro, L. M., & Martins, I. S. (2012). Global biodiversity change: The bad, the good, and the unknown. *Annual Review of Environment and Resources, 37*.

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin III, F. S., Lambin, E. F., . . . Schellnhuber, H. J. (2009). A safe operating space for humanity. *Nature, 461*(7263), 472.

Scheffer, M. (1998). *Ecology of shallow lakes*. London, UK: Chapman and Hall.

Scheffer, M., Bascompte, J., Brock, W. A., Brovkin, V., Carpenter, S. R., Dakos, V., . . . Sugihara, G. (2009). Early-warning signals for critical transitions. *Nature, 461*(7260), 53-59.

Scheffer, M., Carpenter, S., Foley, J. A., Folke, C., & Walker, B. (2001). Catastrophic shifts in ecosystems. *Nature, 413*(6856), 591-596.

Scheffer, M., Hosper, S. H, Meijer, M. L, Moss, B., & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution, 8*(8), 275-279.

Schröder, A., Persson, L., & De Roos, A. M. (2005). Direct experimental evidence for alternative stable states: A review. *Oikos, 110*(1), 3-19.

Søndergaard, M., Jeppesen, E., Lauridsen, T. L., Skov, C., Van Nes, E. H., Roijackers, R., . . . Portielje, R. O. B. (2007). Lake restoration: Successes, failures and long-term effects. *Journal of Applied Ecology, 44*(6), 1095-1105.

Spears, B. M., Futter, M. N., Jeppesen, E., Huser, B. J., Ives, S., Davidson, T. A, . . . Carvalho, L. (2017). Ecological resilience in lakes and the conjunction fallacy. *Nature ecology & evolution, 1*(11), 1616.

Stoks, R., Govaert, L., Pauwels, K, Jansen, B., & De Meester, L. (2016). Resurrecting complexity: The interplay of plasticity and rapid evolution in the multiple trait response to strong changes in predation pressure in the water flea daphnia magna. *Ecology Letters, 19*(2), 180-190. doi: 10.1111/ele.12551

Tilman, D., Reich, P. B., & Knops, J. M. H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature, 441*(7093), 629.

Timms, R. M., & Moss, B. (1984). Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. *Limnology and Oceanography, 29*(3), 472-486.

Van Donk, E., Grimm, M. P., Gulati, R. D., & Breteler, J.P.G.K. (1990). Whole-lake food-web manipulation as a means to study community interactions in a small ecosystem. *Hydrobiologia, 200*(1), 275-289.

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

Wang, R., Dearing, J. A., Langdon, P. G., Zhang, E, Yang, X., Dakos, V., & Scheffer, M. (2012). Flickering gives early warning signals of a critical transition to a eutrophic lake state. *Nature, 492*(7429), 419.

Zimmer, K. D., Hanson, M. A., Herwig, B. R., & Konsti, M. L. (2009). Thresholds and stability of alternative regimes in shallow prairie–parkland lakes of central north america. *Ecosystems, 12*(5), 843-852.

Summary

Ecosystem stability and regime shifts has become a central research topic in ecology and environment over the latest decade. Ponds and shallow lakes have been central to the development of the concept of alternative stable states and regime shifts in ecosystems. Ponds and shallow lakes can exhibit multistate stability and occasionally sudden transitions from regime to another. Since regime shift are common and frequently lead to severe ecological and economic losses, an increasing number of studies has suggested the need to detect the proximity to critical transition to prevent regime shifts.

The first aim of this dissertation is to provide a better understanding of ecosystem stability in freshwater systems at different temporal scales, from among years to seasonal and weekly variation, and both within as well as among habitats. As a second aim, we also explore to what extent an evolutionary response can contribute to top-down control of algae and thus ecosystem stability. Our research used a combination of intensive field data collected in multiple consecutive years (2013, 2014, 2015, 2016 and 2017) and follow-up mesocosm experiment on resurrected *Daphnia magna* population. The findings from study can contribute to the debate on the occurrence of alternative stable state in freshwater system. Moreover, it provides a better insight on how evolution mediate ecosystem stability and functioning.

In the first chapter we presented the difference in phytoplankton biomass (chlorophyll *a* concentration) with in and among years in a set of 25 fish ponds, and relate these difference to a variation in zooplankton body size and macrophyte cover. We found evidence that the 25 interconnected ponds differ strongly in their phytoplankton biomass and that these differences are associated with differences in nitrogen concentration, macrophyte cover in summer and zooplankton body size in spring. The differences in these characteristics among ponds result in repeatable differences in their state. Moreover, our results revealed that zooplankton grazing and macorphyte cover are important determinants for the variation in phytoplankton biomass.

Chapter 2 reports a year to year turnover in environmental conditions and zooplankton community in the same set of ponds during three consecutive years. In this chapter we show the variation in environmental conditions and zooplankton community composition in the same set of ponds, we found repeatable differences among systems that are associated to pond management. Furthermore, environmental variables substantially varied among ponds and these differences are largely linked to the difference in fish pond management (Chapter 2). Chapter 3 focuses in to a higher temporal resolution data by monitoring chlorophyll *a* and phycocyanin loggers that were positioned in four neighboring ponds. In here, we quantify differences among years as well as among systems, and explore early-warning signals for sudden state shifts. In this chapter, we found a substantial variation in overall chlorophyll *a* as well as phycocyanin among years and systems. We also observed considerable variation within a year. We also show a strong association between chlorophyll *a* and phycocyanin, and found an indication of a regime shift in one pond in 2016. In chapter 4, We carried out a follow-up experiment on a resurrection ecology study that documented rapid adaptive change in a natural population of the water flea *Daphnia magna* in response to strong changes in fish predation pressure and study whether the observed genetic trait changes influence population dynamics and top-down control of phytoplankton. We conducted an outdoor mesocosm experiment in which we inoculated D. magna populations derived from three time periods of the same population known to have genetically adaptation to changes in predation pressure. Our results show that the interactions between adults and juveniles strongly impact the dynamics of populations and their top-down effect on algae and can be modulated by rapid evolution, such as here observed in response to changes in predation pressure.

Samenvatting

Ecosysteemstabiliteit en het voorkomen van regimeshifts in ecosystemen zijn belangrijke onderzoeksonderwerpen in hedendaags wetenschappelijk ecologisch onderzoek. Vijvers en ondiepe meren hebben een centrale rol gespeeld bij de ontwikkeling van de theorie van alternatieve stabiele evenwichten en voorkomen van regimeveranderingen in ecosystemen. Vijvers en ondiepe meren worden gekenmerkt door het voorkomen van verschillende stabiele toestanden en het voorkomen van occasionele transities van één stabiele toestand naar een andere stabiele toestand. Dergelijke regimewijzigingen gaan veelal gepaard met aanzienlijke ecologische en economische schade. Een toenemend aantal wetenschappelijke studies wijst op het grote belang om een dergelijke regimeveranderingen en de bijhorende kritieke ecosysteemwijzigingen te detecteren en te voorspellen.

De eerste centrale doelstelling van dit doctoraatsonderzoek is het beter begrijpen van ecosysteemstabiliteit in zoetwater systemen op verschillende temporele schalen, van dagelijkse tot wekelijkse, seizoenale en jaarlijkse variatie, en dit zowel binnen als tussen verschillende systemen. Daarnaast onderzoeken we in welke mate evolutionaire responsen kunnen bijdragen tot top-down controle van fytoplankton en ecosysteemstabiliteit. Het hier gepresenteerde onderzoek maakt gebruik van data verzameld tijdens intensief veldonderzoek overheen vijf opeenvolgende jaren (2013, 2014, 2015, 2016, 2017) en data uit een mesocosm-experiment met verschillende *Daphnia magna* populaties in aan- en afwezigheid van vis. De bevindingen van dit onderzoek kunnen bijdragen tot het debat rond het voorkomen van alternatieve stabiele evenwichten in zoetwatersystemen, en draagt bij tot een beter inzicht in hoe evolutie ecosysteemfunctioneren en –stabiliteit kan beïnvloeden.

In hoofdstuk 1 presenteren we verschillen in fytoplanktonbiomassa binnen en tussen jaren in een set van 25 ondiepe visvijvers. We relateren deze verschillen aan variatie in zoöplanktonlichaamsgrootte en bedekking met ondergedoken waterplanten. Onze data tonen sterke verschillen in fytoplanktonbiomassa tussen vijvers en tonen aan dat deze verschillen geassocieerd zijn met verschillen in stikstofconcentratie en met bedekking met ondergedoken waterplanten tijdens de zomer, alsook met zoöplanktonlichaamsgrootte in het voorjaar. De verschillen tussen vijvers voor deze variabelen resulteren in systematische verschillen in ecosysteemtoestand. Onze resultaten tonen aan dat begrazing door zoöplankton op fytoplankton en bedekking met waterplanten belangrijke factoren zijn voor variatie in fytoplanktonbiomassa tussen vijvers.

In hoofdstuk 2 presenteren we de jaar tot jaar verandering in lokale omgevingscondities en zoöplanktongemeenschapssamenstelling in dezelfde set van vijvers over een periode van 3 jaar. Hier tonen we systematische verschillen in omgevingscondities en zoöplanktongemeenschap tussen vijvers die in sterke mate geassocieerd zijn met het gevoerde vijverbeheer. Daarnaast vonden we dat verschillen in omgevingscondities tussen vijvers en belangrijke mate gelinkt zijn met verschillen in visstandsbeheer.

In hoofdstuk 3 gebruiken we chlorophyll a en phycocyanine data met een hoge temporele resolutie die werden opgemeten met dataloggers in vier verschillende vijvers. We kwantificeren verschillen fytoplanktondynamieken tussen jaren en tussen vijvers, en verkennen de mogelijkheden van “early-warning signals” voor het detecteren van plotse veranderingen in ecosysteem status. In dit hoofdstuk vonden we substantiële variatie in chlorophyll a en phycocyanine tussen jaren en vijvers. We observeerden een aanzienlijke variatie binnen jaar. Daarnaast vonden we een sterke positieve associatie tussen phycocyanine en chlorophyll a, en vonden we een indicatie voor een regimeshift in één vijver in 2016.

In hoofdstuk 4 presenteren we de resultaten van een mesocosm-experiment rond resurrectie ecologie waarbij we de populatiedynamieken van verschillende Daphnia magna populaties met een gekende verschillende genetische adaptatie aan vispredatie onderzoeken. Onze resultaten tonen aan dat de interactie tussen adulten en juvenielen een sterke impact heeft op de populatiedynamieken daarnaast ook een effect heeft op de top-down impact van zoöplankton op fytoplankton. De aard en de sterke van de populatie-interactie worden evenwel bepaald door snelle evolutie als respons op verschillende vispredatie-intensiteit.