

Linking ecological theory with data

How empirical data and theory can inform each other to understand threshold-driven changes in lakes under global change pressure

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List of manuscripts and publications

This thesis is structured as a cumulative work with three manuscripts presented as Chapters 2, 3 and 4. Out of three, one has already been published as a peer reviewed article (Chapter 2) while the other two manuscripts are currently under review (Chapters 3 and 4). A general introduction and discussion is provided in Chapter 1 and 5.

Chapter 2

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Author contributions

US co-designed the study, processed and analysed the data, linked data-driven analysis to regime-shift and dynamic system theory and wrote the manuscript. AM co-designed the study, co-analysed the data and contributed to the text. RA co-designed the study and co-wrote the manuscript.

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US co-conducted the experiment, designed the study, processed and analysed the data, linked data-driven analysis to MTE and wrote the manuscript. EJ co-designed the experiment, advised on data analysis and advised on the preparation of the manuscript. MS co-designed the experiment. AM, MB, MS, DGA, AİÇ, SD, JH, EP, MŠ, KS, AT, PZ co-conducted the experiment. RA co-conducted the experiment, co-wrote the manuscript. All authors contributed to the text.

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ASG designed the study, processed and analysed the data and wrote the manuscript. US co-designed the study and co-analysed the data. DÖ, AW, LA-H, VD and RA co-designed the study. PN, DES, EvD and RA contributed data. All authors contributed to the text.

Appendix

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Author contributions

This method article gives a detailed description of the lake mesocosm experiment which was conducted in the framework of the European project REFRESH. It is included in this thesis solely to provide a comprehensive description of the design and set-up of the cross-European mesocosm experiment. All co-authors coordinated and co-conducted the experiment in one of the 6 countries. FL, MS, EJ designed the experiment.

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Summary

Thresholds highlight points in a driver continuum whose crossing is connected to fundamental changes in the ecosystem. Such threshold-driven changes can either be large abrupt changes in ecosystem components, known as abrupt and discontinuous regime-shifts, but also fundamental changes in the functional status of a lake. In recent years there is a growing awareness that threshold-driven changes might be a wide spread and common phenomenon in complex natural ecosystems. In the light of global change pressures, the possibility that a small change in a driving variable might have the potential to elicit fundamental changes in ecosystem functioning and thus impair important ecosystem services, like carbon sequestration, becomes particularly acute. Anthropogenic activities force changes in many key driving parameters of ecosystems, increasing the probability that such thresholds might be crossed. Since lakes integrate the changes from their catchment, they are thought to be particularly vulnerable to global change pressures, which makes them good sentinels of environmental change. In particular climate change and eutrophication have been identified as leading threats for lakes integrity. While ecological theory hypothesises the existence of such thresholds, their evidence and relevance in real and complex ecosystems, where many processes act at the same time, is an active field of research. Data-driven analyses benefit, from a close link to current ecological theory, because this facilitates causal interpretation and allows the formulation of informed hypotheses and expectations. Importantly, however, results based on empirical data can in return inform theory and help to improve our mechanistic understanding on the functioning of lake ecosystems. In three case studies, this thesis aims to improve our understanding of the relevance of threshold-driven changes in real lake ecosystems under climate change and eutrophication pressure. Thereby the chosen approaches realize the suggested close linking between current ecological theory and empirical data analysis in three slightly different ways.

In the first case study an abrupt shift in the dominance pattern of two coexisting copepod species, *Cyclops vicinus* and *Cyclops kolensis*, was investigated. The driving mechanisms leading to this shift were analysed under particular consideration of the potential role of changing nutrient status and increasing mean water temperatures. The analysis based on long-term observational data from shallow, eutrophic Lake Müggelsee was augmented with the relevant theoretical concepts from population ecology and regime-shift theory to facilitate causal interpretation. Analysis showed that the change in dominance was a result of a complex interplay between the improvement in nutrient status and population dynamics, while changes in temperature did not play a crucial role. Linking results from data analysis with intra-guild predation theory suggested that the abrupt increase in *C. kolensis*, changing it status from a rare to the dominant species as available algal prey declined in the lake, was due to its superior exploitative competition for commonly consumed algal prey. However, *C. kolensis* was only able to thrive in the emerging

low food niche when abundances of competing larger *C. vicinus*, a predator of *C. kolensis* juveniles, fell below a critical abundance threshold. This is consistent with an abrupt regime shift, for which a response variable exhibits an abrupt large change, here *C. kolensis*, after the driver (*C. vicinus*) crosses a threshold. The decline of *C. vicinus*, however, followed quasi-linearly the decline in available algal food. This case study is an important example of the relevance of abrupt regime-shifts, but also intra-guild predation food webs in real shallow lake ecosystems where often simple linear food webs are in the focus of research.

In a second case study the framework of the Metabolic Theory of Ecology (MTE) was used to derive hypotheses on the effects of temperature, nutrient status and light availability on metabolic rates of shallow lakes and the balance between them. The hypotheses were tested based on experimental data from a Pan-European space-for-time experiment. Temperature, nutrient status and light availability are known as important drivers for metabolic rates and all three of them are predicted to be affected by global change. Yet, less is known about their interacting effects on lake metabolism and metabolic status and thus about their influence on the role of shallow lakes in regional and global carbon cycles. The space-for-time approach allowed the set-up of the mesocosms in lakes, thus ensuring natural meteorological regimes and at the same time the utilization of a large climate gradient over six countries from Sweden to Greece. The experimental approach allowed standardized treatment manipulation comprising two nutrient levels (mesotrophic or eutrophic) crossed with two water levels (1 and 2 m) to simulate different light regimes and mixing depths. Average responses showed that all systems switched from net auto- to net heterotrophy over the investigated temperature range. The observed differential temperature dependence of gross primary production and ecosystem respiration was close to that predicted by metabolic theory, thus confirming the potential that increasing temperatures might lead to reduced carbon sequestration capacities. The threshold temperature for the switch in metabolic status was, however, lower under mesotrophic than eutrophic conditions. Overall, we quantified the differential temperature sensitivity of Gross Primary Production (GPP) and Ecosystem Respiration (ER) and found that trophic state is crucial for how much warming a system can tolerate before it switches from net auto- to net heterotrophy, thus highlighting a shortcoming of MTE, which does not explicitly consider essential resources.

In the third case study early warning indicators (EWIs), were tested for their potential to indicate in good time impending discontinuous regime-shifts based on data from standard monitoring schemes. The existence of EWIs in time series from dynamics systems approaching a discontinuous regime-shift is predicted by bifurcation theory. Impending discontinuous regime-shifts might be difficult to predict, since the state variables of complex dynamic systems might show little systematic change prior to the regime-shift. EWIs offer a potential way out, since they signal the increasing loss of system resilience which accompanies systems approaching a discontinuous regime-shift. Four commonly used EWIs were assessed based on long-term datasets of six lake ecosystems that have experienced a regime shift and for which the relevant ecological

mechanisms and drivers were known. While EWIs could be detected in the majority of state variables, often considerable time ahead, consistency among the four indicators was low. This study showed that EWIs are not (yet) an irrefutable, consistent signal of impending critical transitions and detailed system knowledge is needed for their use. As such the study was a timely test of a much hoped for generic alarm clock, and identified important future research needs to advance the concept of EWIs.

The key findings in this thesis show that threshold-driven changes are indeed of relevance in shallow lakes when anthropocentric influence lead to changes in important driving variables. Such abrupt changes can affect all organisational levels of a lake, from populations to whole ecosystems. Further they can affect state variables or the functional state of a lake. The presented results on threshold-driven changes contributes to the improvement of MTE and its predictive power for global change effects on ecosystems in general, but may also inform new concepts such as the Planetary Boundary Concept by identifying and quantifying threshold-driven processes in natural lake ecosystems. Yet, despite the confirmation of the impending risk of sudden changes in lake ecosystems under ongoing global change, the result of this study can not affirm the hope of a soon available generic early warning alarm, which gives managers enough time to prevent the impending changes. As such the results once more highlight the importance of the precautionary principle when dealing with vital complex systems.

Chapter 1

General introduction

In the current epoch of the Anthropocene humankind exerts unprecedented pressure on planetary boundaries by forcing multiple key drivers of ecosystems such that planetary boundaries might be reached or even already been crossed. This leads to a growing concern, as for instance voiced in the IPCC- and the Millennium Ecosystem Assessment reports, that ecosystems including lakes might experience sudden abrupt changes if critical thresholds are crossed, triggering fundamental changes in their functioning. Although there exist several documented cases of such sudden abrupt changes in ecosystems the question about their actual relevance and the involved mechanisms is still an active area of research. For many of the theoretical predicted threshold driven changes, supplementary evidence from natural systems is still needed. The topic of threshold-driven changes has a natural deep root in non-linear system theory, which gives a valuable framework for their analysis. In this thesis I investigate threshold-driven changes in lake ecosystems, including potential ways to predict them. Data driven analysis is conducted with a close link to current ecological theory. The aim of this general introduction is: (1) to introduce threshold-driven changes from a theoretical point of view, (2) to shortly summarise current knowledge about the effects of the two key driving parameters for shallow lake ecosystems: nutrients and climate (3) discuss the consequences of simultaneous changes in a multi parameter space for the prediction of threshold-driven changes, (4) reflect the advancement of scientific knowledge in the field of ecology through a link between theory and empirical data (5) and to finally give a short outline of the thesis.

1.1 Threshold-driven changes in ecology and their theoretical basis

The axiom *Natura non facit saltus* – nature does not make jumps, dates back to the antic philosophers and has long been an important paradigm in natural science and thus in biology. Most prominently in biology this principle was underlying Charles Darwin's theory of evolution by natural selection, where he argues that species diversify by small, gradual changes rather than sudden emergence (Darwin, 1870). Since then, however, compelling evidence has arisen that discontinuous behaviour after all seems to be of great importance on all levels of biological organisation including whole ecosystems. Maybe one of the first and most widely recognised examples for the occurrence of sudden and large change in ecosystems are: the formation of the Sahara Desert, the dominance shift from kelp forests to turf-forming algae in coastal ecosystems, coral bleaching with the loss of hard corals in favour of algae and for freshwater ecosystems the transition from clear, macrophyte to turbid algae dominated lakes (Scheffer, 2009). Common

to all those examples is that the cause for the large and rather abrupt change in the ecosystem state was traced back to a smooth variation in a driving force which passed over a threshold and triggered thus a fundamental reorganisation of the ecosystem.

Large changes in ecosystem state are also referred to as regime shifts. In regime shift theory typically three qualitatively different dynamics are classified as regime shift: (1) a smooth (Figure 1.1 c), (2) an abrupt (Figure 1.1 b) and (3) a discontinuous regime shift (Figure 1.1 a) (Scheffer et al., 2001; Collie et al., 2004; Andersen et al., 2009). Smooth regime shifts are driven by equally large changes in the driving force due to a quasi-linear relationship between ecosystem state and the driving force. As no thresholds are involved, this case is of less interest from the perspective of threshold-driven changes. Abrupt regime shifts, on the other hand, arise from a strong non-linear relationship between the driving force and the ecosystem state. In this case a threshold arises as point in the driver continuum where the system is most sensitive and a transgression of the threshold triggers a large and abrupt but continuous change in the ecosystem state. In contrast, the term “discontinuous regime shift” describes a jump from one ecosystem state into a contrasting. This jump happens if a critical threshold is surpassed.

The existences of such thresholds is predicted by bifurcation theory, which is concerned with the analysis of the qualitative and quantitative changes of the equilibrium states of a dynamic system over the parameter space of the system. A dynamical system is generally described by a set of differential equations, which parametrise the processes leading to gains and losses for each state variable of the system. In equilibrium state the state variables of the dynamic system do not change over time, indicating that all processes are balanced. In mathematical terms this means that the partial derivative of the state variable with respect to time is zero. However, after a perturbation by external forcing or a change in driving parameters, the equilibrium state is not achieved until some time after the disturbance. In this case the system is in a non-equilibrium state or transient state. In bifurcation theory a critical threshold is the point in the continuum of a driving parameter, where a stable and an unstable equilibrium collide and annihilate each other, a so called saddle-node bifurcation. Since after this collision the stable equilibrium ceases to exist, the system is forced into another equilibrium state for parameter values beyond the bifurcation point. If two such saddle-node bifurcations combine, the phenomenon of bistability can arise. This means that for a certain interval of the driving parameter, two alternative stable states exist with an unstable one in-between. The starting and end point of this interval of coexistence are delimited by the respective critical thresholds of the two saddle-node bifurcations. Each of the two stable equilibria is reinforced by positive feedback interactions and thus inducing a memory (hysteresis) into the system. As a result of hysteresis the transition paths from stable state A into stable state B with increasing driver values is distinct from the one of the return path from the state B to A, induced by decreasing driver values (Figure 1.1 a). From an ecological point of view alternative stable states with hysteresis are thus of great relevance not only because of the abruptness with which large and fundamental changes can take place once a critical threshold

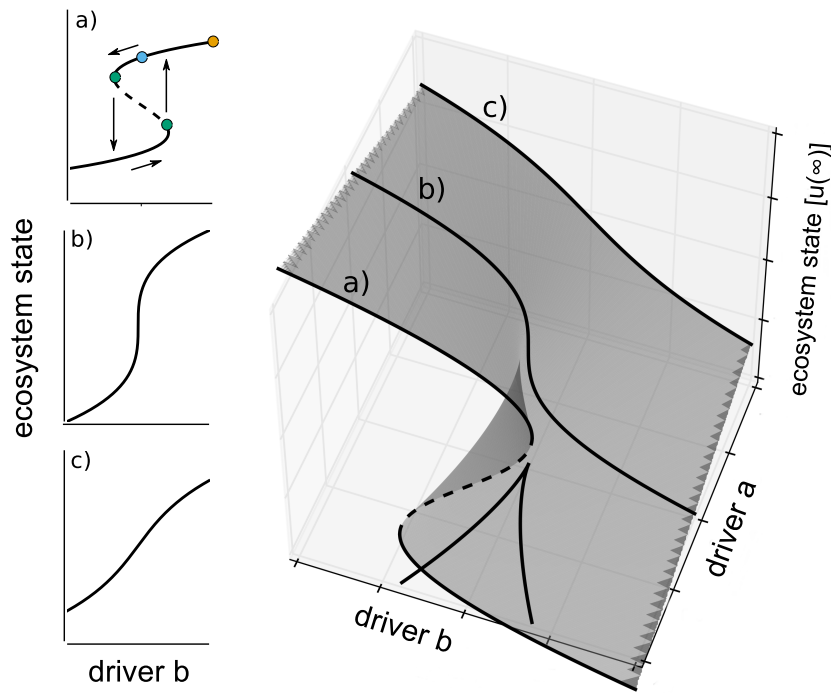


Figure 1.1: Bifurcation plots of the three commonly distinguished regime-shifts in response to an environmental driver b and their relation with each other with regard to a second environmental driver a . The large picture to the right depicts how the same generic two parameter dynamic model ($du/dt = b * u - u^3 + a$) can exhibit all three types of regime-shifts in dependence of a second driver a . If driver a is reduced the parameter range where alternative stable states exist gets smaller (triangle on the bottom) until the cusp bifurcation is met (peak of the triangle). The cusp bifurcation marks the transition between discontinuous- and abrupt regime-shift. Further reduction of driver a leads to a gradual transition between abrupt- and discontinuous regime-shift. a) Discontinuous regime-shift, the solid lines depicts the two stable equilibria (alternative stable states), the dashed line unstable ones (separatrix); the green dots depict the bifurcation points of the two saddle-node bifurcations; the blue dot depicts an arbitrary point close to the bifurcation point, in an parameter range where two alternative stable states exist and critical slowing down is high; the yellow dot depicts an arbitrary point far from the bifurcation in an parameter range where the system has only one stable equilibrium and critical slowing down is low (see also Figure 1.3). The arrows depict the movement on the two equilibrium branches, depending on if driver b is increased or decreased (hysteresis; for detailed information see text). b) Abrupt regime shift accompanied by large changes in ecosystem state, but no hysteresis. c) Smooth change in ecosystem state.

is surpassed but also because much higher efforts (i.e. changes in driver values) are needed to return the system back to its former state. It is also important to note that before the critical threshold is reached state variables might exhibit very little change, which makes the sudden change from a management point of view unpredictable.

In population ecology dynamical system analysis leads to the identification of several important mechanisms with the potential of alternative stable states and thus critical thresholds for a certain parameter ranges of an environmental driver, including competition, trophic cascades and intra-guild predation. Already simple two competitor models, like for example the classic Lotka-Volterra model, can exhibit alternative stable states if competition within a species is less strong than the competition between species (Beisner et al., 2003). If this situation is met depends strongly on the carrying capacity for the two species and thus on environmental

conditions. Likewise resource harvesting or overexploitation models, which depict simplified trophic interaction models, are known for their potential for alternative stable states (May, 1977; Noy-Meir, 1975; Scheffer, 2009). Overexploitation models predict a discontinuous regime-shift of a prey population from an under to an over exploited state in dependence of the consumer density. Consumer density in turn can increase with the general productivity of the environment if the prey population is not the only food source. Such a mechanism, for example, has been suggested to be responsible for the breakdown of daphnid populations and ensuing algae dominance following gradual increases in the dominance of planktivorous fish caused by rising phosphorus concentrations (Jeppesen et al., 1997; Scheffer, 2004). Also more elaborate multi equation models describing interacting predator-prey dynamics, cascading effects over several trophic levels in linear food chains, or the inclusion of omnivory are known to exhibit alternative stable states for certain parametrisations (Scheffer, 1989; Scheffer et al., 1997; Mylius et al., 2001). Intra-guild predation (IGP) food webs, for example, consisting of an omnivorous predator which competes with its prey for a common food source, have been shown to possess alternative stable states if the environment changes from intermediate to high productivity levels (Mylius et al., 2001). Predictions from IGP models have been shown to match closely with results from laboratory experiments (Morin, 1999; Diehl and Feiße, 2000), but to the best of our knowledge still lack confirmation from real ecosystems. In **Chapter II** we contribute to filling this gap and present an IGP based regime shift observed in long-term observational data. Albeit, more complex multi equation models can have alternative stable states, their dynamics might easily become much more involved, including a variety of different bifurcation types like for instance transitions between a stable equilibrium and an oscillation (Scheffer et al., 1997; Heath et al., 2014).

Since abrupt and even more so discontinuous regime-shifts have been recognized by the scientific community as important and a potentially wide spread mechanism in ecology (Reid et al., 2016), they are in the focus of the discussion on threshold-driven changes. This is not surprising as they raised the general awareness that non-linear and discontinuous processes in ecosystems are possible and might rather be the rule than the exception.

To focus on the dynamics of equilibrium states is a valuable approach to population ecology and in particular plankton ecology, where generation cycles are short and populations can reasonably be assumed to be in stable state or at least fluctuate around it. The response times in earth systems, however, can be years, decades and longer. Thus the understanding of non-equilibrium dynamics gains importance. Earth's dynamical systems, like for instance biogeochemical cycles, are often conceptualised as box models. Each box represents the stored mass of, for example, carbon, water, or nitrogen, for a distinct compartment of the system, such as the atmosphere, oceans or lakes and is equivalent to a state variable. The boxes are connected by rate processes which mediate the transport of mass in and out of these reservoirs (Slingerland and Kump, 2011). Such rate processes are in turn dependent on driving parameters, which most often include

temperature. In equilibrium state the same amount of mass enters and leaves the box and the mass of the substance in the box stays constant over time. Changes in the driving parameters of the rate processes can, however, induce imbalance between those processes which forces the system into non-equilibrium state (Figure 1.2).

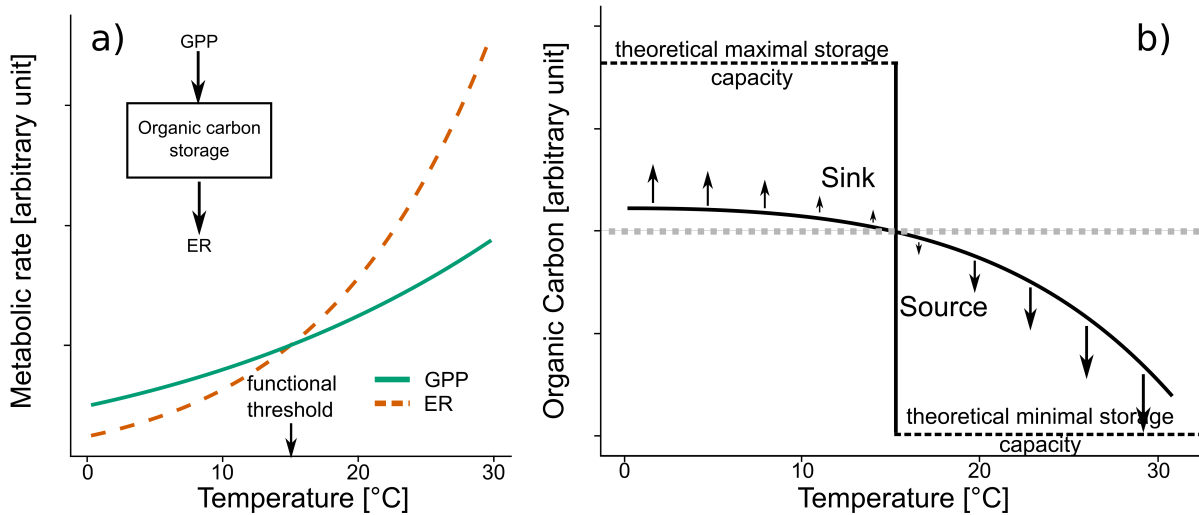


Figure 1.2: Illustration of the functional threshold between Gross primary production (GPP) and Ecosystem respiration (ER), and its relation to the non-equilibrium and equilibrium dynamics based on simplified box model for the organic carbon storage in lakes (insert). a) Dynamics of GPP and ER in dependence of temperature, as described by MTE based on the Arrhenius law (equation 1.1 and 1.4). The functional threshold (here 15 °C) marks the point where both rate processes are equal and the system is in equilibrium. To the left of the functional threshold (lower temperatures) $GPP > ER$ and the system is net autotrophic, to the right $ER > GPP$ and the system is net heterotrophic. b) At the functional threshold (here 15 °C) the system is in equilibrium and the amount of stored carbon does not change, independent on the present value (solid vertical line). If the temperature deviates from the temperature at the function threshold the system either adds OC (sink) until a theoretical maximal values is reached or loses OC (source) until a theoretical minimal storage is reached. Exemplary, with an arbitrary starting value of OC (grey dashed line), the non-equilibrium dynamics and their different speeds are depicted by the bended horizontal line. The straight dashed horizontal lines depict some maximal or minimal carbon storage as new theoretical equilibrium states.

Hence, to understand the non-equilibrium dynamics of a system one needs to know how the balance between the rate processes change with regard to their drivers. In the parameter ranges of the driver where the input rates get larger than the output rates the reservoir will act as a net sink. Contrary, in the parameter ranges where the output rate outpaces the input rate the reservoir acts as net source. Thus, the point in a driver continuum where the process rates switch dominance is a third type of threshold, which we term here functional threshold. Thus, functional thresholds, even so rarely explicitly considered in the discussion about threshold-driven changes, are of considerable interest since they determine when a reservoir acts as either net source or net sink of mass. Yet, functional thresholds are also connected to regime shifts, considering that they separate in a simplified minimal model two stable states of theoretical minimal or maximal mass in the reservoirs. This is so, because the trajectory of the system during non-equilibrium state will head either to a theoretical maximal or minimal reservoir capacity as new stable state, depending on whether the system resides left or right of the functional threshold. The speed of

this dynamics depends on the distance to the functional threshold of the rate processes (Figure 1.2).

Lakes have been recognised as active and important sites of carbon turn over (Tranvik et al., 2009). Following the idea of box modelling, the stored mass of organic carbon (OC) in a lake can be seen as such a reservoir. The synthesis of organic carbon from inorganic carbon by photosynthesis is the rate process adding to the organic carbon reservoirs, while the degradation of organic carbon to inorganic carbon via respiration is an important, even so not the only, output flux (Lovett et al., 2006). Both photosynthesis and respiration are known to be temperature dependent. The metabolic status of a lake as either net autotrophic ($GPP > ER$) or net heterotrophic ($GPP < ER$) can be decisive in determining if a lake acts as sink or source of carbon (Andersson and Sobek, 2006; Brothers et al., 2013; Pacheco et al., 2014). Thus, the effect of temperature on the metabolic status is not only of basic but also of applied ecological interest. It depicts a potential feedback mechanism to atmospheric CO_2 levels (Figure 1.2).

The Metabolic Theory of Ecology (MTE) provides a theoretical framework to assess temperature dependencies of metabolic rates (Brown et al., 2004). MTE is a deductive framework which uses first principles of physics, chemistry and biology to link metabolic rates from the level of single metabolic units to individuals (Gillooly, 2001; Brown et al., 2004) and whole ecosystems (Enquist et al., 2003; Allen et al., 2005; Yvon-Durocher et al., 2010b). On the level of metabolic units (MU), which are the chloroplasts for photosynthesis and the respiratory complexes for respiration, the temperature dependence of the biochemical rates is described by the Arrhenius equation:

$$v = v_0 \exp\left(\frac{-E}{k \cdot T}\right) \quad (1.1)$$

where v is the metabolic rate from individual chloroplasts or respiratory complexes measured as carbon turn-over of the metabolic unit per unit time ($gC \times MU^{-1} \times Time^{-1}$); v_0 ($gC \times MU^{-1} \times Time^{-1}$) is a proportionality constant, depicting a basic flux independent of temperature; T is the temperature measured in Kelvin (K); k is the Boltzmann's constant ($8.63 \times 10^{-5} \text{ eV K}^{-1}$) and E (eV) is the activation energy, which expresses the strength of the temperature effect on the metabolic rate v . The temperature dependence of respiration is characterized by the kinetics of ATP synthesis in the respiratory complex with an activation energy of ~ 0.65 eV (Gillooly, 2001). The temperature dependence of light saturated photosynthesis is controlled by Rubisco carboxylation in chloroplasts with an activation energy of ~ 0.32 eV, determined for C3 terrestrial plants (Bernacchi et al., 2001). Strictly speaking, the temperature dependence of photosynthesis is more complicated, since functional curves of photosynthetic rates against temperature commonly have an optimal temperature (Farquhar et al., 1980; Allen et al., 2005). Likewise, enzyme degeneration at high temperatures limits the range of exponential rate increase in the respiratory chain. The approximation, described by the Arrhenius law, is commonly accepted to be valid in

the temperature range of 0 – 30 °C (Allen et al. 2005).

On the level of individual organisms the density of metabolic units per unit of mass (M), p_i (MU g C⁻¹), is modelled as a power-law relationship, originating from Kleiber's $\frac{3}{4}$ power law. The quarter-power scaling reflects fundamental constraints on the number of metabolic units that can be supplied with energy and materials through a fractal-like distribution network (West, 1997):

$$p_i = p_0 M^{-\frac{1}{4}} \quad (1.2)$$

where p_0 (MU g C^{-3/4}) is a proportionality constant independent of mass and M has the unit of g C^{-1/4}.

The metabolic rate Q_i (g C Time⁻¹) of an organism i in dependence of its mass and temperature is then given by the combined Arrhenius- and Kleiber laws:

$$Q_i = \underbrace{p_0 v_0}_{q_0} \exp\left(\frac{-E}{k \cdot T}\right) M^{\frac{3}{4}} \quad (1.3)$$

where q_0 has the unit of g C^{1/4} Time⁻¹ and M the unit of g C^{3/4}.

On the ecosystem level, metabolic rates per unit Area (A), Q_{eco} (g C Area⁻¹ Time⁻¹), i.e. GPP and ER, are determined by the sum of all metabolic rates of all constituent individuals $i = [1, J]$ of the ecosystem :

$$\begin{aligned} Q_{eco} &= \frac{1}{A} \sum_i^J Q_i \\ &= \frac{1}{A} q_0 \exp\left(\frac{-E}{k \cdot T}\right) \sum_i^J M_i^{\frac{3}{4}} \end{aligned} \quad (1.4)$$

Hence, at the ecosystem level, the absolute metabolic rates depend on the density of metabolic units which are determined by the size and abundance distribution of the constituent organisms. The physiological temperature dependence, however, is still controlled by the biochemical rates, i.e. by Rubisco carboxylation for light saturated photosynthesis and ATP synthesis for respiration. From this follows that based on MTE we expect, also on the ecosystem level, a higher physiological temperature sensitivity of ER than GPP, implying that autotrophic systems have the potential to switch from autotrophy to heterotrophy if ER is unconstrained from GPP. Heterotrophy in already heterotrophic systems may increase. This prediction might have important consequences for the role of lakes in regional and global carbon cycles since they not only have often considerable carbon stores in the sediment (Anderson et al., 2014) but receive also additional organic carbon inputs from their catchment. This makes it likely that ER is unconstrained by GPP in the non-equilibrium state. The carbon stores, as well as the

allochthonous carbon from the catchment are, however, mainly recalcitrant, and it is unclear how much they are available for biochemical degradation. Yet, evidence suggests that recalcitrant dissolved organic carbon (DOC) can become increasingly available as energy source at higher temperatures (Jenkinson et al., 1991; Gudas et al., 2010; Ylla et al., 2012). Predictions made by MTE regarding the physiological temperature dependence of metabolic rates and the increasing imbalance between GPP and ER, have been confirmed for aquatic ecosystems in mesocosm experiments (Yvon-Durocher et al., 2010a,b), Icelandic river systems with natural temperature differences (Demars et al., 2011; Perkins et al., 2012) and epipelagic oceans (López-Urrutia et al., 2006). Yet, single systems can have the potential for a wide variance in temperature dependence (De Castro and Gaedke, 2008; Dell et al., 2011) or in some cases experiments even failed to confirm a significant temperature dependence of metabolic rates at all (Davidson et al., 2015). Deviation from physiological temperature dependence could be caused by: interactions with (Cross et al., 2015); limitation by (Staehr and Sand-Jensen, 2006; Kirk, 2010; Huryn et al., 2014) or covariation with (Huryn et al., 2014; Welter et al., 2015) other driving variables or processes. Failures to confirm temperature effects could be caused by stronger signals from more dominant driving variables, overriding the weaker temperature effect as was concluded by Davidson et al. (2015), who compared eutrophication and climate warming effects on metabolic rates and gas fluxes. Moreover, the influence of acclimation and adaptation on the level of individuals, species, or communities on metabolic rates is still under research (Atkin and Tjoelker, 2003; Perkins et al., 2012; Wilken et al., 2013). Important drivers for metabolic rates of shallow lake ecosystems are light, nutrients and OC. Interaction with temperature or limitation by one of those factors could either affect the physiological temperature dependence on the single cell level (Davidson et al., 2012) or the apparent temperature dependence on the ecosystem level (Huryn et al., 2014; Welter et al., 2015). Thus, MTE is a powerful framework to investigate temperature effects on metabolic rates, but missing inclusion of limiting resources poses, a potentially serious short-coming for its use to assess global change effects on metabolic rates. Hence, the interplay between temperature and other important drivers remains an active field of research (Anderson-Teixeira and Vitous, 2012; Huryn et al., 2014; Welter et al., 2015; Cross et al., 2015). In **Chapter III** of this thesis I present a case study where we investigated the combined effect of temperature, nutrient and water level change, with its influence on light availability, for shallow lake ecosystems based on a pan-European mesocosm experiment.

1.2 Global change pressure, threshold determination and early warnings

At all times ecosystems were forced to adapt to changing conditions in the Earth system, caused either by external forcing like changes in solar radiation (e.g. Milankovitch cycles), impacts by meteorites or by internal forcing like fluctuations in ocean circulation, volcanic activity or even changes in the biosphere itself. The Holocene, known for its relative stable climate conditions,

which are thought to be the vital framework for the development of human civilisation, has seen, yet, the rise of an other forcing, humanity itself. Throughout their history human activity always influenced their local or regional environment, industrialisation and accompanying massive population increase in the last 100 years, however, lead to an unprecedented human impact on ecosystems. The resulting simultaneous change in several control variables of important Earth system processes is also referred to as global change pressure (Steffen, 2005; Steffen et al., 2007). In the 'planetary boundary' framework Rockström et al. (2009) and Steffen et al. (2015) identified several of such Earth system processes and their associated control variables which are under pressure, including among others: rate of biodiversity loss, chemical pollution, change in land use, freshwater use, interference with the nitrogen and phosphorus cycles and climate change. Furthermore they quantified planetary boundaries as critical values of the control variables, whose crossing might lead to irreversible environmental changes.

Global change pressure affects all ecosystems and lakes are no exception. On the contrary, lakes, since they integrate various influences over a wide spatial scale through their intimate link with their catchment, make particular good sentinels of environmental change (Adrian et al., 2009). Current studies and future prognoses identified climate change (Adrian et al., 2009; Nickus et al., 2010; Jeppesen et al., 2012a) and eutrophication (Vitousek et al., 1997; Carpenter et al., 1998; Smith et al., 1999; Smith, 2003; Jeppesen et al., 2010) as two of the leading threats for lake integrity (Finlayson et al., 2005). Primary components of climate, which is defined as the long-term prevailing weather in an area, are temperature and precipitation (Stevens 2010). Climate is driven by the balance between the energy that the earth receives from sunlight and radiates back to space. This balance, however, is influenced by several forcing mechanisms including greenhouse gases (e.g. CO₂, CH₄, N₂O). The current increase in the atmospheric CO₂ concentrations are with high certainty ascribed to increasing anthropogenic CO₂ release from fossil fuel burning and land use change (Ciais et al., 2014). As a result, global average surface temperature (land and ocean) already underwent a warming trend of 0.85 (0.65 – 1.06) °C, over the period from 1880 – 2012. Predictions from climate models expect a further increase of 0.3 to 1.7 °C for the lowest and 2.6 to 4.8 °C for the highest emission scenario until the end of the 21st century (Stocker et al., 2013). Considering the increase in global average surface temperature it is not surprising that warming trends have also been confirmed for lakes. In an extensive worldwide survey of lakes' summer surface temperatures O'Reilly et al. (2015) found an average warming trend of 0.34 °C per decade for the period 1985 until 2009. Yet, due to interactions between climatic and lake specific drivers (morphometry, winter ice cover) the between-lake variance was huge (-0.7 to 1.3 °C per decade). Contrary to temperature, there is much less confidence about ongoing and future changes in precipitation patterns. A large scale tendency, however, is that spatial contrasts in precipitation patterns are expected to increase and dry areas become drier (e.g. the Mediterranean) and wet areas becoming wetter (e.g. subpolar latitudes). Similar, also the contrast between seasons is expected to increase, including more extreme precipitation

events (IPCC, 2013).

Major source of nutrients to lakes are modern agriculture and urban waste waters (Carpenter et al., 1998; Finlayson et al., 2005; Withers and Haygarth, 2007), and in the light of a growing human population the problem of eutrophication is predicted to increase further (Hurt et al., 2011; Krausmann et al., 2013).

Both, temperature and nutrient availability are key variables and have profound impact on lake functioning on a physical, chemical and biological level, including biogeochemical cycles, community composition, biodiversity and trophic structure (Nickus et al., 2010; Jeppesen et al., 2010). Higher water temperatures have been shown to amplify eutrophication (Jeppesen et al., 2010; Moss, 2011). Stratification, for instance, partly decouples the epilimnion from the hypolimnion of a lake, thus facilitates oxygen depletion of the hypolimnion. Hypolimnetic oxygen depletion could occur, however, faster and be more severe in eutrophic lakes due to higher availability of organic material in the hypolimnion and faster and more complete decomposition at higher temperatures (Jenkinson et al., 1991; Gudas et al., 2010; Ylla et al., 2012). The hypolimnetic oxygen depletion, in turn, can further fuel eutrophication due to phosphorus release from the sediments (Carpenter et al., 1999; Søndergaard et al., 2003; Hupfer and Lewandowski, 2008). On community level prolonged stratification in combination with high nutrient availability has been shown to promote cyanobacteria dominance (Wagner and Adrian, 2009a). Further, high nutrient environments as well as warmer waters tend to have fish communities dominated by omnivorous small fish. This leads to an increased top-down control of zooplankton and consequently to a decreased grazing pressure on algae, also stimulating algae and, in particular, cyanobacteria dominance (Jeppesen et al., 1997; Scheffer, 2004). Scenarios like this are coherent with findings from space for time studies indicating that regime shifts from clear water to turbid state occur at lower nutrient concentrations at higher water temperature (Kosten et al., 2009). In contrast, as discussed in the previous section, while higher temperature is expected to decrease net ecosystem production ($NEP = GPP - ER$), high nutrient availability is expected to increase it.

Precipitation, on the other hand, regulates water levels, but also the transport of nutrients from the catchment. Thus, increased and extreme precipitation can boost eutrophication by increased nutrient run-off from the catchment, and at the same time negatively affect light availability under water due to higher water levels and dissolved organic carbon loads from the catchment (Jeppesen et al., 2009, 2011). Moreover, decreased precipitation particular in combination with increased evaporation and water abstraction for irrigation, can severely reduce lake water levels and even lead to their temporary or permanent desiccation. While under low precipitation nutrient and DOC run off from the catchment are limited, nutrient and in addition salts can concentrate in the remaining water, while lower water levels can mitigate adverse effects on the light climate (Bucak et al., 2012; Özcan et al., 2010). Thus, increased temperatures, rising nutrient levels and changes in precipitation patterns interact in complex ways at several levels of lakes. It is expected that these factors mutually intensify their effect on many lake processes (Moss, 2010,

2011); variability and uncertainty is, however, still high (Jeppesen et al., 2010).

The simultaneous changes in multiple key driving parameters and their potential interaction with particular lake characteristics, but also among each other, have severe implications for threshold driven changes and threshold determination. Under these circumstances thresholds might be highly system specific and thresholds for particular lake categories with similar stability properties might be difficult to establish due to the enormous data requirement of such an undertaking. Thus, the stability properties found in one system might not be generally applicable to other systems (Scheffer, 2009).

Returning to the framework of dynamical systems this can be easily understood, since the position of critical bifurcation points always depends on all driving parameters in a dynamic model. In addition, not only the location of the bifurcation point might change in dependence of other driving parameters, but also the stability landscape and thus the transition type itself. In a two-parameter model, for example, the bifurcation points of two saddle-node bifurcation can move gradually closer together in dependence of the second parameter, indicating a diminishing influence of the involved feedback mechanisms. The bifurcation points eventually merge and disappear in a so called cusp bifurcation (co-dimension-2 points). Thus the cusp bifurcation point marks the transition from a system with a potentially discontinuous to a system with potentially abrupt regime shift (Figure 1.1). Changing the second parameter even further, might finally induce smooth transitions. Hence, the same dynamic system can in theory exhibit all three types of regime shifts.

Even so the determination of thresholds might be of high theoretical value and of great practical importance for the management of well-studied systems, availability of more generic indicators notifying in good time impending sudden transitions are desirable. Theoretically such early warning indicators (EWI), independent of the precise mechanisms involved, exist due to some generic changes in the stability landscape around an equilibrium approaching a critical bifurcation. To understand the generic nature of EWIs stability landscapes are best envisioned with the famous ball cup analogy, where the ball depicts the state of the system and the bottom of the cup correlates to the equilibrium. The width of the cup corresponds to the basin of attraction around the equilibrium and the steepness of the cup to the rate of change of the system and thus the rate with which the system returns to the equilibrium after a small perturbation. For a system far from bifurcation this cup is deep and steep, indicating high resilience and only very large perturbation could move it into an alternative state. In response to random small perturbations the system returns quickly to its equilibrium. The more the system approaches the bifurcation the shallower this cup becomes indicating increasingly slower return times to its equilibrium (critical slowing down) and reduced resilience (Scheffer, 2009). Since natural systems are constantly exposed to small perturbations the nature of the fluctuations around the equilibrium should reflect those changes in the stability landscape. Due to critical slowing down the system spends more and more time on its basin boundaries after perturbation and the recovery time increases.

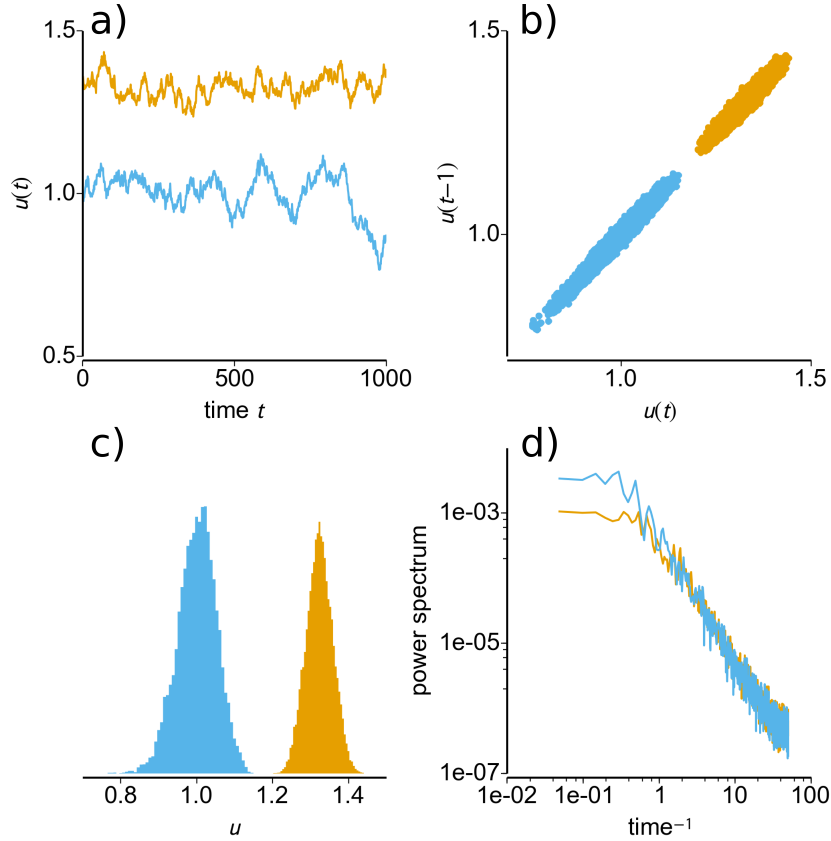


Figure 1.3: Four generic early-warning indicators assessed on a equilibrium point far from (yellow) and close to a critical transition (blue; see Figure 1.1 a). The statistical properties: a) Variance, b) Lag-1 autocorrelation, c) Skewness and d) Density ratio for time series are expected to increase when the system approaches a critical thresholds and thus experiences critical slowing down.

This behaviour is reflected in certain statistical properties of the resulting time series of the dynamic system, including among others increased autocorrelation, variance, skewness as well as shifts from high to lower frequencies (Dakos et al., 2012a) (Figure 1.3). Thus, trends in those quantities over time could serve as generic EWI. EWIs have been successfully shown to precede discontinuous regime shifts in modelled (Ditlevsen and Johnsen, 2010; Dakos et al., 2012a; Carpenter et al., 2008), experimental (Veraart et al., 2011; Batt et al., 2013b; Dai et al., 2012; Carpenter et al., 2001) and reconstructed paleo-climate time series (Lenton et al., 2012; Dakos et al., 2008). Yet, a test of their potential and reliability based on data collected with standard monitoring schemes is still missing. However in practise, only this type of data is of relevance for timely detection of impending discontinuous regime shifts in ecosystems. To fill this gap I present in **Chapter IV**, the first comprehensive assessment of EWIs based on long-term observational data from five lake ecosystems.

1.3 Linking theory and empirical data

Ecosystems consist of both biotic and abiotic components. While both components are governed by the fundamental laws of physics and chemistry, the biotic components are additionally

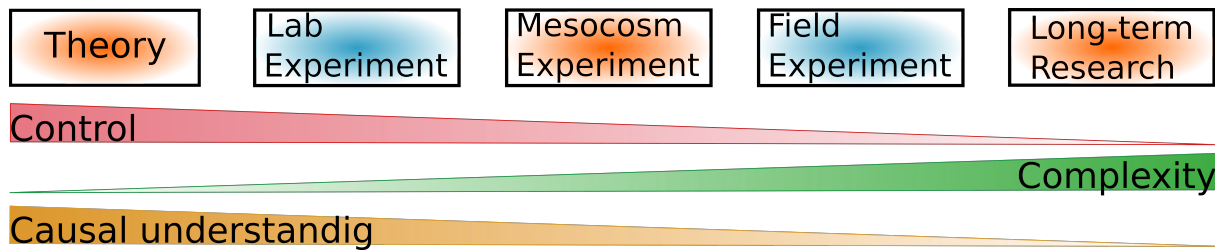


Figure 1.4: Schematic drawing of the five tools in the scientific toolbox which are available to build up knowledge about ecosystems. Each of the five tools has strengths and weaknesses due to their inherent trade-off between control, causal understanding and complexity. While theory provides high control and formulates causal understanding, long-term research captures the large complexity of a natural ecosystem but detective work is needed to identify the 'experimental conditions'.

characterised by diversity and complexity of interaction (National Research Council (U.S.) and Committee on Research at the Intersection of the Physical and Life Sciences, 2010). The complexity of biological systems arises amongst others from their hierarchical organization, whereby each level of integration is characterised by many interactions among the constituent parts, leading to emergent properties at each level of organisation. Thus, even complete knowledge of the properties of the parts can only give a partial explanation of the system as a whole (Mayr, 2004; Schellnhuber et al., 2014). The level of the entire ecosystem represents not only the highest level of integration for biological systems, but is also characterised by strong interactions between abiotic and biotic components. This complexity makes it a challenging task to gain causal understanding of ecosystem functioning, not least because of chaotic behaviour and computational irreducibility (Huisman and Weissing, 2001; Beckage et al., 2011). In the light of global change pressure, however, causal knowledge on how specific drivers change lake ecosystem composition and function is absolutely essential. Only such knowledge allows us to predict the effect of changes in drivers and supports the prevention of potential adverse effects or at least their mitigation. For the analysis of ecosystems various scientific tools are at hand, including theoretical approaches, laboratory-, mesocosm- and field-experiments, as well as long-term research. Albeit each of those tools has its particular possibilities but also shortcomings. There exists an inherent trade-off between the degree of control and immediate causal understanding a method provides and the degree of complexity of the ecosystem it considers (Figure 1.4). Theoretical approaches are located at the end of highest control and immediate causal understanding, including the formulation of laws, concepts, but also process based models. Theoretical approaches are concerned with the direct formulation of causal relations based on first principles and current knowledge; often in the language of mathematics. Based on theoretical approaches we attempt to explain as much of the observed facts as possible. However, in order to achieve this, we need to abstract from the large diversity, variation and uniqueness which characterises biological systems and focus on particular aspects of the system. Thus, simplification and reduction of the system is inevitable.

Opposed to theoretical approaches, long-term ecological research captures the development

of an ecosystem under the full complexity of its abiotic and biotic interactions. Importantly, long-term research is unique in its capability to allow the detection of long-term trends. Other than in theory or experiments long-term ecological research requires detective skills to identify the ‘experimental conditions’ under which the observed pattern have developed (Figure 1.4). As the analysis of long-term empirical data is bound to correlational relationships only approximate causality can be identified. In between theory and long-term research the experiment is located. Experiments allow hypothesis testing within a varying degree of control and complexity of the system.

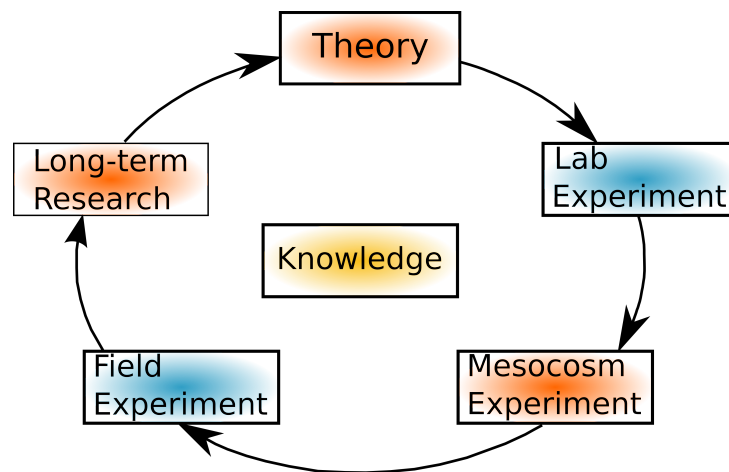


Figure 1.5: Schematic drawing on how all five tools, available in the scientific toolbox should mutually inform each other to reach the ultimate aim of ecosystem science, i.e. to gain causal knowledge. Integrating the five tools in this view, might bridge existing reluctance between representatives of either approach to acknowledge the usefulness of the respective other tools.

Between the ends of the need for causal understanding and the complexity of biological systems on the one hand and the inherent trade-off between control, causality and complexity in scientific methods on the other hand (Figure 1.4), there is a tendency between the representatives of either approach to doubt the usefulness of the respective other approaches (Whitfield, 2004; Fussmann, 2008; Clarke, 2004). This reluctance between the different approaches for the analysis of ecosystems functioning can be bridged, if one acknowledges, that scientific knowledge is the collection of theories, laws and, in particular in biology, of concepts supported by the scientific consensus. This consensus is the result of many tests, including accurate predictions e.g. based on theory, the replication of the most important experiments by multiple independent groups and the thus growing conviction that new evidence (Mayr, 2004) or falsification (Popper, 1963) is unlikely to alter those theories, laws or concepts substantially. From this definition it gets immediately clear that scientific knowledge and the growth of it, is precisely achievable by reiterating the diverse set of methods (Figure 1.5). Thus, observations from long-term research and results from experiments can confirm and inform theoretical concepts but can also stimulate their improvement. By modifying theory or by developing new theories we may then be able to capture more of the complexity of the empirical evidence - a route rarely taken. However,

testing model outputs with long-term observational or experimental data has gained impetus since modelling is an important tool to project responses toward global environmental change. Theoretical concepts, on the other hand, are capable to provide valuable frameworks to derive new hypothesis and facilitate and substantiate the interpretation of the complex patterns found in long-term observational data. Data analysis in the context of current theory also stimulates cross-disciplinary interactions, facilitates the use of a common nomenclature and consistent definitions and thus aids to the coherent development of a research field. In this way the available scientific methods can mutually inform each other and complement the strength and weaknesses of each other, to gradually improve and broaden scientific knowledge.

1.4 Objectives and outline of this thesis

Ecosystem theory predicts the existence of threshold driven changes for both ecosystem state variables (Scheffer, 2009) but also flux balances (Brown et al., 2004; Yvon-Durocher et al., 2010b). The global environmental change in key abiotic driving forces is expected to increase the probability that such thresholds may be crossed (Finlayson et al., 2005). As a consequence, fundamental changes in ecosystems which affect their functioning and hence the ecosystem services they provide can be expected (Rockström et al., 2009). This might be particular true for lakes, since they integrate changes from their catchment leading to their particular vulnerability. As such lakes make good sentinels of environmental change (Adrian et al., 2009). This makes it important to better understand the actual relevance of threshold-driven changes for lake ecosystems, identify the mechanisms causing them and develop ways to predict such changes. Long-term ecological research is a promising avenue towards projections, but data driven proof of theory is still rare.

In the present thesis I investigated on the level of communities (**Chapter II**) and whole ecosystems (**Chapter III**) threshold-driven changes in lakes which were simultaneously affected by changing climate and nutrient status. I further accessed the potential to predict discontinuous regime shifts based on generic early warning signals (**Chapter IV**). The investigations were based on long-term observational data (**Chapter II** and **IV**) or experimental data from a pan European mesocosm experiment (**Chapter III**). A central theme of my thesis is the close link between data analysis and current ecosystem theory. I thereby contribute to filling the gap between theoretical frameworks of threshold driven processes and their relevance in lake ecosystems. Importantly the studies contribute to the further development of ecological theory.

In **chapter II** I investigated the mechanisms leading to an observed dominance shift between two coexisting copepod species *Cyclops kolensis* and *Cyclops vicinus* in shallow lake Müggelsee (Germany). In this analysis we link regime-shift theory on the level of population ecology with observations from 30 years of long-term observational data, demonstrating how environmental change can fundamentally restructure the community composition in a shallow lake due to a

sudden large change in a state variable. While the abundance of *C. vicinus* mainly declined gradually over the study period, a sudden large increase in abundance changed the status of *C. kolensis* from a rare to the most dominant copepod species in the winter community of lake Müggelsee. In the period of the study, lake Müggelsee not only improved in trophic status with a subsequent decline in algal food, but also experienced a significant increase in average surface water temperatures. *C. vicinus* and *C. kolensis* are linked in an intraguild predation food web. Intraguild predation models predict the coexistence of a predator (*C. vicinus*) and a consumer (*C. kolensis*) at intermediate productivity levels of the environment, if the consumer is superior in exploitative competition for the common resource, which is the case for *C. kolensis*. In highly productive systems the competitive exclusion of the consumer is expected. In addition the transition between the consumer-predator equilibrium and predator equilibrium is marked by a transition zone with alternative stable states. Combining the predictions from intraguild predation theory with correlation analysis and food niche partitioning was successful in determining the nature of the changes of the two copepod species, the identification of the major drivers causing the abrupt changes, along with the quantification of the critical threshold of *C. vicinus* abundance to release *C. kolensis* from predation pressure. This study is an example where regime shift theory along with models of intraguild predation theory facilitated the data driven identification of the mechanisms underlying sudden abrupt changes as observed in long-term observational data. At the same time, we build a successful case for the relevance of those concepts in shallow lake ecosystems.

In **Chapter III** I investigated the combined effects of temperature, nutrient status and changes in water level on shallow lake's gross primary production (GPP) and ecosystem respiration (ER) as well as the balance between them. In this analysis we compared findings from a pan-European space-for-time mesocosm experiment with predictions made in the theoretical framework of Metabolic Theory of Ecology (MTE). Due to the strong linkage between experiment and MTE we not only had a valuable framework to derive hypothesis and test our results against, but could also identify explicit short-comings and formulate needed improvements of the MTE.

Due to stored or allochthonous carbon sources ER of lakes is not necessarily limited by its GPP. In this situation MTE predicts that with increasing water temperature lakes might become increasingly heterotrophic or even switch from net autotrophy to net heterotrophy, due to the higher physiological temperature sensitivity of respiration compared to photosynthesis. Contrary, increased nutrient availability, has been found to benefit GPP more than ER and thus has been shown to be accompanied by increasing net ecosystem production ($NEP = GPP - ER$). Yet, the nutrient status of ecosystems is not explicitly considered in the MTE. Moreover, even so water level has profound effects on the light climate and mixing depth in shallow lakes, little is known, how it effects metabolic rates and their balance. To increase our understanding on the combined effects of temperature, nutrients and water level on metabolic rates we conducted a pan-European space-for-time mesocosm experiment which ran from May until November in six lakes, covering

a temperature gradient from Sweden to Greece. The experiment comprised two nutrient levels (mesotrophic or eutrophic) crossed with two water levels (1 and 2 m).

This study is an example where the combination of theory and large scale experiments was successful in the quantification of trophic state specific temperature thresholds for the switch from one regime (autotrophy) to another (heterotrophy) and by that pointing to the need to include nutrients into the framework of MTE.

In **Chapter IV**, I assessed the reliability of early-warning indicator (EWIs) to signal critical transitions in long-term observational data. The simultaneous change in key driving variables might increase the probability for discontinuous regime shifts. The determination of thresholds in a multi-parameter space is, however, challenging and most likely highly system specific. Yet, the timely anticipation of an impending discontinuous regime shift and its potential prevention is highly desirable, since the reversal of the shift can be difficult and costly or even impossible due to hysteresis. EWIs are derived from bifurcation theory as generic characteristics caused by the loss of resilience of a complex dynamic system prior to a discontinuous regime shift. So far EWIs have been shown to precede discontinuous regime shifts in modelled, experimental, reconstructed paleo-climate time series and whole-lake experiments. Up to now, however, an assessment of the generality and the detection power of EWIs on long-term observational data in aquatic systems is lacking. To fill this gap we tested four commonly used EWIs (increase in autocorrelation at lag-1, standard deviation, density ratio and skewness) on long-term observational data of five lake ecosystems that have experienced a critical transition and for which the relevant ecological mechanisms and drivers are known. Based on these data we assessed the reliability of the EWIs to signal the regime shift, their consistency among each other, their sensitivity to computational decisions (window size), their significance and finally how much in advance they are able to signal the impending transition. We further tested if inconsistency in the detection of the four EWIs can be linked to either the regime shift generation mechanisms, the state variable category (species, group, ecosystem), the magnitude of the change, the length of the pre-breakpoint time series or the sampling interval.

This study is an example where intuitive expectations implied by a concept such as the EWIs do not match up with observational data. Other than intuitively implicated, EWIs can, with the current knowledge, not be applied to just any time series of sufficient length for statistical analysis but only to case studies where expert knowledge is extensive; i.e. knowing that the case study at hand has experienced a critical transition for which the relevant mechanisms and drivers are known. By disclosing those critical shortcomings of the current concept of EWIs we identified important future research needs to advance the concept of EWIs – which would be otherwise at stake to not fulfil expectations implied by the concept.

Chapter 2

Threshold-driven shifts in two copepod species: Testing ecological theory with observational data

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Chapter 3

Effects of trophic status, water level and temperature on shallow lake metabolism and metabolic balance: A standardised pan-European mesocosm experiment

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3.1 Abstract

The balance between gross primary production (GPP) and ecosystem respiration (ER) determines the metabolic status of lakes. As an integrative quantity, the metabolic status is an important indicator of lake function and can have a decisive influence on the role of lakes in regional and global matter cycling. Lake metabolism is influenced by environmental conditions such as light, mixing depth, nutrients and temperature – drivers predicted to be affected by climate change. To improve our understanding of these driving factors on the metabolic status of shallow lakes and their role in the carbon cycle, a pan-European space-for-time mesocosm experiment was conducted from May until November 2011 in six lakes, covering a temperature gradient from Sweden to Greece. The experiment comprised two nutrient levels (mesotrophic or eutrophic) crossed with two water levels (1 and 2 m) to simulate different light regimes and mixing depths. In situ GPP and ER were estimated using the O₂ free-water method. GPP and ER were significantly higher in the eutrophic mesocosms than in mesotrophic ones, whereas the shallow mesocosms had significantly higher volumetric metabolic rates but lower area-based metabolic rates than the deep mesocosms. GPP and ER increased exponentially with temperature. Temperature gains of ~0.53 eV for GPP and ~0.65 eV for ER were comparable with those predicted by metabolic theory. All systems switched from autotrophy to heterotrophy over the investigated temperature range. The threshold temperature for the switch in metabolic status was, however, lower under mesotrophic (~16 °C) than eutrophic conditions (~22 °C). Contrary to expectations, no significant interactions between temperature, nutrients and depth were observed for GPP and ER. Overall, we quantified the differential temperature sensitivity of GPP and ER and found that trophic state is crucial for how much warming a system can tolerate before it switches from net auto- to net heterotrophy.

3.2 Introduction

The balance between gross primary production (GPP) and ecosystem respiration (ER) determines the metabolic status of a lake and has a decisive influence on its role in regional and global matter and energy cycles, either as a source or sink of carbon (Andersson and Sobek, 2006; Brothers et al., 2013; Pacheco et al., 2014). Shallow lakes, the most numerous lake type on Earth (Downing et al., 2006), have been recognised as hotspots of carbon turnover (Cole et al., 2007; Tranvik et al., 2009). While oligotrophic lakes with high allochthonous carbon inputs tend to be predominantly net heterotrophic ($GPP < ER$), many eutrophied lakes have been found to be net autotrophic ($GPP > ER$) (del Giorgio and Peters, 1994; Cole et al., 2000; Balmer and Downing, 2011). On daily, weekly and seasonal timescales, however, lakes can switch between net autotrophy and net heterotrophy (Staehr and Sand-Jensen, 2007; Sadro et al., 2011; Coloso et al., 2011; Laas et al., 2012), and the extent of net autotrophy, particularly in spring and summer, may become decisive for the annual metabolic status, especially in temperate lakes (Staehr et al., 2010b; Laas et al., 2012). Autotrophic and heterotrophic metabolic pathways are susceptible to changes in light regime, nutrient status, external organic matter loading and temperature. All these drivers are predicted to be affected by climate change due to alterations in water levels, nutrient cycling and runoff from the catchment (Coops et al., 2003; Tranvik et al., 2009; Jeppesen et al., 2015). Theory and evidence from several studies indicate that the metabolic rates of both photosynthesis and respiration increase with increasing temperatures, where photosynthetic gains are smaller than those for respiration (Gillooly, 2001; López-Urrutia et al., 2006; Yvon-Durocher et al., 2010b). Thus, lakes might become increasingly heterotrophic or even switch from net autotrophy to net heterotrophy in a warmer world if stored or allochthonous carbon sources are or become available (Yvon-Durocher et al., 2010a; Laas et al., 2012). A shift towards heterotrophy implies a reduction in the carbon sequestration capacity or even loss of this important ecosystem service as a carbon sink, if this is not off-set by sedimentation rates.

The metabolic theory of ecology (MTE) provides a theoretical basis for investigating temperature dependence of metabolic rates (Brown et al., 2004). Its deductive framework allows scaling of metabolic rates from individual biochemical reactions to the level of intracellular metabolic units (chloroplasts and respiratory complexes) to whole organisms (Gillooly, 2001; Brown et al., 2004) and ecosystems (Enquist et al., 2003; Allen et al., 2005; Yvon-Durocher et al., 2010b). At the ecosystem level, the temperature-independent, absolute metabolic rate is mainly determined by the size and abundance of the constituting organisms per unit area or volume. The physiological temperature dependence of the metabolic rate is, on the other hand, governed by rate-limiting processes at the biochemical level and maintained over the organisational levels (Bernacchi et al., 2001; Gillooly, 2001; Allen et al., 2005). The physiological temperature dependence of metabolic rates is approximated by the Arrhenius equation. For non-limiting conditions, MTE assumes activation energies of ~ 0.3 eV (photosynthesis) and ~ 0.65 eV (respiration) and hence predicts a shift towards heterotrophy with increasing temperatures. The temperature at which a

net autotrophic system switches to net heterotrophy, however, depends both on the difference in the temperature response of GPP and ER, and the ratio of the absolute GPP and ER rates. Theoretically, the further GPP exceeds ER at a given reference temperature, the more extreme are the temperature increases that can be buffered before ER outpaces GPP.

Since temperature together with eutrophication are regarded as the two major stressors for lake ecosystems, multiple studies document their effects on GPP, ER and the balance between them (see for example (Kosten et al., 2010; Yvon-Durocher et al., 2010a; Solomon et al., 2013)). Several of these studies have confirmed the occurrence of positive effects of temperature on ER and GPP but negative effects on net ecosystem production ($NEP = GPP - ER$). Moreover, there is a general agreement that elevated nutrient concentrations promote metabolic rates but have greater impact on GPP than ER. Thus, increased nutrient availability has been shown to be accompanied by increased NEP or decreased ER:GPP ratios (del Giorgio and Peters, 1994; Hanson et al., 2003; Duarte et al., 2004; Staehr et al., 2010b) as well as a stronger coupling between ER and GPP in oligotrophic than in eutrophic lakes (Solomon et al., 2013). Yet, the interacting effects of temperature and trophic state on ecosystem metabolism within the context of already observed and predicted changes in lake water levels with global warming are not well understood (Anderson-Teixeira and Vitous, 2012; Cross et al., 2015; Welter et al., 2015).

A further complicating factor is that changes in water level affect the light conditions. A reduction in water level could thus promote benthic primary production and at the same time lead to increased relative importance of benthic respiratory processes. Furthermore, shallow lakes are most of the time fully mixed; in this way, the whole water column is part of the mixed layer where most of the production is assumed to take place (Staehr et al., 2010b). Under these conditions, a reduction in water level and thus a reduction in mixing depth imply that even if metabolism remains constant on a volumetric basis, area-based rates would decrease, thereby reducing the contribution of the waterbody to the regional and global carbon budget.

It is particularly unclear how temperature-nutrient and temperature-light interactions, the factors that we focus on here, affect the observed activation energies at ecosystem level, resulting in deviations from the canonical activation energies predicted by MTE. Yet, obtaining an understanding of this seems essential since changes in differential temperature sensitivities of ER and GPP affect their balance and, thereby, the threshold temperature at which a shallow lake switches from autotrophy to heterotrophy. Models combining Arrhenius and Michaelis-Menten kinetics show, for instance, that substrate limitation and trophic structure can influence the intrinsic temperature sensitivity (Davidson et al., 2012, 2015). In addition, resource-temperature interactions are known for photosynthesis-irradiance relations where both the maximum rate and the half saturation constant have been found to increase with temperature (Kirk, 2010). Temperature-dependent increases in the photosynthetic rate, however, might be dampened if phosphorus is limiting (Wykoff et al., 1998; Kirk, 2010). In accordance with this, (Staehr and Sand-Jensen, 2006) found a reduced metabolic response of a natural algae assembly to increased temperatures under

nutrient-limiting conditions. Yet, results from laboratory experiments are difficult to scale to ecosystem level at which temperature-driven processes, which in turn influence metabolic rates, or covariates driven by the same seasonal forces as temperature, might influence the observed temperature sensitivity (i.e. “apparent” or “effective” activation energies (Cross et al., 2015). Such processes would, for example, include accelerated nutrient re-cycling or temperature dependence of N₂ fixation, hence increased nutrient availability (Anderson-Teixeira and Vitous, 2012; Welter et al., 2015), reduction in water level, leading to improved light conditions and acclimation and adaptation at species or community level (Atkin and Tjoelker, 2003).

This study aims to improve the understanding of the combined effects of water temperature, water level and nutrient status on metabolic rates in shallow lake ecosystems, as based on a space-for-time substitution experiment across Europe. A synchronised mesocosm experiment was conducted along a latitudinal gradient from Sweden to Greece, covering a temperature range from 7 – 29 °C between July and November. The mesocosms were set up with local flora and fauna to ensure the presence of communities adapted to the particular climates. We investigated temperature effects on GPP, ER and the ratio of ER:GPP under eutrophic and mesotrophic nutrient conditions. A treatment with two depth levels simulated different light and mixing depth conditions.

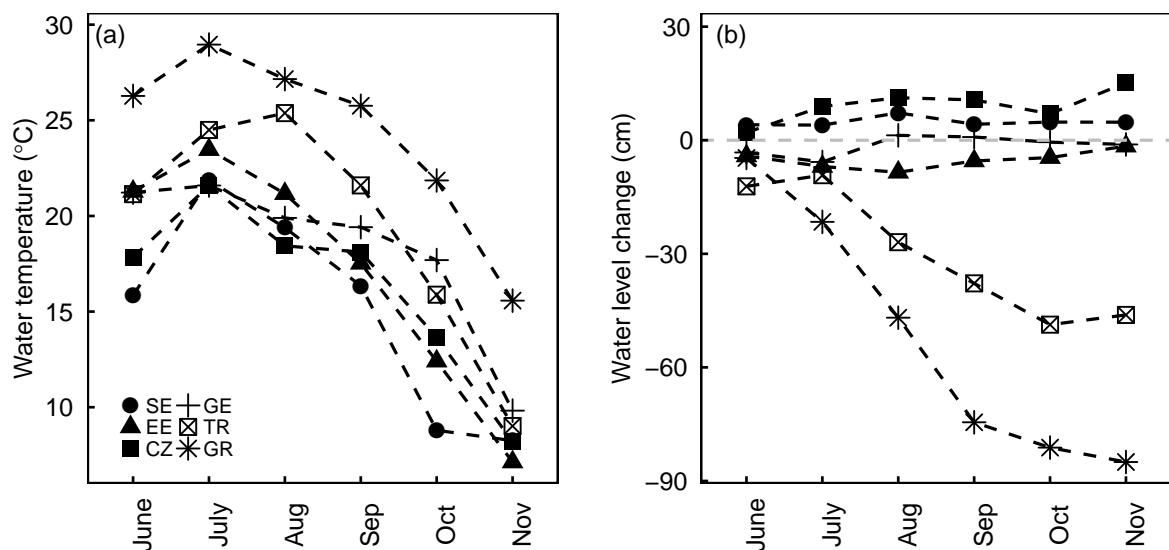


Figure 3.1: a) Country-wise development of water temperature and b) change in water level due to evaporation losses and precipitation gains over the experimental period from June to November.

Both volumetric and area-based metabolic rates were considered. While volumetric rates provide information on the biological process, area-based rates are important for regional and global carbon budgets by integrating the metabolic rate of the actual available water column. We compared our experimental findings with predictions made within the theoretical framework of MTE. We tested the following hypotheses: (1) Both area-based and volumetric metabolic rates are

lower at reduced nutrient availability. Lower water levels, on the other hand, promote volumetric metabolic rates due to higher light availability but simultaneously diminish area-based rates by reducing the water column contributing to production. (2) A shift towards heterotrophy occurs with increasing temperature due to the predicted higher physiological temperature sensitivity of ER compared to GPP. (3) The apparent temperature effect on ER, GPP and the ER:GPP ratio will be modified by light and nutrient interactions with temperature. We anticipate a less pronounced response of GPP to increased temperatures under reduced light and nutrient conditions, while at the same time ER might be either substrate-limited, unaffected or even augmented due to the need to burn excess carbon caused by reduced food quality in low-nutrient environments (increased C:P ratios) (McFeeters and Frost, 2011). (4) Switches from auto- to heterotrophy occur at lower temperature if the magnitude of ER and GPP is similar (NEP reaches towards zero). This situation is expected if lower nutrient and light levels sustain only smaller GPP.

3.3 Material and Methods

Experimental set up and sampling – The mesocosm experiment was conducted simultaneously in six European countries from May until November 2011, encompassing a climate gradient from Sweden to Greece (Table 3.1). The deployed fibreglass mesocosms were all produced by the same manufacturer and had a diameter of 1.2 m and heights of 1.2 or 2.2 m. The mesocosms were set up in lakes to ensure a natural and ambient water temperature regime but were otherwise isolated from the surrounding water. In each country, the experiment involved a 2 x 2 factorial design with 4 replicates and monthly repeated measurements. The first factor comprised two different water levels: 1 m (shallow – S) and 2 m (deep – D) deep mesocosms. The two different depths coincided with different mixing depths since the water in the mesocosms was constantly circulated from bottom to top by standard aquariums pumps, entailing different light conditions (Figure S3.1). Water levels were allowed to fluctuate with precipitation and evaporation. The second factor involved nutrient manipulation to simulate mesotrophic (low – L) and eutrophic (high – H) conditions. Nutrients were adjusted to the two conditions by monthly nutrient addition aiming at initial concentrations after loading of 25 μg phosphate (P) L^{-1} (Na_2HPO_4) and 0.5 mg nitrogen (N) L^{-1} ($\text{Ca}(\text{NO}_3)_2$) in the mesotrophic and 200 μg P L^{-1} and 2 mg N L^{-1} in the eutrophic treatment. The experiment was synchronised by a common protocol to facilitate comparability (Landkildehus et al., 2014).

The mesocosms contained a 10 cm sediment layer of 90% washed sand and 10% natural sediment from a local oligotrophic lake. In order to prevent prolonged internal P loading (low nutrient conditions) or P retention (high nutrient conditions) at the start of the experiment, the sediment was acclimatised to the phosphate concentrations targeted in the experiment for at least two months in the laboratory prior to the experiment. Filtered (500 μm mesh) lake water was used in the mesocosms in all countries except Germany and the Czech Republic. In these two countries, tap water was used as the P level of the lakes exceeded the 25 μg TP L^{-1} threshold

Table 3.1: Location of the experimental sites and average temperatures. Average air temperatures were calculated based on daily average air temperatures of the periods leading up to the 24-hour measurements as defined in Table S1.2. Water temperatures are daily averages based on 24-hour point measurements (Table S1.2).

Experimental site	Coordinates	Altitude (m a.s.l.)	Mean air temperature (°C)			Water temperature (°C)		
			Mean	Min	Max	Mean	Min	Max
Sweden (SE) - Erken	59°49'59"N 18°33'55"E	11	14.5	8.7	18.8	14.9	8.1	22.0
Estonia (EE) - Võrtsjärv	58°12'17"N 26°06'16"E	35	15.1	7.5	19.9	16.3	6.8	24.0
Germany (GE) - Müggelsee	52°26'0"N 13°39'0"E	32	16.0	9.6	18.4	17.7	9.7	21.7
Czech Republic (CZ) - Vodňany	49°09'14"N 14°10'11"E	395	15.2	7.5	18.8	16.0	8.1	22.0
Turkey (TR) - ODTÜ-DSİ Gölet	39°52'38"N 32°46'32"E	998	20.0	10.4	26.2	19.3	8.2	25.6
Greece (GR) - Lysimachia	38°33'40"N 21°22'10"E	16	23.8	15.0	27.9	24.8	15.4	29.1

of the low-nutrient treatment. The initial P and N loadings were adjusted in all high-nutrient mesocosms to obtain the nutrient concentration targeted by the experiment.

Development of a natural flora and fauna, with the potential to adapt to the specific climate and nutrient conditions, was ensured by using an inoculum of plankton and sediment collected from five different local lakes in each country, covering a nutrient gradient from 25 to 200 $\mu\text{g TP L}^{-1}$. Macrophytes (*Myriophyllum spicatum*) and planktivorous fish were added to all mesocosms. Monthly samples were analysed for water chemistry and chlorophyll a in laboratories and on site applying comparable standard methods (see Table S2. 1). Concomitantly, macrophyte biomass was quantified as plant volume inhabited (PVI (%)). Subsequent to each sampling event, a 24-hour measurement of dissolved oxygen and water temperature at a two-hour interval was conducted using a multi-parameter probe (for detailed sampling dates, see Table S1.1). In addition to the 24-hour measurements, at midday light profiles of the water column (10 cm interval) were measured. For details on the set up and sampling, see Landkildehus et al. (2014) .

Data preparation – Data from July and onwards were used, assuming that until then all systems had had enough time to adjust to the experimental manipulation. Seven mesocosms were excluded from the analysis (2 DH, 1 DL and 1 SH mesocosm in Germany and 2 SH and 1 SL mesocosm in the Czech Republic) as they were lost during storm events. The analysis is thus based on five measurements, including data from 89 mesocosms per measurement occasion. In Greece, massive water loss due to evaporation prevented sampling in the shallow mesocosms (Figure 3.1 b), involving light profiles (from September onwards), water chemistry (from October onwards) and the 24-hour measurement in November. Since visual inspection of these shallow mesocosms indicated high light attenuation, we assumed the same high attenuation for the remaining sampling occasions. Light profiles were also missing for August and September for the Estonian mesocosms. Here, the missing attenuation coefficients were linearly interpolated

since none of the attenuation coefficients from the other countries indicated strong seasonality. All data were visually inspected at raw data level and outliers were identified using box-plots. Only extreme outliers (larger than 3 times the interquartile range) were removed from the data (O_2 : 36x (1%) and water temperature: 4x (0.1%)) and replaced by interpolated values. Single gaps in the 24-hour data were substituted by values from a polynomial model of degree 4 of time; for all other data linear interpolation was used. Reported average values, like for example average air temperature, correspond to the sampling periods as listed in Table S1.2.

Meteorological data – For the Swedish site, data on air temperature (AT), air pressure (AP) and global radiation (E) or photosynthetically active radiation (PAR) were provided by the Erken Laboratory Weather Station at Lake Erken, Department of Ecology and Genetics, Uppsala University (AT) and the Swedish Meteorological and Hydrological Institute (AP (measured at Lake Erken), PAR (Norrköping)); for the Estonian site by the Centre of Limnology of the Estonian University of Life Sciences measured at Lake Võrtsjärv; for the German site by the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) measured at Lake Müggelsee; for the Czech site by České Budějovice - Czech Hydrometeorological Institute (AP) and Institute of Hydrobiology (AT, E, (situated at the Římov Reservoir)); for the Turkish site by Turkish State Meteorology Service (Ankara) and for the Greek site by the Hellenic National Meteorological Service. Global radiation was converted to PAR using the transformation given in Kirk (2010).

Estimation of reaeration coefficient (K_{20} (h^{-1})) at 20 °C for O_2 – Gas exchange was measured after the last sampling in late October or early November, when the water temperature was low, to ensure minimal influence from respiration. At each experimental site in two randomly chosen shallow and deep mesocosms, the oxygen concentration was lowered to ca. 30% by bubbling N_2 into the systems under continuous mixing. At nightfall, the oxygen reduction was completed and the oxygen recovery was monitored overnight (reaeration). For each mesocosm included in the gas exchange experiment, a K_{20} value was estimated following Liboriussen et al. (2011). Because of the still relatively high temperatures in Turkey and Greece, an Arrhenius type temperature model was added for those two countries to account for respiration. Two different respiration models ($R_{20\text{ °C}} \cdot 1.047^{(T-20)}$ and $R_{20\text{ °C}} \cdot 1.07^{(T-20)}$) were tested, and finally an average K_{20} value based on both models was calculated. An overall reaeration coefficient was calculated as a mean for all mesocosms, excluding values from the Czech mesocosms because the O_2 reductions were too low to permit proper calculations.

Estimation of primary production (GPP) and ecosystem respiration (ER) rates – Metabolic rates were estimated based on the 24-hour O_2 measurements using the free-water method following Jeppesen et al. (2012b). Since the main focus of our investigation is to analyse the temperature response of the metabolic rate, Arrhenius type corrections based on a-priori Q10 values from the literature were avoided.

To assess the uncertainty of the estimated metabolic rates, we used a bootstrap approach similar

to the one described in [Solomon et al. \(2013\)](#). Estimates with standard errors larger than the estimate itself, and estimates explaining <5% of the variability of the 24-hour dissolved oxygen curve, were excluded from further analysis. Overall, 374 data points remained. For an overview of the distribution of data points per country, month and treatment, see Table [S1.1](#). We derived area-based measures for GPP and ER by multiplication by the actual water column height, which is equal to the mixing depth in our systems. To obtain day length-corrected GPP values, GPP_{dl} ($mg\ L^{-1}\ hd^{-1}$), GPP per day was divided by the average daylight period, LP (hd^{-1}), per month and country. For further details on the estimation of the metabolic rates, see supplement [SI.B](#).

Estimation of light attenuation coefficient, K_d , mean available light and effective light period – For each light profile, an attenuation coefficient, K_d , was estimated based on the Beer-Lambert law. Mean available light over the water column (MAL) was estimated following [Staehr et al. \(2010b\)](#), and effective light period (LP_{eff}), describing the effective light period due to mixing, was calculated following [Shatwell et al. \(2012\)](#). For more details, see supplement [SI.B](#).

Hypotheses generation based on MTE for the expected temperature dependence of the ER:GPP ratio and the temperature thresholds for the switch between autotrophy and heterotrophy – We used the framework of MTE to formally derive the expected temperature dependence of the measured metabolic rates and the ratio between them, against which our experimental findings were tested. Following MTE, the temperature dependence of metabolic rates can be fairly well approximated by the Arrhenius equation within a temperature range of 0 – 30 °C ([Gillooly, 2001](#); [Brown et al., 2004](#); [Allen et al., 2005](#)). At ecosystem level, MTE is formulated as:

$$M(T) = M_0 \exp\left(\frac{-E}{kT}\right) \quad (3.1)$$

where $M(T)$ is the temperature-dependent metabolic rate, M_0 at ecosystem level can be interpreted as the size-dependent basic metabolic flux summed over all autotrophs or heterotrophs, respectively, per unit volume or area ([Allen et al., 2005](#)), E is the activation energy and expresses the strength of the temperature effect on the metabolic rate, k is the Boltzmann constant ($8.62 \times 10^{-5}\ eVK^{-1}$) and T is the absolute temperature in Kelvin. The above formulated temperature effect can be conveniently analysed and plotted with so-called Arrhenius plots which are based on the logarithmised version of equation [3.3](#). In Arrhenius plots, the natural logarithm of the metabolic rate is plotted against the inverse and scaled temperature $1/(kT)$ such that the slope of this linear relationship represents the activation energy and the intercept the absolute metabolic rate of a particular metabolic process. Commonly, the absolute metabolic rate is shifted to a biological meaningful reference temperature (T_c), here following [Demars et al. \(2011\)](#) and [Yvon-Durocher et al. \(2010b\)](#) to 15 °C. Thus, the MTE equation used to analyse the temperature dependence of the metabolic rates reads:

$$\ln M(T) = \ln M_{T_c} - E \frac{1}{k} \left(\frac{1}{T} - \frac{1}{T_c} \right) \quad (3.2)$$

At ecosystem level, resource availability could either affect the absolute metabolic rate or the apparent activation energy, E . To derive the expected temperature effects for the ER:GPP ratio, we assumed, following [Yvon-Durocher et al. \(2010b\)](#), that our systems are in a non-steady state and that ecosystem respiration is mainly driven by heterotrophic metabolism, unconstrained by net primary production. Thus, the temperature-driven change of the ratio between ER and GPP can be expressed in a simplified manner as:

$$\frac{ER}{GPP}(T) = \frac{ER_0}{GPP_0} \exp \left(\frac{E_p - E_r}{kT} \right) \quad (3.3)$$

where ER:GPP(T) is the temperature-dependent metabolic ratio, ER_0 and GPP_0 are the absolute metabolic rates according to the definition of M_0 , and E_p and E_r are the activation energies for GPP and ER, respectively.

Again, the Arrhenius plot can be used to analyse and depict the relation in logarithmic terms:

$$\ln \frac{ER}{GPP}(T) = \ln \frac{ER_{T_c}}{GPP_{T_c}} + \frac{E_p - E_r}{k} \left(\frac{1}{T} - \frac{1}{T_c} \right) \quad (3.4)$$

where GPP_{T_c} and ER_{T_c} are the GPP and ER rate at the reference temperature T_c , and E_p and E_r are the activation energies for GPP and ER, respectively. Equation 3.2 and 3.4 show that metabolic rate-dependent changes in carbon sequestration capacity due to changes in temperature depend solely on the amount of differential temperature sensitivity between ER and GPP, while the actual carbon sequestration capacity also depends on the relative difference between ER and GPP at reference conditions. Even though metabolic theory does not consider the effects of resources, several studies indicate the occurrence of resource-dependent changes in ER:GPP, i.e. that nutrient and light status influence the absolute carbon sequestration capacity.

Based on MTE, we derive an expectation about the temperature at which the switch from auto- to heterotrophy occurs. Formally, this is the point of equality between GPP and ER and is given by:

$$T = \frac{1}{\frac{k \ln \frac{GPP_{T_c}}{ER_{T_c}}}{E_p - E_r} + \frac{1}{T_c}} \quad (3.5)$$

For fixed E_p and E_r , the switch point solely depends on the ratio between GPP_{T_c} and ER_{T_c} , i.e. the smaller the ER_{T_c} relative to GPP_{T_c} , the higher the temperature at which the system switches from auto- to heterotrophy (Figure 3.5b). Thus, assuming resource-dependent absolute ER_{T_c} : GPP_{T_c} values, the switch point from auto- to heterotrophy is expected to be lower in nutrient-reduced environments.

Statistical analysis – All statistical analyses were conducted using R version 3.1.3 (R Core Team, 2015). We analysed temperature and treatment effects based on monthly data from all countries and months using linear mixed effect models in the “lme4” package (Bates et al., 2014). Mixed effect regression can accommodate missing data and is robust with unbalanced data as is the case in our experiment (Table S1.1). In addition, since mixed effect models include a mixture of fixed and random effects, they allow control of level-specific effects. The following basic model was applied:

$$\ln M_{c,(s,m),i}^{(DxN)}(T) = \overline{\ln M_{Tc}^{DxN}} + \epsilon_R^c + \epsilon_R^{c,s} + \epsilon_R^{c,m} - \left(\overline{E^{(DxN)}} + \epsilon_E^c + \epsilon_E^{(c,m)} \right) \frac{1}{k(T-T_c)} + \epsilon_{c,(s,m),i} \quad (3.6)$$

where $\ln M_{c,(s,m),i}^{(DxN)}$ and $\epsilon_{c,(s,m),i}$ are the temperature-dependent metabolic rate and associated random error for measurement i , of mesocosm m in month s and country c ; k is the Boltzmann constant and T_c is the reference temperature set to 15 °C as in equation 3.2 and 3.4. $\overline{\ln M_{Tc}^{DxN}}$ and $\overline{E^{(DxN)}}$ are the logarithmic average metabolic rate at T_c and the average apparent activation energy, respectively, for each treatment. For the average metabolic rate at 15 °C, random effects at the level of country (ϵ_R^c), month ($\epsilon_R^{c,s}$) and mesocosm ($\epsilon_R^{c,m}$) were considered, where both month and mesocosm are nested in country. For the average apparent activation energy random effects on the level of country (ϵ_E^c) and mesocosm nested in country ($\epsilon_E^{(c,m)}$) were taken into account. All random effect terms are expected to be normally distributed with mean zero. This is particularly useful to control for the effect that a potential covariation of $\ln M_{Tc}^{DxN}$ with temperature over time might have on the apparent activation energy. Yvon-Durocher et al. (2012) demonstrated that if the level-specific random deviations follow a normal distribution with a mean of zero, the average apparent activation energy, is predicted to approximate the physiological temperature dependence of respiration and photosynthesis. The same basic model as in equation 3.6 was used for the analysis of area-based and volumetric GPP, day length-corrected GPP_{dl}, ER and the ER:GPP ratio. Initially, all fixed effects (nutrient, depth, temperature and all interactions) were used since all terms are connected with our research hypotheses. In a model selection process, however, we reduced the random and fixed effect structure, if justified by a stepwise backward elimination process as implemented in the step function of the “lmerTest” package (Kuznetsova et al., 2014). The fixed effects of the reduced model included as minimum the main effects of depth, nutrient and temperature since we – apart from significance – sought to elucidate effect size and conduct comparisons between estimated and predicted values based on MTE. Model parameters in terms of standard error, t-value and p-value were estimated using the summary function of the package “lmerTest” (Kuznetsova et al., 2014). Effect sizes for all predictors were calculated using standardised predictors following the recommendation of Gelman (2008). Standard errors for the activation energies and intercepts were estimated using the R function “InteractionMeans” of the “phia” package (De Rosario-Martinez, 2015) and the

“ref.grid” function of the “lsmeans” package (Lenth and Maxime, 2015). Model validation was carried out based on graphical inspection of the distribution of the Pearson residuals and plots of those residuals against all predictors. The model fit was assessed by conditional and marginal coefficients of determination (R function “r.squaredGLMM” of package “MuMIn” (Bartoń, 2015)), and while the former captures the variance explained by the fixed effects only, the latter includes both the variance explained by fixed and by random effects.

To determine potential covariation of other parameters (TP, TN, K_d , MAL, LP_{eff} , Chl a , PVI) with temperature, depth and nutrient levels, we used the same basic mixed effects model as given in equation 3.6; instead of the scaled inverse water temperature, however, water temperature centred to 15 °C was used. Box-Cox transformation was applied for the dependent variable to meet model assumptions (R function “boxcox” of the “MASS” package (Ripley et al., 2015)). Model selection and validation were done as described above. Factor covariate interaction was probed using two-tailed t tests for pairwise comparisons of least-square-means over the temperature gradients in intervals of one degree. The tests were conducted using the standard settings of the “lsmeans” package (Lenth and Maxime, 2015), i.e. p-values were adjusted for multiple testing based on the Tukey method and the significance level was set to 0.05. The models were then used to calculate treatment-wise adjusted means (R function “InteractionMeans” of the “phia” package (De Rosario-Martinez, 2015)) as well as the direction and amount of average change between the lower (7 °C) and upper (29 °C) end of the temperature gradient.

To assess the differential influence of potential important covariates like MAL, LP_{eff} , daylight length, chlorophyll a and PVI and inverse and scaled water temperature on GPP, ER and the ER:GPP ratio, semipartial Spearman correlation was used (R function “spcor” of the package “ppcor” (Kim, 2012)). Semi-partial Spearman correlation coefficient r and percentile 95% confidence intervals were bootstrapped over mesocosms (R function “boot” and “boot.ci” of the “boot” package (Canty and Brian, 2015)). To further disentangle the influence of different day lengths on GPP, we analysed day length-corrected GPP (Allen et al., 2005).

Assessment and interpretation of the variability in absolute metabolism at 15 °C, as well as temperature sensitivity among countries and months, which might be systematically influenced by acclimatisation and adaptation processes, were only possible in a cautious and limited way since the data set is highly unbalanced for the factors of month and country (Table S1.1). The analysis and results from country- and month-wise mixed effect regression can be found in the supplementary material S1.D.

3.4 Results

Water temperatures revealed a distinct seasonal pattern in all countries. In the period July to November 2011, the temperature ranged between 6.8 to 29.1 °C (all countries included). The

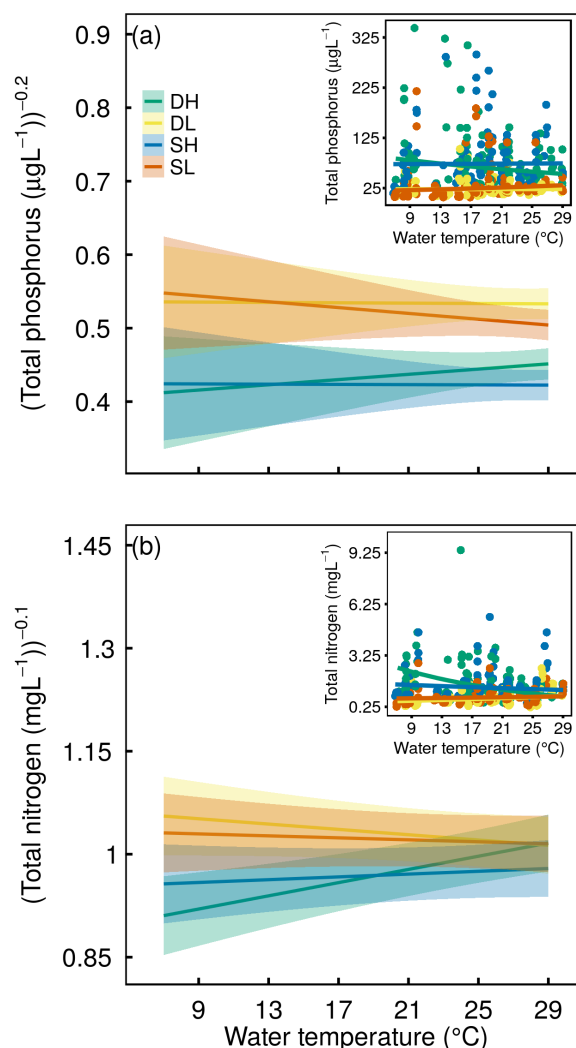


Figure 3.2: Co-variation of nutrients with water temperature. Treatment-wise co-variation of monthly a) total phosphorus (TP) and b) total nitrogen (TN) levels with water temperature. Main images show least-square means of the treatment factors over the temperature gradient with 95% confidence intervals at the scale of the transformed variable. The inserts depict TP and TN at original scale with treatment-wise average TP or TN concentrations as estimated by mixed effects regression (Table S3.1). DH = deep high nutrient, SH = shallow high nutrient, DL = deep low nutrient and SL = shallow low nutrient treatment.

highest temperatures occurred in July in Greece and the lowest in November in Estonia (Table 3.1, Figure 3.1 a). The water level decreased drastically in the two southern countries in autumn, while changes were modest overall in the mid- and northern European countries (Figure 3.1b). Water temperatures were highly correlated with monthly mean air temperatures ($r = 0.88$ with a 95% confidence interval of 0.85 – 0.90), confirming that the monthly point measurements in our enclosures represented the overall seasonal temperature conditions (Table 3.1).

The differential monthly loading of phosphate resulted in significantly (<0.05) different TP levels between the high- and low-nutrient mesocosms over the entire temperature gradient (Figure 3.2a, Table S3.1). TN levels between deep eutrophic mesocosms and both low-nutrient treatments, however, lost significance for temperatures above $\sim 25^\circ\text{C}$ (Figure 3.2b, Table S3.1). Light conditions, as measured by mean available light (MAL) and effective light period (LP_{eff}), were

most favourable in the shallow mesotrophic followed by the shallow eutrophic mesocosms, the deep mesotrophic and lastly the deep eutrophic mesocosms (Figure S3.1b, c). Over the entire temperature gradient, MAL differed significantly (<0.05) between all treatments (for DL – SH above 9 °C). Effective light period (LP_{eff}) was significantly shorter in the deep eutrophic mesocosms compared to all other treatments. The deep mesotrophic mesocosm had shorter LP_{eff} compared to the shallow mesocosms for temperatures above 15 °C (SL) and 21 °C (SH), while LP_{eff} values between the shallow mesocosm were never significantly different (Figure S3.1, Table S3.1).

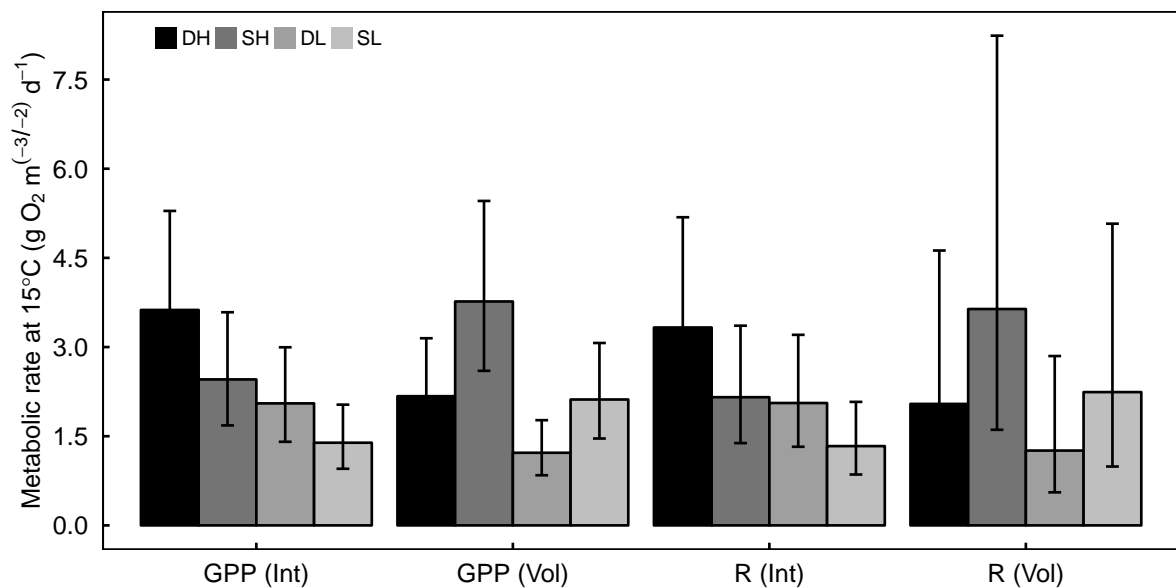


Figure 3.3: Average treatment-wise volumetric and area-based metabolic rates at 15 °C as estimated by mixed effects regression and back transformed to original scale (Table 3.3). Error bars represent the 95% confidence interval and have also been back transformed to original scale. DH = deep high nutrient, SH = shallow high nutrient, DL = deep low nutrient and SL = shallow low nutrient treatment.

Nutrient and light effects on average metabolic rates (Hypothesis (1)) – On average, volumetric GPP and ER were significantly higher in the eutrophic than in the mesotrophic systems and significantly higher in the shallow than in the deep mesocosms (Table 3.2, Figure 3.3). In line with our assumptions, the eutrophic shallow mesocosms with ample light had the highest metabolic rates, followed by eutrophic deep systems with reduced light availability, shallow mesotrophic systems with ample light and deep mesotrophic light-reduced systems. Interactions between nutrients and depth were only significant for ER per unit area, indicating that elevated nutrient availability had a stronger increasing effect on ER in the deep mesocosms than in the shallow mesocosms. However, the interaction lost significance in the simplest model containing only the significant terms of the full regression (Table 3.2).

Effect of mixing depth on average metabolic rates (Hypothesis (1)) – For the volumetric metabolic rate estimates, the better light conditions in the shallow mesocosms had a significantly increasing

Table 3.2: Results from minimal linear mixed effect regressions. The impacts of normalised inverse temperature (invT), depth (D) and nutrients (N), as well as their interactions, were tested on: volumetric gross primary production per day (GPP_{Vol}), volumetric ecosystem respiration per day (ER_{Vol}), volumetric primary production per daylight hour (GPP_{Vol-dl}), area-based gross primary production per day (GPP_{Area}), area-based gross primary production per daylight hour ($GPP_{Area-dl}$), area-based ecosystem respiration per day (ER_{Area}) and ratio between ecosystem respiration and gross primary production ($ER : GPP$). Effect size is given as regression coefficients from standardised predictors (Shallow = -0.5, Deep = 0.5, Low = -0.5, High = 0.5). The first R^2 value refers to the marginal R^2 (variance explained by fixed factors) and the second to the conditional R^2 (variance explained by fixed and random factors).

Response	Predictor	Effect size	Std. error	T-value	P-value	R^2
ln(GPP_{Vol})	Int	11.34	0.14	77.99	<0.01	0.50, 0.8
	invT	-0.91	0.16	-3.29	0.02	
	D	-0.55	0.03	-9.93	<0.01	
	N	0.58	0.03	10.52	<0.01	
ln(ER_{Vol})	Int	11.37	0.32	34.99	<0.01	0.33, 0.88
	invT	-1.05	0.24	-2.54	0.06	
	D	-0.58	0.03	-9.37	<0.01	
	N	0.48	0.03	7.91	<0.01	
ln(GPP_{Vol-dl})	Int	8.71	0.18	46.68	<0.01	0.34, 0.78
	invT	-0.53	0.15	-2.03	0.10	
	D	-0.54	0.03	-9.74	<0.01	
	N	0.58	0.03	10.49	<0.01	
ln(GPP_{Area})	Int	11.38	0.15	76.07	<0.01	0.50, 0.81
	invT	-0.91	0.15	-3.49	0.02	
	D	0.39	0.03	7.33	<0.01	
	N	0.57	0.03	10.82	<0.01	
ln(ER_{Area})	Int	11.37	0.17	64.89	<0.01	0.51, 0.85
	invT	-1.11	0.20	-3.35	0.02	
	D	0.43	0.03	8.34	<0.01	
	N	0.48	0.03	9.29	<0.01	
ln($GPP_{Area-dl}$)	Int	8.75	0.14	62.23	<0.01	0.41, 0.74
	invT	-0.53	0.14	-2.18	0.09	
	D	0.40	0.03	7.44	<0.01	
	N	0.57	0.03	10.79	<0.01	
ln($ER:GPP$)	Int	-0.01	0.14	-0.41	0.71	0.04, 0.60
	invT	-0.22	0.10	-1.27	0.27	
	D	-0.01	0.02	-0.20	0.85	
	N	-0.09	0.02	-1.99	0.05	

effect on both GPP and ER. This trend changed when rates were calculated per unit of area, likely reflecting the greater mixing depth in the deep mesocosms (all mesocosms were fully mixed). Thus, the deep eutrophic mesocosms had higher metabolic rates per unit of area than the shallow eutrophic mesocosms, and the rates in the deep mesotrophic mesocosms exceeded those in the shallow mesotrophic mesocosms (Table 3.2, Figure 3.3).

Temperature effects and interactions with nutrient and light regimes (Hypotheses (2) and (3))

– Both log-transformed GPP and ER (volumetric and area-based) increased significantly with increasing temperatures as predicted by the Arrhenius equation 3.3 and 3.2 (Figure 3.4, Table 3.2). Contrary to our expectations, however, we did not find significant interacting effects between water temperature and the different light and nutrient regimes on GPP and ER. The average temperature sensitivity of volumetric and area-based ER in all treatments was 0.62 eV and 0.65 eV, respectively, predicting a 13.5- (volumetric) and 15.4- (area-based) fold in-

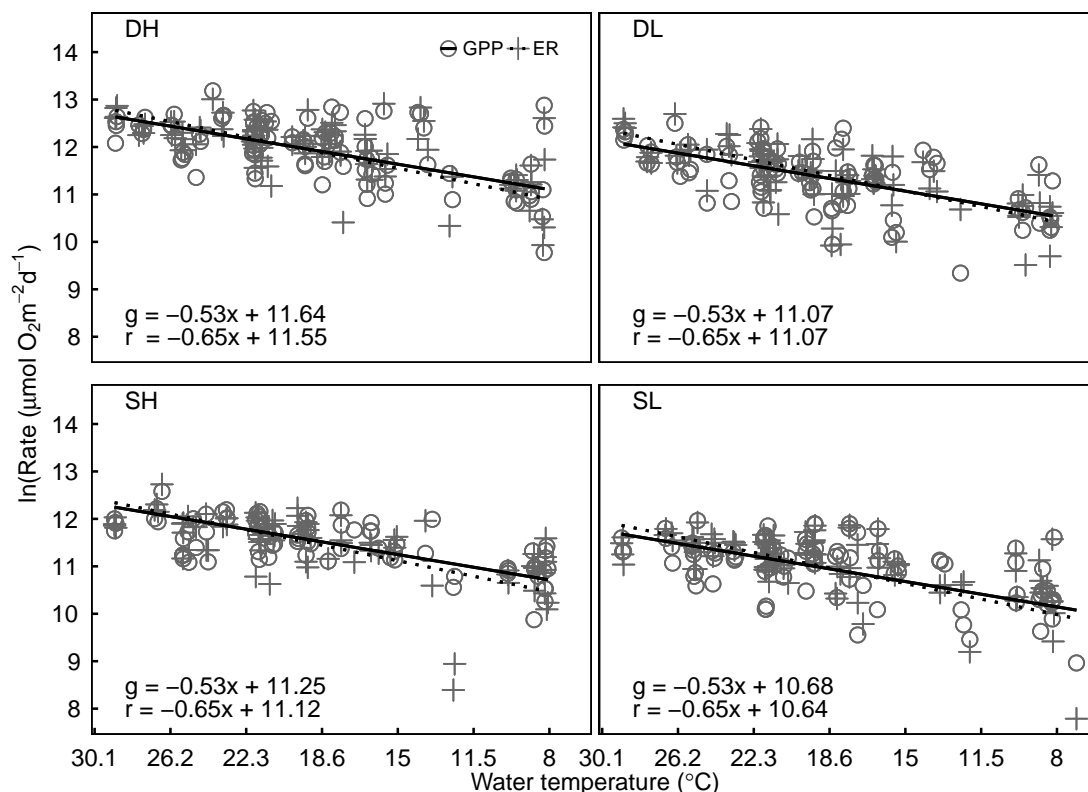


Figure 3.4: Arrhenius plot of area-based GPP and ER (Arrhenius plot of volumetric data shows a similar relationship (Table 3.3)) for each treatment (DH = deep high nutrient, SH = shallow high nutrient, DL = deep low nutrient and SL = shallow low nutrient). The plot is based on monthly measurements from July to November along a temperature gradient from Sweden to Greece. The solid line represents estimated average GPP and the dotted line average ER as estimated by mixed effects regression. Note that the actual units of the x-axis of the Arrhenius plot are $1/k (1/T - 1/T_c)$ in units of electron volt and a reference temperature, T_c , of 15 °C; for the sake of easier interpretation corresponding temperatures in degree Celsius are depicted.

crease in ER over a temperature range from 0 to 30 °C. The average temperature sensitivity for volumetric and area-based GPP amounted to 0.54 eV and 0.53 eV, respectively (Figure 3.4, Table 3.3), predicting a 9.7- (volumetric) and 9.3- (area-based) fold increase in GPP over a temperature range from 0 to 30 °C. Thus, as predicted from metabolic theory, ER increased more with temperature than did GPP. Consequently, according to MTE (equation (3) and (4)), the activation energy of the ER:GPP ratio is expected to be, on average, around 0.08 eV (volumetric) to 0.12 eV (area-based). This corresponds to a 1.4- (volumetric) to 1.5- (area-based) fold increase of the ratio over a temperature range from 0 to 30 °C. Due to lower absolute ER:GPP ratios in eutrophic compared to mesotrophic systems, however, the mesotrophic mesocosms had an about 9% lower metabolic-driven carbon sequestration capacity than the eutrophic mesocosms. Nevertheless, the actual estimated average activation energy of 0.13 eV for the ER:GPP ratio, although close to theoretically predicted values, was not significant (Figure 3.5a, Table 3.3).

Covariation with temperature – The average temperature sensitivity for daylight length-corrected GPP had an estimated average activation energy of 0.31 eV for both area-based and volumetric metabolic rates and was thus close to the canonical temperature dependence of photosynthesis,

Table 3.3: Slope, intercept and temperature at which the systems switch from auto- to heterotrophy. Slope and intercept values are derived from minimal mixed effect models, i.e. models from which all insignificant terms are removed, but which contain at least the inverse scaled temperature and the main effects of the depth and nutrient treatment (Table 3.2). DH = deep high nutrient, SH = shallow high nutrient, DL = deep low nutrient and SL = shallow low nutrient treatment.

		GPP _{Vol}	ER _{Vol}	GPP _{Vol-dl}	GPP _{Area}	ER _{Area}	GPP _{Area-dl}	ER:GPP
Slope (eV)	DH	-0.54± 0.16	-0.62± 0.24	-0.31±0.15	-0.53± 0.15	-0.65±0.20	-0.31± 0.14	-0.13±0.10
	SH							
	DL							
	SL							
Intercept at 15 °C ln(μ mol O ₂ m ⁻³ or ⁻² d ⁻¹) or ln(μ mo O ₂ m ⁻³ or ⁻² LP ⁻¹)	DH	11.13±0.15	11.06±0.32	8.60± 0.19	11.64±0.15	11.55±0.18	9.10± 0.14	-0.11±0.15
	SH	11.68±0.15	11.64±0.32	9.14± 0.19	11.25±0.15	11.12±0.18	8.70± 0.15	-0.10±0.15
	DL	10.55±0.15	10.58±0.32	8.03± 0.19	11.07±0.15	11.07±0.18	8.53± 0.14	-0.02±0.15
	SL	11.10±0.15	11.16±0.32	8.57± 0.19	10.68±0.15	10.64±0.18	8.14± 0.14	-0.01±0.15
Switch point temperature (°C)	DH	21			20			21
	SH	19			23			21
	DL	12			15			16
	SL	10			17			16

but lost significance at 0.05 per cent level (Table 3.2).

Results from semi-partial Spearman correlations for the criterion variables GPP, ER and the ER:GPP ratio and predictor variables such as water temperature, MAL, LP_{eff}, daylight length, chlorophyll a and PVI confirmed the importance of temperature (Table 3.4). In addition to temperature, ER was strongly correlated with GPP and GPP with daylight length. Correlation with chlorophyll a and PVI was significant where their levels were highest, i.e. in the eutrophic deep and mesotrophic shallow mesocosms, respectively (Figure S3.2, Table 3.4).

Table 3.4: Bootstrapped semi-partial Spearman correlation coefficient *r* over all treatments (all) and treatment wise (S = shallow, D = deep, H = high nutrient, L = low nutrient) for the three criterion variables area-based GPP (GPP_{area}), area-based ER (ER_{area}) and ER:GPP and the predictor variables: inverse-scaled temperature (invT), chlorophyll a (Chl *a*), PVI (plant volume inhabited), effective light period (LP_{eff}), mean available light (MAL), daylight length (DayL), ER:GPP ratio, GPP_{area}, ER_{area}. Only *r* values ≥ 0.10 are reported, “*” denotes values where the 95% confidence interval did not include zero, “-” denotes cells of variables not used as predictors for the particular variable.

		invT	Chl <i>a</i>	PVI	LP _{eff}	MAL	DayL	ER:GPP	GPP _{area}	ER _{area}
GPP _{Area}	all	-0.27*	0.18*	-0.13*	-0.15*		0.20*	-	-	-
	DH	-0.10	0.21*		-0.12	0.18	0.24*	-	-	-
	SH	-0.24*					0.25*	-	-	-
	DL	-0.25*	0.10		-0.16*	0.17*	0.18	-	-	-
	SL	-0.14		0.15			0.15	-	-	-
ER _{Area}	all	-0.24*			-	-	-	-	0.50*	-
	DH	-0.24*			-	-	-	-	0.54*	-
	SH	-0.36*			-	-	-	-	0.29*	-
	DL	-0.35*			-	-	-	-	0.39*	-
	SL	-0.34*			-	-	-	-	0.37*	-
ER:GPP	all	-0.15*					-0.10	-	-	-
	DH	-0.16	-0.11			-0.14		-	-	-
	SH	-0.20*			-0.12			-	-	-
	DL	-0.29*				-0.16	-0.11	-	-	-
	SL	-0.24			-0.17			-	-	-

Temperature-specific switch from auto- to heterotrophy (Hypothesis (4)) – In the mesotrophic mesocosms, based on equation 3.5 and average values from the mixed effects regression for

GPP and ER (Table 3.3), the switch from auto- to heterotrophy generally occurred at lower temperatures (12 °C and 10 °C (volumetric), 15 °C and 17 °C (area-based)) than in the eutrophic mesocosms (21 °C and 19 °C (volumetric), 20 °C and 23 °C (area-based)) (Figure 3.5b). This is in line with our prediction. In comparison, light regime and mixing depth had only a minor influence on the switch point and predictions are inconsistent between area and volumetric-based estimates. This is confirmed by a significant nutrient effect ($p = 0.05$) but an insignificant depth effect ($p = 0.85$) for the ER:GPP ratio (Figure 3.5a).

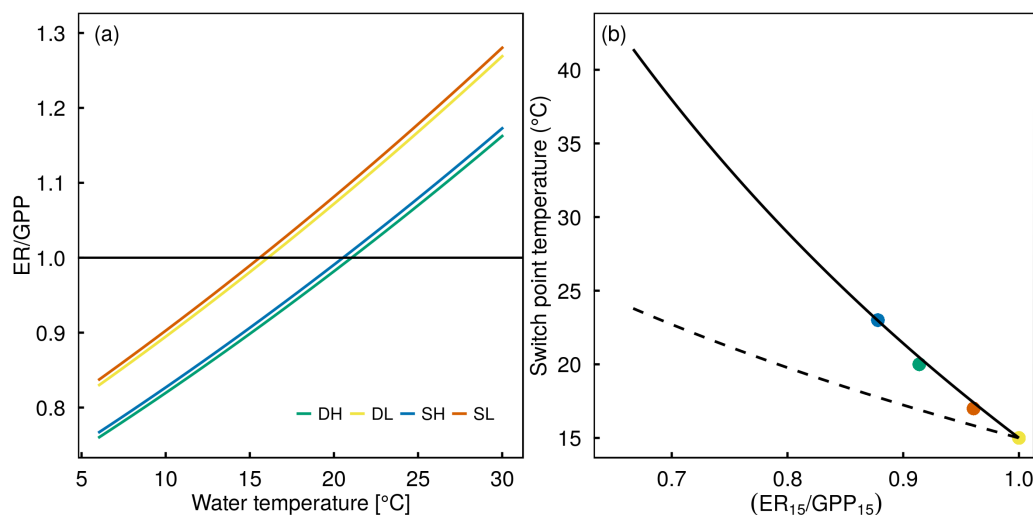


Figure 3.5: Water temperature-dependent switch from net auto- to net heterotrophy. a) Average treatment-wise change in ER/GPP ratio over the temperature gradient as estimated by mixed effect model (Table 3.3). b) Theoretically predicted switch point temperatures from auto- to heterotrophy depending on the ER/GPP ratio at a reference temperature of 15 °C (equation 5). The solid line depicts the switch point temperature for activation energies of 0.53 eV and 0.65 eV for GPP and ER, respectively. The dashed line represents the relation for an activation energy of 0.31 eV for GPP as suggested by the metabolic theory of ecology and established as average apparent activation energy for daylight length-corrected primary production. Superimposed are the treatment-wise average switch point temperatures based on area-based metabolic values as established by mixed effects regression (Table 3.3).

3.5 Discussion

It is anticipated that climate change will affect shallow lake metabolism and thereby the ability of shallow lakes to sequester carbon due to direct and indirect impacts on major drivers like temperature, nutrients, water level and light conditions (Tranvik et al., 2009; Nickus et al., 2010; Jeppesen et al., 2015). We confirmed the existence of a positive relationship between nutrient concentrations, light availability, temperature and metabolic rates in shallow lakes. The ER:GPP ratio increased with increasing temperature and all systems switched from autotrophy to heterotrophy over the temperature gradient. Lower temperature thresholds for the switch were, however, observed under mesotrophic than under eutrophic conditions due to lower ER:GPP ratios for eutrophic than for mesotrophic systems. Both increasing ER:GPP ratios and the switch points could be predicted within the framework of the metabolic theory. Contrary to our expectations, however, no significant interactions between the main drivers (temperature,

nutrients and water level) were observed.

The observed differential temperature sensitivity between GPP and ER adds support to the anticipated shift (Cole et al., 2000; Staehr and Sand-Jensen, 2006; Yvon-Durocher et al., 2010a,b) towards heterotrophy in shallow lake ecosystems with increasing temperatures. Since the observed average activation energy for ER was higher than for GPP, ecosystem respiration increased more than production, leading to a predicted increase in ER:GPP over the tested temperature gradient. Since proportionally more of the carbon fixed by primary production was released again as CO₂ at increasing temperatures, the carbon sequestration capacity likely decreased and the mesocosms became potential carbon sources in the upper temperature range (Figure 3.4). The average temperature sensitivity of the ER:GPP ratio itself was not significant, perhaps due to the inherent variance of both the GPP and the ER estimates. Nonetheless, the estimated average activation energy of 0.13 eV is in accordance with the predicted values of 0.08 eV for volumetric and 0.12 eV for area-based values, respectively, based on equation (4) (Figure 3.5b). The established apparent average temperature sensitivities for area-based metabolic rates of 0.65 eV and 0.31 eV for ER and GPP per daylight hour, respectively, match well with predictions for the physiological temperature dependence for respiration (0.6 eV) and photosynthesis (0.3 eV) predicted by the metabolic theory (Allen et al., 2005). The observed activation energy of GPP per day, however, exceeds with 0.54 eV the predicted physiological temperature dependence of 0.3 eV established for terrestrial C3 plants (Bernacchi et al., 2001). Still, this value is in good agreement with findings from other aquatic environments (0.50±0.18 eV (Wilken et al., 2013), 0.54±0.24 eV (Demars et al., 2011), 0.45 eV (95% CI 0.38-0.53) (Yvon-Durocher et al., 2010b)). While the higher activation energies in aquatic ecosystems could have physiological causes (Demars et al., 2011), here it seems best explained by a positive correlation between daylight length and temperature since daylight length-corrected estimates led to the physiologically predicted temperature sensitivity of 0.31 eV. Still, it is interesting to note that for regions with seasonal temperature fluctuations, a correlation between daylight length and temperature is natural and might mitigate the anticipated shift towards heterotrophy.

Assuming that direct temperature effects on both GPP and ER can be approximated by the Arrhenius equation within the range of 0 to 30 °C (Gillooly, 2001; Allen et al., 2005), the temperature at which a system switches from autotrophy to heterotrophy can be modelled (equation 3.5). The model suggests that the temperature threshold at which a system switches from being net autotrophic to net heterotrophic depends on the extent of the differential temperature sensitivity between GPP and ER (E_p and E_r , respectively) and on the log ratio between GPP and ER at a reference temperature, T_c (here 15 °C). This temperature threshold turned out to be affected by trophic state (Figure 3.5a): higher nutrient availability in the eutrophic mesocosms led not only to significantly higher GPP and ER but also to significantly lower ER to GPP ratios (0.9 (DH) and 0.91 (SH)) than under mesotrophic conditions (0.98 (DL) and 0.99 (SL)). This apparently small difference was, however, large enough to cause an average temperature threshold increase

of approximately 4 °C (area-based calculations). Thus, high-nutrient systems are likely to have lower risk of becoming net heterotrophic under the predicted 1.5 to 5 °C warming scenarios until 2100 (Rogelj et al., 2012; Stocker et al., 2013) than systems with lower nutrient concentrations (Figure 3.5b, Table 3.3). The direct effect of water depth on the ER:GPP ratio was not significant and the effect of depth on the threshold temperature was low (2.5 °C difference averaged for both shallow and deep mesocosms and for both mesotrophic and eutrophic systems), indicating that reduced water level is of minor importance for the switch from auto- to heterotrophy in these overall shallow systems.

Co-variation between major driving forces and local- and seasonal-specific mechanisms has the potential of modulating the apparent temperature sensitivity of GPP and ER and may thus have a huge influence on the metabolic balance and the threshold temperature at which lakes switch to heterotrophy (Equation 3.5, Figure 3.5b). Co-variation of daylight length with temperature modulated, for instance, the difference between the activation energy of GPP and ER per day, which turned out to be smaller than expected from the physiological temperature dependence predicted by MTE (Brown et al., 2004). While the confirmation of MTE was strong when aggregating data from all countries and seasons, the variability between countries and seasons was huge (Table S4.1, S4.2, S4.3, S4.4). Obviously, imbalance in metabolisms with increasing temperature is a strong signal at large spatial scale, while it may not necessarily be significant for a given lake (De Castro and Gaedke, 2008; Davidson et al., 2015). Local and seasonal specific mechanisms may modulate the temperature response of metabolism (Anderson-Teixeira and Vitous, 2012). Most notably, acclimatisation and adaptation processes are suggested to induce reduced temperature sensitivity with increasing average temperature (Atkin and Tjoelker, 2003; Hikosaka et al., 2005; Hartley et al., 2008; Angilletta, 2009; Smith and Dukes, 2013), while Perkins et al. (2012) reported consistent Q10 temperature coefficient values for ER regardless of the thermal history or community composition of biofilms. Neither among countries nor months could we confirm a consistent decrease in the temperature sensitivity of GPP and ER with increasing average temperature (Table S4.2, S4.4). Yet, we suggest that co-variation of factors like light availability, WLC, Chl *a*, PVI, nutrient availability and community composition with temperature may have induced the variation in the apparent temperature sensitivity between months and countries (Anderson-Teixeira and Vitous, 2012; Welter et al., 2015), as being well-demonstrated in experimental mesocosms studies (Davidson et al., 2015). Apart from co-variation between driving forces, true interactions between trophic state and lake depth with temperature also have the potential of modifying temperature sensitivity and thus adjust the predicted loss of carbon sequestration capacity based on physiological temperature sensitivity (Davidson et al., 2012; Cross et al., 2015; Davidson et al., 2015).

In contrast to our expectations, however, we did not observe significant interactions between the temperature sensitivity of both GPP and ER and nutrient levels or depth. We can only speculate about the underlying mechanisms. Perhaps the missing depth-temperature interaction reflects

light adaptation, rendering photosynthesis primarily dependent on the maximum photosynthetic rate. The lack of TP-temperature interaction indicates that either TP affinity was not a function of temperature or shifts in community composition in the mesotrophic mesocosms towards species with higher phosphate affinity prevented limitation of photosynthesis by phosphorus (De Senerpont Domis et al., 2014). Lack of sensitivity to depth and nutrients may, however, also reflect the relatively modest variations in these variables in the experiment.

A decline in water level, as already reported and as further anticipated within the context of global warming for lakes in the Mediterranean region (Coops et al., 2003; Jeppesen et al., 2015; Beklioğlu et al., 2006, 2007), affects mixing depth and light availability. We found a significantly lower volumetric GPP and ER in deep than in shallow mesocosms (Table 3.3). The difference in production levels was most likely generated by the influence of depth on the light availability, while impacts on gas exchange due to a lower surface-to-volume ratio were most likely negligible since all mesocosms were fully mixed. Light saturation for photosynthesis is algal species specific and ranges around 60 – 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Lampert and Ulrich, 1999). This classified the deep mesocosms to the lower end of the range and makes them more prone to being light limited, while the shallow mesocosms are close to or above the upper end of the range (Table S3.1) and thus most likely light saturated. Additionally, only in the eutrophic deep systems, the sediment layer was generally below the euphotic zone; thus, only these mesocosms had a considerably shorter average L_{Peff} compared to all other treatments (Table S3.1). Therefore, a reduction in water level considerably improves the light availability and might allow benthic primary production where it was not possible before. However, in order to quantify the importance of shallow lakes as a carbon sink or source within the context of regional or global carbon budgets, area-based measures are needed given that gas exchange of lakes with the atmosphere is inherently an areal process. Area based measures also allow comparability of different sized lakes. Despite the less favourable light conditions in the deep mesocosms, the effect of depth on the response of GPP and ER per unit of area changed its direction compared with the volumetric data, while still being significant (Figure 3.3, Table 3.2). The difference in water column between the shallow and deep mesocosms seemingly overruled the adverse effect of the less favourable light conditions in the deep mesocosms. This ambiguous role of depth for the metabolic rate estimates, as seen when comparing volumetric or area-based rates, is interesting as it demonstrates that increased production in the shallow mesocosms, probably reflecting better light availability, can be outweighed by the lower depth. For polymictic shallow lakes, this implies that even though a water level drop might improve light availability, and thus stimulate production and indirectly respiration, the importance of the lake in the local carbon cycle diminishes. Yet, it remains to be seen if this contradictory effect of decreasing mixing depth on volume vs. area-based metabolism estimates is of relevance in natural lakes, which exhibit a much more complicated morphometry than our cylindrical mesocosms.

Shallow lakes are known to play an important role in the local and global carbon cycling as they

are the most numerous lake type in the world (Downing et al., 2006; Tranvik et al., 2009). Given that a differential temperature sensitivity of ER and GPP poses a potential feedback mechanism to atmospheric CO₂ levels in a warming scenario, understanding the metabolic processes of shallow lake ecosystems and how they will be affected by a changing climate is not only of basic but also of applied ecological interest. Here, we confirmed and quantified the differential temperature sensitivity of GPP and ER and showed that trophic state is crucially important for the question of how much warming a system can buffer before it switches from net auto- to net heterotrophy. Our study also encourages future metabolism models to include a temperature effect for GPP, which is often neglected (see, for instance, (Cole et al., 2000; Holtgrieve et al., 2010; Staehr et al., 2010b; Solomon et al., 2013)). This might particularly improve inverse modelling approaches when estimating lake metabolism based on diel oxygen curves. We linked our experimental findings to the framework of MTE and tested theoretically derived predictions on our data. In line with earlier studies, we found good agreement between theory and practice, which affirmed the potential of MTE. Yet, we also found that the balance between ER and GPP not only depends on the energy supply, as in MTE, but also on the availability of nutrients, most notably nitrogen and phosphorus. Thus, we conclude that quantitative inclusion of these aspects in MTE would greatly add to its predictive power for ecosystems.

3.6 Acknowledgements

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Chapter 3 – SI Appendix

SI.A Tables

Table S1.1: Available data points for the statistical analysis of GPP, ER and metabolic balance (ER:GPP) and their distribution over countries, months and treatments. Imbalance in the data is due to loss of mesocosms during storm events and removal of GPP and ER estimates with standard errors larger than the estimated value or less than 5% explained variability by the model.

Country	Treatment	June	July	Aug	Sep	Oct	Total
SE	DH	4	4	4	4	3	76
	DL	4	4	4	2	4	
	SH	4	4	4	3	4	
	SL	4	4	4	4	4	
EE	DH	4	3	4	2	0	50
	DL	4	4	3	1	0	
	SH	4	3	1	2	0	
	SL	4	4	3	3	1	
GE	DH	2	2	2	2	2	60
	DL	3	3	3	3	3	
	SH	3	3	3	3	3	
	SL	4	4	4	4	4	
CZ	DH	4	4	3	4	2	46
	DL	1	3	3	4	1	
	SH	1	1	0	2	2	
	SL	3	3	1	3	1	
TR	DH	3	4	4	4	4	73
	DL	2	3	4	4	3	
	SH	3	4	4	4	4	
	SL	4	4	4	4	3	
GR	DH	4	4	4	4	4	69
	DL	4	4	4	4	4	
	SH	4	4	4	2	0	
	SL	4	4	4	3	0	
Total		81	84	78	75	56	374

Table S1.2: Country-wise measurement dates for the 24-h measurement and associated month and period. Monthly 24-h measurements for measurement 3 – 7 were planned to take place on 12.07, 09.08, 06.09, 04.10 and 1.11. However, the actual measurements deviated slightly from this date; thus, average values for each country, for instance monthly air temperature, are calculated over the individual period for each country leading up to a 24-h measurement.

Measurement no.	Associated month	Sweden		Estonia		Germany		Czech Republic		Turkey		Greece	
		Date	Period	Date	Period	Date	Period	Date	Period	Date	Period	Date	Period
3	July	11.07 12.07	14.06 – 11.07	12.07 13.07	09.06 – 12.07	11.07 12.07	15.06 – 11.07	11.07 12.07	14.06 – 11.07	12.07 13.07	15.06 – 12.07	15.07 16.07	16.06 – 15.07
4	August	15.08 16.08	12.07 – 15.08	09.08 10.08	13.07 – 09.08	08.08 09.08	12.07 – 08.08	08.08 09.08	12.07 – 08.08	08.08 09.08	13.07 – 08.08	13.08 14.08	16.07 – 13.08
5	September	13.09 14.09	16.08 – 13.09	05.09 06.09	10.08 – 05.09	05.09 06.09	09.08 – 06.09	05.09 06.09	09.08 – 05.09	06.09 07.09	09.08 – 06.09	11.09 12.09	14.08 – 11.09
6	October	18.10 19.10	14.09 – 18.10	03.10 04.10	06.09 – 03.10	04.10 05.10	07.09 – 04.10	03.10 04.10	06.09 – 03.10	04.10 05.10	07.09 – 04.10	10.10 11.10	12.09 – 10.10
7	November	07.11 08.11	19.10 – 07.11	31.10 01.11	04.10 – 31.10	31.10 01.11	05.10 – 31.10	30.10 31.10	04.10 – 31.10	01.11 02.11	05.10 – 01.11	08.11 09.11	11.10 – 31.10

Table S1.3: Country- and month-wise average air temperature. For each month, column 5 shows the difference between the warmest and the coldest country along the climate gradient as well as the standard deviation between countries.

Month	Country	Average air temperature (°C)	Monthly average air temperature (°C)	Range (°C) and Std (°C)
July	SE	16.86	20.01	9.56 3.52
	EE	18.75		
	GE	18.43		
	CZ	17.98		
	TR	21.64		
	GR	26.42		
August	SE	18.81	21.29	10.11 4.33
	EE	19.92		
	GE	18.37		
	CZ	17.16		
	TR	26.23		
	GR	27.27		
September	SE	16.7	20.18	11.51 4.44
	EE	16.35		
	GE	18.4		
	CZ	18.79		
	TR	22.99		
	GR	27.86		
October	SE	11.64	16.01	11.04 4.04
	EE	13.2		
	GE	15.32		
	CZ	14.49		
	TR	18.74		
	GR	22.68		
November	SE	8.71	9.78	7.50 2.79
	EE	7.47		
	GE	9.61		
	CZ	7.5		
	TR	10.44		
	GR	14.97		

SI.B Methods

Estimation of primary production (GPP) and ecosystem respiration (ER) rates

Temporal dynamics of dissolved oxygen were modelled according to [Jeppesen et al. \(2012b\)](#):

$$\frac{dDO}{dt} = K_{20}\Phi^{(T_t-20)}(DO_{sat} - O_2) + \rho \frac{I_t}{\eta + I_t} - \pi$$

where DO (mg L⁻¹) is the measured dissolved oxygen concentration, t (h) is time, K₂₀ (h⁻¹) is the reaeration coefficient as established in the gas exchange experiment, T (°C) is the water temperature, I_t (mol photons m⁻² s⁻¹) is the surface photosynthetically active radiation, Φ_K = 1.0241 is the coefficient for the Arrhenius type temperature dependence of the reaeration coefficient and DO_{sat} (mg L⁻¹) is the saturation concentration of dissolved oxygen estimated following [Benson and Krause \(1980, 1984\)](#). Corrections for atmospheric pressure were made based on monthly mean air pressure data for each experimental site. The model uses a Michaelis Menten type relation to describe light saturation of primary production. We did not use the Arrhenius type temperature model suggested by [Jeppesen et al. \(2012b\)](#) since we were particularly interested in investigating the temperature responses of the metabolic rates to the climate gradient. The parameter ρ (mg l⁻¹ h⁻¹) is interpreted as the maximum obtainable production rate, η (mol photons m⁻²s⁻¹) is the light intensity at which the primary production reaches half its maximum (half saturation constant) and π (mg l⁻¹ h⁻¹) is the average rate of ecosystem respiration. GPP and ER per day are then calculated as follows:

$$GPP \left[\frac{mg}{ld} \right] = \sum_t \frac{I_t}{\eta + I_t} \Delta t$$

$$ER \left[\frac{mg}{ld} \right] = \pi \cdot 24$$

where Δt(h) is the time difference between two consecutive measurements.

For estimation of ρ, π and η, we used the shuffled complex evolution optimisation algorithm (SCE) as implemented in the R package "hydromad" ([Andrews and Guillaume, 2015](#)) to minimise the sum of the squared residuals. Starting values for all three parameters were found using a brute force grid search within the range 0.0001 to 5 applying the nls2 R package ([Grothendieck, 2013](#)). To assess the uncertainty of the estimated production and respiration rates, we used a bootstrap approach similar to that in [Solomon et al. \(2013\)](#) where the autocorrelation of lag 1 and the variance from the residuals of the original data are used to construct bootstrapped residuals with the same autocorrelation and variance as the original data. Those bootstrapped residuals are then added to the fitted values and the estimation, using the same starting values, is repeated for

the so generated pseudo dDO replicates.

Estimates with standard errors larger than the estimate itself:

$$\frac{\sqrt{\left(\frac{1}{500} \sum_i^{500} (e_i - e)^2\right)}}{e} > 1$$

where e_i is the bootstrapped estimates and e is the estimate from the raw data; estimates explaining less than 5% of the variability of the 24-h dissolved oxygen curve were excluded from further analysis. Overall, 374 data points remained. For an overview of the distribution of data points per country, month and treatment, see Table S1.2. We derived integrated (area) measures for GPP and ER by multiplication by the actual water column height, which is equal to the mixing depth in our systems. To obtain day length-corrected GPP values, GPP_{dl} ($\text{mg L}^{-1} \text{hd}_{\text{L}}$), GPP per day was divided by the average daylight period, LP (hd^{-1}), per month and country.

Estimation of light attenuation coefficient, K_d , mean available light and effective light period

Global radiation was converted to PAR using the transformation given in [Kirk \(2010\)](#):

$$\text{PAR} \approx E \cdot \gamma \cdot 0.45$$

where PAR is given in $\mu\text{mol m}^{-2}\text{s}^{-1}$ and E in W m^{-2} ; $\gamma = 4.6 \mu\text{mol J}^{-1}$ is a conversion factor based on the centre wavelength of 550 nm of the 400 – 700 nm waveband ([McCree, 1981](#)).

For each light profile (d) and each concurrent light intensity measurement (i), the attenuation coefficient K_{di} (m^{-1}) was estimated based on the Beer-Lambert law:

$$K_{\text{di}} = \frac{\ln \frac{I_i}{I_{i+1}}}{z_{i+1} - z_i}$$

where I_i and I_{i+1} are PAR values at depth z_i and z_{i+1} . Values with $I_{i+1} > I_i$ were removed. K_d (m^{-1}) was then taken as the mean of all K_{di} .

Mean available light ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) was estimated as:

$$I = \frac{I_0}{z \cdot K_d} (1 - \exp(-K_d \cdot z))$$

where I_0 is the incident light just beneath the surface. If only incident light measurements were available, we corrected values for 10% backscatter ([Kirk, 2010](#); [Staehr et al., 2010b](#)).

Effective light period, LP_{eff} , was calculated as described in [Shatwell et al. \(2012\)](#):

$$LP_{eff} = \frac{z_{1\%}}{z_{mix}} \cdot LP$$

where $z_{1\%}$ (m) is the euphotic depth (depth at which 1% of surface light intensity is left), z_{mix} is the mixing depth, coinciding in our fully mixed mesocosms with the water column height, and LP is the mean light period (hd^{-1}) per month and country. Values were limited to one since the experienced LP cannot be larger than the actual LP. If $z_{1\%}$ is above than z_{mix} , the effective day length is shorter than the actual LP due to mixing.

Table S2. 1: Overview of the used methods for water chemistry and chlorophyll a analysis.

	TP	TN	Chl a
Sweden	EN ISO 6878	EN ISO 11905-1 Tecator AN 5202-SE with a FIAstar 5000 system	Spectrophotometrically at a wavelength of 665 nm from acetone extraction. Material gained on GF/F glass microfiber filters by filtering of the water samples.
Estonia	ISO 15681-2	ISO 29441	Spectrophotometrically (Edler, 1979) at a wavelength of 665 nm from 96% ethanol extracts. Material gained on GF/F glass microfiber filters by filtering of the water samples.
Germany	EN ISO 6878 (DEV, D11)	EN 12260 (DEV, H 34)	High-performance liquid chromatography (Waters, USA) following Fietz and Nicklisch (2004) .
Czech Republic	TP was determined spectrophotometrically with a molybdate method after perchloric acid digestion according to Kopáček and Hejzlar (1993)	EN 12260 (DEV, H 34) Elementar vario TOC cube analyser (Elementar Analysensysteme GmbH, Germany).	Spectrophotometrically (Edler, 1979) at a wavelength of 665 nm from 96% ethanol extracts. Material gained on glass-fibre filters of 0.4- μ m nominal pore size (GF-5, Macherey-Nagel, Düren, Germany) by filtering of the water samples.
Turkey	ISO 6878:2004	ISO 29441:2010	Spectrophotometrically (Edler, 1979) at a wavelength of 665 nm from 96% ethanol extracts. Material gained on GF/F glass microfiber filters by filtering of the water samples.
Greece	ISO 6878:2004	ISO 11905-1 and then use of a Shimadzu TOC-VCS/CP analyzer, equipped with TNM-1 TN unit.	Spectrophotometrically (Edler, 1979) at a wavelength of 665 nm from 96% ethanol extracts. Material gained on GF/F glass microfiber filters by filtering of the water samples.

SI.C Treatment differences and covariation with temperature for: Nutrients, light conditions, chl *a* and PVI

A proxy to assess the effects of the differential monthly loading of phosphate and nitrogen is TP and TN levels. Average TP levels in deep mesotrophic and shallow eutrophic mesocosms were almost constant over the entire temperature gradient. Whereas TP concentrations in shallow mesotrophic mesocosms increased with temperature (on average $10.2 \mu\text{g L}^{-1}$ over 7 to 29 °C), they decreased in the deep eutrophic mesocosms (on average $30.6 \mu\text{g TP L}^{-1}$ over 7 to 29 °C). Despite these dynamics, all treatments exhibited significantly different TP levels over the entire temperature gradient (Figure 3.2, Table S3.1).

The average TN concentration showed only slight changes over the temperature gradient for all treatments except in the deep eutrophic mesocosms where they decreased with, on average, 1.5 mg TN L^{-1} along the whole temperature gradient from 7 to 29 °C. Due to these differences in dynamics, TN levels between deep eutrophic and both mesotrophic treatments were no longer significantly different for temperatures above 25 °C (SL) and 26 °C (DL) (Figure 3.2, Table S3.1).

Average light attenuation (K_d) was significantly higher in the eutrophic than in the mesotrophic mesocosms. For the contrast between shallow mesotrophic and deep eutrophic mesocosms, this is, however, only true for temperatures above 10 °C. Generally, average light attenuation showed only small changes over the temperature gradient, with the exception of the shallow eutrophic mesocosms where attenuation decreased with increasing temperatures (1.1 m^{-1} from 7 to 29 °C) (Figure S3.1a, Table S3.1).

Average mean available light (MAL) was highest in the shallow mesotrophic mesocosms (adjusted mean: $111 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) along the entire temperature gradient, followed by the shallow eutrophic, deep mesotrophic mesocosms and, finally, the deep eutrophic mesocosms (adjusted mean: $41 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). MAL increased strongly with temperature for the shallow mesocosms and slightly less for the deep mesocosms, leading to more pronounced differences between treatments with increasing temperature. MAL differed significantly for all pairwise treatment comparisons over the temperature gradient. However, for the deep mesotrophic and shallow eutrophic mesocosms, this was only true for temperatures higher than 9 °C (Figure S3.1b, Table S3.1).

The average effective light period (LP_{eff}) was longest in the shallow mesotrophic mesocosms (adjusted mean: 13.6 h d^{-1}); however, LP_{eff} in the shallow eutrophic and deep mesotrophic mesocosms was only slightly shorter. LP_{eff} in the deep mesotrophic mesocosms was only significantly shorter than in the shallow mesocosms for temperatures above 15 °C (SL) and 21 °C (SH). Along the entire temperature gradient, the deep eutrophic mesocosms had a significantly shorter LP_{eff} (adjusted mean: $11.47 \text{ h LP}_{\text{eff}} \text{ h d}^{-1}$) (Figure S3.1c, Table S3.1).

The coinciding increase in temperature with daylight hours was more pronounced in the northern and mid-European than in the southern European countries (Figure S3.1d).

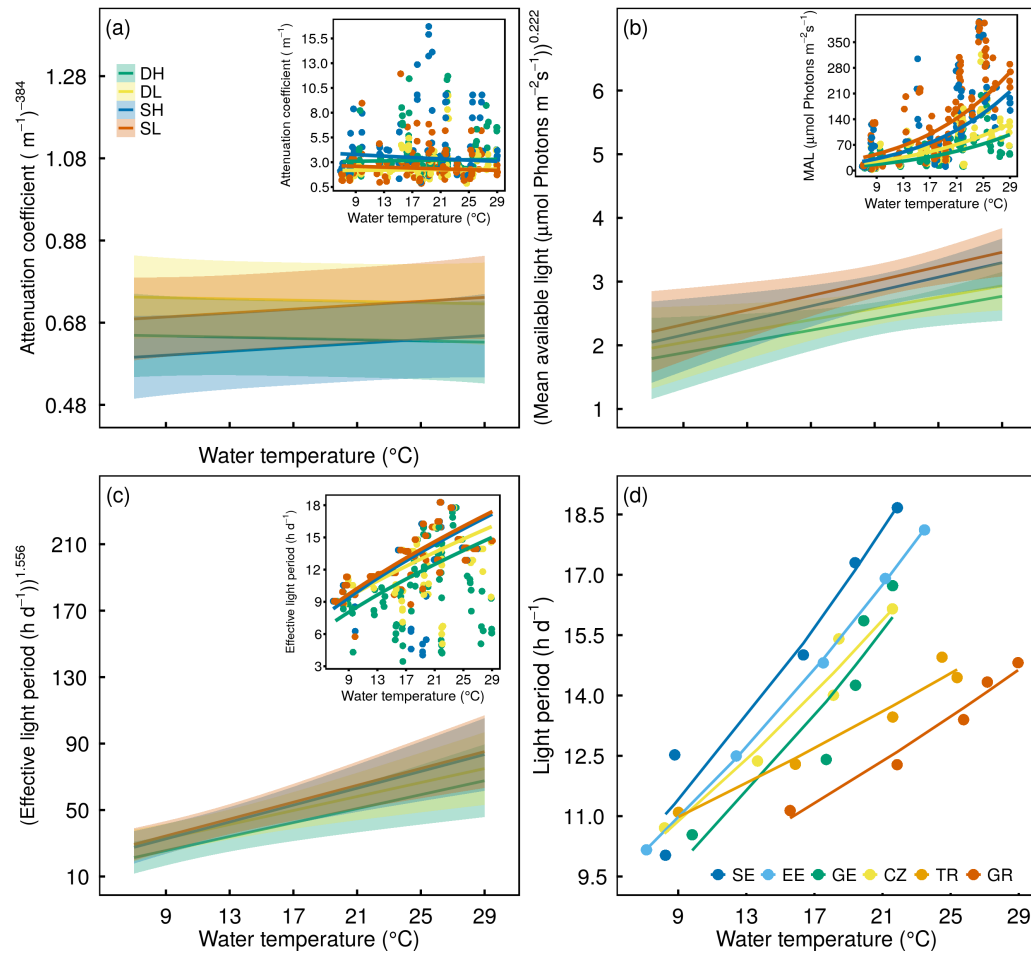


Figure S3.1: Co-variation of light conditions with water temperature. Treatment-wise co-variation of a) attenuation coefficient (K_d), b) mean availability light (MAL) and c) effective light period (LP_{eff}). Main images in a), b) and c) show treatment-wise least square means with 95% confidence intervals at the scale of the transformed variable. The insets depict the same variable at original scale with treatment-wise average values as estimated by mixed effects regression (Table S3.1). Lines in d) depict country-wise average daylight hours per day estimated by linear regression. DH = deep high nutrient, SH = shallow high nutrient, DL = deep low nutrient and SL = shallow low nutrient treatment.

Average chlorophyll *a* levels were significantly higher under eutrophic (adjusted mean: 25.35 (DH) and 15.57 (SH $\mu\text{g L}^{-1}$) than mesotrophic (6.54 (DL), 7.25 (SL) $\mu\text{g L}^{-1}$) conditions. In the eutrophic mesocosms, chlorophyll *a* decreased significantly along the temperature gradient but remained almost constant in the mesotrophic mesocosms. Despite these dynamics, average chlorophyll *a* levels remained significantly higher in the eutrophic than in the mesotrophic mesocosms along the entire temperature gradient (Figure S3.2a, Table S3.1).

PVI levels showed a trend opposite to that of chlorophyll *a* and were highest in the shallow mesotrophic mesocosm (adjusted mean: 8.09%), followed by the shallow eutrophic and deep mesotrophic mesocosms with comparable values (adjusted mean: 3.51% (SH) and 3.89% (DL)) and, finally, the deep eutrophic mesocosms (0.71%). PVI decreased strongly with increasing temperatures in all treatments but the deep, eutrophic mesocosms where similar low average

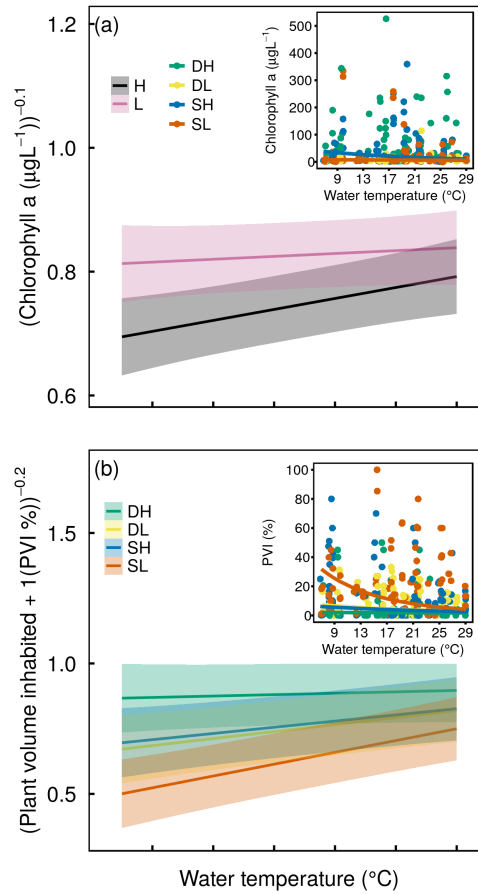


Figure S3.2: Co-variation of primary producers with water temperature. Main image shows least-square means of significant factors with 95% confidence intervals on the scale of the transformed variable. The inserts depict values at original scale with average values transformed to original scale. Treatment-wise co-variation of a) phytoplankton measured as chlorophyll a and b) macrophytes measured as plant volume inhabited (PVI). DH = deep high nutrient, SH = shallow high nutrient, DL= deep low nutrient and SL = shallow low nutrient treatment. Lines in a) and b) depict treatment-wise average PVI and chlorophyll a levels in dependence of water temperature as estimated by mixed effects regression (Table S3.1)

values were observed along the entire temperature gradient (Figure S3.2a, b, Table S3.1).

Table S3.1: Mixed effects regression for potential covariates. The influences of water temperature (T), depth (D) and nutrients (N), as well as their interactions, were tested on: total phosphorus (TP), total nitrogen (TN), attenuation coefficient (Kd), mean available light (MAL), effective light period (LP_{eff}), chlorophyll a (Chl a) and plant volume inhabited (PVI). Only p-values ≤ 0.05 are reported. The first R² value refers to the marginal R² (variance explained by fixed factors) and the second to the conditional R² (variance explained by fixed and random factors). The next four columns show treatment-wise adjusted means and average differences over the water temperature gradient, i.e. the difference between average values at 7 °C and 29 °C. The last 6 columns show the results of pairwise comparison of least-square means over the temperature gradient in one degree intervals. Temperatures in brackets indicate treatment differences from where on significant treatment differences were found at 0.05 significance level.

		Adjusted mean and gradient difference						Contrasts over temperature gradient					
		R ²	SH	DH	SL	DL		SL - SH	DL - DH	SL - DH	DL - SH	SL - DL	DH - SH
TP ($\mu\text{g L}^{-1}$)			68.92, 0.43	71.02, -30.63	26.32, 10.24	21.90, 0.30		p = <0.05	p = <0.05	p = <0.05	p = <0.05	p = <0.05	p = <0.05
TN (mg L^{-1})			1.37, -0.29	1.43, -1.54	0.79, 0.11	0.71, 0.28		p = <0.05	p = <0.05 (<26 °C)	p = <0.05 (<25 °C)	p = <0.05	n.s.	p = <0.05 (<13 °C, >24 °C)
K _d (m^{-1})			3.45, -1.12	3.17, 0.03	2.38, -0.18	2.25, 0.22		p = <0.05	p = <0.05	p = <0.05 (>10 °C)	p = <0.05	p = <0.05 (<14 °C)	p = <0.05 (<14 °C)
MAL ($\mu\text{mol Photons m}^{-2} \text{s}^{-1}$)	0.02		85.90, 177.41	41.29, 76.95	110.56, 213.53	57.30, 100.48		p = <0.05	p = <0.05	p = <0.05	p = <0.05 (>9 °C)	p = <0.05	p = <0.05
LP _{eff} (h d^{-1})	<0.01		13.23, 7.74	11.47, 6.89	13.60, 8.20	12.76, 7.07	0.03	n.s.	p = <0.05	p = <0.05	p = <0.05 (>21 °C)	p = <0.05 (>15 °C)	p = <0.05
Chl a ($\mu\text{g L}^{-1}$)			15.57, -20.21	25.35, -32.57	7.25, -2.33	6.54, -2.03		p = <0.05 for H - L (depth was not in the final model)					
PVI (%)	0.01		3.51, -4.98	0.71, -0.04	8.09, -16.86	3.89, -5.83		p = <0.05	p = <0.05	n.s.	p = <0.05	p = <0.05	p = <0.05

SI.D Country- and month-wise treatment effects on metabolism

To obtain an idea of the variability between countries for absolute metabolism at 15 °C and the activation energy, we included the factor country and the interaction between country and inverse-scaled temperature in the fixed effects of the model (6). The country-wise analysis of volumetric and integrated GPP, ER and ER/GPP ratio was based on the following mixed effect model:

$$\ln(\text{Parameter}) = \text{invT} + D + N + C + \text{invT} \times C + (\text{invT}|C : Id) + (1|C : M)$$

, where invT is the inverse-scaled water temperature, D and N are the treatment factors for depth and nutrient level, respectively, C stands for the factor country, Id uniquely identifies each mesocosm and M stands for month. An intercept and slope model for the random factor Id nested in country and an intercept model for only the random factor month nested in the country were considered. Model selection, validation and fit are assessed as described in the method part of the main text. Following the hypothesis that warm adaptation leads to lower temperature sensitivity than cold adaptation (Atkin and Tjoelker, 2003), we would expect a systematic decrease in activation energy with increasing average country temperature and, possibly, a decrease in average absolute metabolic rate at the reference temperature. We tested for systematic increases or decreases in country-wise activation energies and average absolute metabolic rates using a Helmert contrast with the factor variable country following the same order as the country-wise average air temperature (Table 3.1) over the experimental period (SE, EE, CZ, GE, TR, GR). The results are summarised in Table S4.1. In addition, Spearman correlations between average temperature of the countries and estimated country-wise absolute metabolic rates and activation energies were calculated (Table S4.2).

In a second set of models, to examine the variability in temperature sensitivity along the gradient, while excluding seasonal adaption processes, the factor month as well as the interaction between month and inverse-scaled water temperature were included in the fixed effects model (6), leading to the following model:

$$\ln(\text{Parameter}) = T + D + N + M + T \times M + (1|C).$$

In this model, again assuming a potentially lower temperature sensitivity and absolute metabolism at 15 °C for warm-adapted communities (Atkin and Tjoelker, 2003), lower temperature sensitivity could be expected if either large differences exist between the average temperature of the countries or if average temperature levels are relatively high in all countries. We tested for systematic increases or decreases in activation energies and absolute metabolic rates between months using a Helmert contrast with the factor variable month (Nov, Oct, June, July, August) ordered relative to average monthly temperature for all countries (Table S1.3). The results are summarised in

Table S4.3. We further tested for systematic differences using Spearman correlation between average monthly temperature and metabolic rate at 15 °C as well as temperature sensitivity (Table S4.4). Yet, it is important to note that, theoretically, sampling at different temperatures from different communities with different temperature sensitivity and reference metabolic rates does not necessarily lead to a linear relation, as depicted by the Arrhenius plot.

However, even though acclimation and adaptation processes are an important aspect of how ecosystem metabolism will be affected by increasing temperatures, due to the unbalanced nature of the data set, the results are subject to high uncertainty and require careful interpretation.

Table S4.1: Statistical results for models with country as fixed effect. The following abbreviations are used: Int = Intercept at 15 °C (Tc), invT = Inverted and scaled water temperature T in Kelvin ($k^{-1} T^{-1} - k^{-1} T_c^{-1}$), D = Depth, N = Nutrient, C = Country (SE = Sweden, EE = Estonia, CZ = Czech Republic, GE = Germany, TR = Turkey, GR = Greece). Systematic differences between countries were tested using a Helmert contrast, for instance $C_SE_EE : CZ$ is the difference between average metabolic rates at 15 °C of SE and EE against the average rate in CZ, and $invT \times C_SE_EE : CZ$ is the difference in average activation energy of SE and EE against the difference in average activation energy of CZ.

Response	Predictor	Estimate	Std. error	T-value	P-value	R ²
ln(GPP _{Vol})	Int	11.40	0.07	160.69	<0.01	0.65, 0.80
	invT	-0.78	0.07	-6.69	<0.01	
	D	0.57	0.03	-9.88	<0.01	
	N	-0.55	0.03	10.45	<0.01	
	C_SE:EE	-0.08	0.10	-2.10	0.04	
	C_SE_EE:CZ	0.04	0.06	3.52	<0.01	
	C_SE_EE_CZ:GE	0.04	0.04	1.22	0.23	
	C_SE_EE_CZ_GE:TR	0.02	0.03	1.91	0.07	
	C_SE_EE_CZ_GE_TR:GR	0.09	0.05	2.31	0.03	
	invT x C_SE:EE	-0.40	0.12	-2.08	0.05	
	invT x C_SE_EE:CZ	0.50	0.08	4.17	<0.01	
	invT x C_SE_EE_CZ:GE	0.03	0.06	0.34	0.74	
	invT x C_SE_EE_CZ_GE:TR	0.13	0.03	2.46	0.02	
	invT x C_SE_EE_CZ_GE_TR:GR	0.06	0.03	1.04	0.31	
ln(ER _{Vol})	Int	11.43	0.09	127.04	<0.01	0.64, 0.86
	invT	-0.94	0.09	-6.86	<0.01	
	D	-0.58	0.03	-9.40	<0.01	
	N	0.48	0.03	7.93	<0.01	
	C_SE:EE	-0.24	0.13	-4.15	<0.01	
	C_SE_EE:CZ	0.00	0.08	1.87	0.07	
	C_SE_EE_CZ:GE	0.05	0.06	1.91	0.07	
	C_SE_EE_CZ_GE:TR	0.08	0.04	3.18	<0.01	
	C_SE_EE_CZ_GE_TR:GR	0.20	0.05	5.04	<0.01	
	invT x C_SE:EE	-0.97	0.14	-4.25	<0.01	
	invT x C_SE_EE:CZ	0.44	0.09	3.13	0.01	
	invT x C_SE_EE_CZ:GE	0.19	0.07	1.73	0.10	
	invT x C_SE_EE_CZ_GE:TR	0.16	0.04	2.53	0.02	
	invT x C_SE_EE_CZ_GE_TR:GR	0.22	0.04	3.53	<0.01	
ln(GPP _{Vol-dl})	Int	8.74	0.07	116.72	<0.01	0.61, 0.78
	invT	-0.43	0.08	-3.47	<0.01	
	D	-0.54	0.03	-9.71	<0.01	
	N	0.58	0.03	10.43	<0.01	
	C_SE:EE	-0.04	0.11	-1.72	0.10	
	C_SE_EE:CZ	0.06	0.06	3.67	<0.01	
	C_SE_EE_CZ:GE	0.06	0.05	1.69	0.10	
	C_SE_EE_CZ_GE:TR	0.05	0.04	2.31	0.03	
	C_SE_EE_CZ_GE_TR:GR	0.12	0.05	2.86	0.01	

Table S4.1 Continued from previous page

Response	Predictor	Estimate	Std. error	T-value	P-value	R ²
	invT x C_SE:EE	-0.43	0.13	-2.11	0.05	
	invT x C_SE_EE:CZ	0.48	0.08	3.76	<0.01	
	invT x C_SE_EE_CZ:GE	0.04	0.06	0.41	0.68	
	invT x C_SE_EE_CZ_GE:TR	0.09	0.04	1.59	0.13	
	invT x C_SE_EE_CZ_GE_TR:GR	0.04	0.04	0.69	0.52	
ln(GPP _{Area})	Int	11.43	0.06	198.29	<0.01	0.64, 0.77
	invT	-0.91	0.06	-9.71	<0.01	
	D	0.39	0.03	7.33	<0.01	
	N	0.57	0.03	10.79	<0.01	
	C_SE:EE	-0.13	0.08	-2.89	0.01	
	C_SE_EE:CZ	0.05	0.05	4.58	0.00	
	C_SE_EE_CZ:GE	0.03	0.03	1.15	0.26	
	C_SE_EE_CZ_GE:TR	-0.05	0.03	-1.01	0.32	
	C_SE_EE_CZ_GE_TR:GR	-0.08	0.04	-2.71	0.01	
	invT x C_SE:EE	-0.34	0.10	-2.21	0.04	
	invT x C_SE_EE:CZ	0.51	0.06	5.19	<0.01	
	invT x C_SE_EE_CZ:GE	0.02	0.05	0.28	0.78	
	invT x C_SE_EE_CZ_GE:TR	0.06	0.03	1.53	0.14	
	invT x C_SE_EE_CZ_GE_TR:GR	-0.05	0.03	-1.18	0.25	
ln(ER _{Area})	Int	11.43	0.07	165.69	<0.01	0.63, 0.82
	invT	-1.08	0.07	-10.32	<0.01	
	D	0.44	0.03	8.38	<0.01	
	N	0.48	0.03	9.26	<0.01	
	C_SE:EE	-0.27	0.10	-5.39	<0.01	
	C_SE_EE:CZ	0.00	0.06	2.55	0.02	
	C_SE_EE_CZ:GE	0.04	0.04	2.19	0.04	
	C_SE_EE_CZ_GE:TR	0.02	0.03	1.41	0.17	
	C_SE_EE_CZ_GE_TR:GR	0.00	0.04	0.50	0.62	
	invT x C_SE:EE	-0.86	0.11	-4.86	<0.01	
	invT x C_SE_EE:CZ	0.44	0.07	3.99	<0.01	
	invT x C_SE_EE_CZ:GE	0.16	0.05	2.04	0.06	
	invT x C_SE_EE_CZ_GE:TR	0.09	0.03	1.92	0.07	
	invT x C_SE_EE_CZ_GE_TR:GR	0.06	0.03	1.25	0.23	
ln(GPP _{Area-dl})	Int	8.77	0.06	145.29	<0.01	0.56, 0.73
	invT	-0.56	0.06	-5.65	<0.01	
	D	0.40	0.03	7.45	<0.01	
	N	0.57	0.03	10.75	<0.01	
	C_SE:EE	-0.09	0.09	-2.43	0.02	
	C_SE_EE:CZ	0.08	0.05	4.80	<0.01	
	C_SE_EE_CZ:GE	0.05	0.04	1.76	0.09	
	C_SE_EE_CZ_GE:TR	-0.02	0.03	-0.31	0.76	
	C_SE_EE_CZ_GE_TR:GR	-0.04	0.04	-1.74	0.10	
	invT x C_SE:EE	-0.37	0.10	-2.27	0.03	
	invT x C_SE_EE:CZ	0.49	0.06	4.72	<0.01	
	invT x C_SE_EE_CZ:GE	0.03	0.05	0.38	0.71	
	invT x C_SE_EE_CZ_GE:TR	0.02	0.03	0.51	0.62	
	invT x C_SE_EE_CZ_GE_TR:GR	-0.07	0.03	-1.53	0.14	
ln(ER:GPP)	Int	0.01	0.06	-0.72	0.48	0.23, 0.58
	invT	-0.16	0.07	-1.47	0.15	
	D	-0.02	0.02	-0.33	0.74	
	N	-0.09	0.02	-2.01	0.05	
	C_SE:EE	-0.14	0.10	-3.04	<0.01	
	C_SE_EE:CZ	-0.05	0.05	-1.17	0.25	
	C_SE_EE_CZ:GE	0.00	0.04	1.18	0.25	
	C_SE_EE_CZ_GE:TR	0.06	0.03	2.19	0.04	
	C_SE_EE_CZ_GE_TR:GR	0.08	0.04	3.02	0.01	
	invT x C_SE:EE	-0.50	0.11	-2.72	0.01	

Table S4.1 Continued from previous page

Response	Predictor	Estimate	Std. error	T-value	P-value	R ²
	invT x C_SE_EE:CZ	-0.06	0.07	-0.51	0.62	
	invT x C_SE_EE_CZ:GE	0.13	0.05	1.61	0.12	
	invT x C_SE_EE_CZ_GE:TR	0.02	0.03	0.42	0.68	
	invT x C_SE_EE_CZ_GE_TR:GR	0.11	0.03	2.30	0.03	

Table S4.2: Table of country-wise activation energies and metabolic rates at 15 °C plus/minus standard error. In addition, Spearman correlation coefficients (r) are calculated between country-wise average water temperature and activation energies and metabolic rates at 15 °C.

		GPPVol	GPPVol_dl	GPPArea	GPPArea_dl	ERVVol	ErArea	ER/GPP
Slope (eV)	SE	-0.69 ± 0.15	-0.41 ± 0.16	-0.69 ± 0.12	-0.41 ± 0.13	-0.62 ± 0.18	-0.61 ± 0.13	0.08 ± 0.14
	EE	-1.19 ± 0.18	-0.95 ± 0.19	-1.11 ± 0.15	-0.87 ± 0.16	-1.82 ± 0.22	-1.69 ± 0.18	-0.54 ± 0.18
	CZ	0.00 ± 0.19	0.23 ± 0.21	0.05 ± 0.16	0.27 ± 0.16	-0.39 ± 0.22	-0.33 ± 0.17	-0.34 ± 0.18
	GE	-0.55 ± 0.21	-0.28 ± 0.22	-0.53 ± 0.16	-0.26 ± 0.17	-0.48 ± 0.24	-0.46 ± 0.18	0.07 ± 0.13
	TR	-0.20 ± 0.14	-0.07 ± 0.15	-0.37 ± 0.11	-0.25 ± 0.12	-0.33 ± 0.17	-0.48 ± 0.12	-0.12 ± 0.13
	GR	-0.31 ± 0.19	-0.15 ± 0.20	-0.73 ± 0.15	-0.57 ± 0.16	0.11 ± 0.22	-0.49 ± 0.17	0.26 ± 0.17
	Correlation coefficient with mean air temperature	r = 0.54 p = 0.30	r = 0.54 p = 0.30	r = 0.14 p = 0.8	r = 0.14, p = 0.8	r = 0.88 p = 0.3	r = 0.37, p = 0.5	r = 0.37 p = 0.50
Intercept at 15 °C (average of treatments)	SE	10.94 ± 0.13	8.27 ± 0.14	11.25 ± 0.11	8.58 ± 0.11	11.02 ± 0.17	11.33 ± 0.13	0.08
	EE	10.52 ± 0.16	7.90 ± 0.17	10.77 ± 0.14	8.16 ± 0.14	9.92 ± 0.21	10.22 ± 0.17	-0.52
	CZ	11.33 ± 0.14	8.74 ± 0.15	11.65 ± 0.12	9.07 ± 0.12	10.89 ± 0.19	11.22 ± 0.15	-0.41
	GE	11.14 ± 0.16	8.61 ± 0.17	11.38 ± 0.13	8.86 ± 0.13	11.04 ± 0.20	11.30 ± 0.15	-0.09
	TR	11.30 ± 0.15	8.79 ± 0.16	11.13 ± 0.12	8.62 ± 0.13	11.40 ± 0.20	11.24 ± 0.15	0.10
	GR	11.67 ± 0.27	9.29 ± 0.28	10.64 ± 0.22	8.26 ± 0.23	12.48 ± 0.31	11.18 ± 0.24	0.56
	Correlation coefficient with mean air temperature	r = 0.77, p = 0.10	r = 0.89, p = 0.03	r = 0.37, p = 0.5	r = 0.09, p = 0.92	r = 0.83, p = 0.06	r = -0.26 p = 0.66	r = 0.66 p = 0.18

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Table S4.3: Statistical results for models with month as fixed effect. The following abbreviations are used: Int = Intercept at 15 °C (Tc), invT = Inverted and scaled water temperature T in Kelvin ($k^{-1} T^{-1} - k^{-1} T_c^{-1}$), D = Depth, N = Nutrient, M = Month (N = November, O = October, J = July, S = September, A = August). Systematic differences between countries were tested using a Helmert contrast, for instance $M_{NO} : J$ is the difference between average metabolic rates at 15 °C in November and October against the average rate in July, and $invT \times M_{NO} : J$ is the difference in average activation energy in November and October against the difference in average activation energy in July.

Response	Predictor	Estimate	Std. error	T-value	P-value	R ²
ln(GPPVol)	Int	11.47	0.08	134.00	<0.01	0.61, 0.63
	invT	-1.10	0.10	-6.55	<0.01	
	D	-0.56	0.02	-11.21	<0.01	
	N	0.58	0.02	11.96	<0.01	
	M_N:O	0.08	0.09	0.23	0.82	
	M_NO:J	-0.02	0.09	0.99	0.33	
	M_NOJ:S	-0.04	0.03	-2.50	0.01	
	M_NOJS_A	-0.10	0.03	-3.96	<0.01	
	InvT x M_N:O	-0.20	0.10	-1.18	0.24	
	InvT x M_NO:J	0.32	0.06	3.31	<0.01	
	InvT x M_NOJ:S	-0.14	0.04	-2.35	0.02	
	InvT x M_NOJS_A	-0.07	0.03	-1.63	0.10	
ln(ERVol)	Int	11.55	0.11	101.48	<0.01	0.57, 0.61
	invT	-1.48	0.13	-6.84	<0.01	
	D	-0.56	0.03	-9.87	<0.01	
	N	0.51	0.03	9.22	<0.01	
	M_N:O	-0.12	0.10	-1.03	0.31	
	M_NO:J	-0.25	0.11	-1.47	0.15	
	M_NOJ:S	-0.07	0.04	-2.40	0.02	
	M_NOJS_A	-0.08	0.03	-1.66	0.10	
	InvT x M_N:O	0.06	0.12	0.31	0.76	
	InvT x M_NO:J	0.30	0.07	2.65	0.01	
	InvT x M_NOJ:S	-0.07	0.04	-1.09	0.28	
	InvT x M_NOJS_A	0.08	0.03	1.52	0.13	
ln(GPPVol_dl)	Int	8.79	0.09	88.90	<0.01	0.57, 0.61
	invT	-1.11	0.12	-5.94	<0.01	
	D	-0.56	0.02	-11.55	<0.01	
	N	0.58	0.02	12.24	<0.01	
	M_N:O	-0.07	0.09	-0.12	0.90	
	M_NO:J	-0.14	0.09	-0.73	0.47	
	M_NOJ:S	-0.04	0.03	-2.65	0.01	
	M_NOJS_A	-0.12	0.03	-5.20	<0.01	
	InvT x M_N:O	-0.26	0.10	-1.61	0.11	
	InvT x M_NO:J	0.22	0.06	2.27	0.02	
	InvT x M_NOJ:S	-0.15	0.04	-2.65	0.01	
	InvT x M_NOJS_A	-0.10	0.03	-2.36	0.02	
ln(GPPArea)	Int	11.45	0.15	75.86	<0.01	0.56, 0.70
	invT	-1.13	0.14	-5.10	<0.01	
	D	0.38	0.02	8.01	<0.01	
	N	0.57	0.02	12.33	<0.01	
	M_N:O	-0.06	0.09	-0.40	0.69	
	M_NO:J	-0.05	0.10	-0.70	0.49	
	M_NOJ:S	0.00	0.03	0.21	0.83	
	M_NOJS_A	-0.07	0.03	-3.58	<0.01	
	InvT x M_N:O	0.07	0.10	0.41	0.68	
	InvT x M_NO:J	-0.05	0.06	-0.56	0.57	
	InvT x M_NOJ:S	0.03	0.03	0.55	0.58	
	InvT x M_NOJS_A	-0.13	0.03	-2.99	<0.01	
ln(ERArea)	Int	11.53	0.11	96.56	<0.01	0.59, 0.65
	invT	-1.36	0.13	-6.34	<0.01	

Table S4.3 Continued from previous page

Response	Predictor	Estimate	Std. error	T-value	P-value	R ²
	D	0.39	0.03	7.61	<0.01	
	N	0.49	0.02	9.96	<0.01	
	M_N:O	-0.21	0.09	-1.30	0.20	
	M_NO:J	-0.23	0.10	-2.46	0.02	
	M_NOJ:S	-0.02	0.04	0.17	0.86	
	M_NOJS_A	-0.04	0.03	-1.08	0.28	
	InvT x M_N:O	0.29	0.11	1.66	0.10	
	InvT x M_NO:J	-0.05	0.06	-0.54	0.59	
	InvT x M_NOJ:S	0.09	0.04	1.59	0.11	
	InvT x M_NOJS_A	0.03	0.03	0.61	0.54	
ln(GPPArea_dl)	Int	8.78	0.15	56.79	<0.01	0.51, 0.68
	invT	-1.21	0.14	-5.57	<0.01	
	D	0.38	0.02	8.16	<0.01	
	N	0.57	0.02	12.61	<0.01	
	M_N:O	-0.09	0.09	-0.94	0.35	
	M_NO:J	-0.19	0.10	-2.50	0.01	
	M_NOJ:S	-0.01	0.03	-0.06	0.95	
	M_NOJS_A	-0.10	0.03	-5.03	<0.01	
	InvT x M_N:O	0.01	0.10	0.09	0.92	
	InvT x M_NO:J	-0.17	0.06	-1.80	0.07	
	InvT x M_NOJ:S	0.02	0.03	0.32	0.75	
	InvT x M_NOJS_A	-0.15	0.03	-3.73	<0.01	
ln(ER/GPP)	Int	0.08	0.10	-0.52	0.62	0.10, 0.23
	invT	-0.42	0.12	-2.20	0.03	
	D	0.00	0.02	0.04	0.97	
	N	-0.07	0.02	-1.65	0.10	
	M_N:O	-0.22	0.08	-1.59	0.11	
	M_NO:J	-0.25	0.09	-2.81	0.01	
	M_NOJ:S	-0.03	0.03	-0.42	0.68	
	M_NOJS_A	0.01	0.03	2.07	0.04	
	InvT x M_N:O	0.26	0.10	1.70	0.09	
	InvT x M_NO:J	-0.03	0.06	-0.31	0.76	
	InvT x M_NOJ:S	0.06	0.03	1.19	0.23	
	InvT x M_NOJS_A	0.15	0.03	3.74	<0.01	

Table S4.4: Table of month-wise activation energies and metabolic rates at 15 °C plus/minus standard error. The month refers to the periods defined in Table S1.2 and they are listed relative to increasing average temperature (Table S1.3) as used with the Helmert contrast. In addition, Spearman correlation coefficients (r) are calculated between month-wise average water temperature and activation energies and metabolic rates at 15 °C.

		GPP _{Vol}	GPP _{Vol-dl}	GPP _{Area}	GPP _{Area-dl}	ER _{Vol}	ER _{Area}	ER:GPP
Slope (eV)	November	-0.63 ± 0.20	-0.51 ± 0.21	-0.65 ± 0.22	-0.58 ± 0.22	-1.15 ± 0.24	-1.07 ± 0.23	-0.54 ± 0.20
	October	-0.88 ± 0.11	-0.83 ± 0.11	-0.57 ± 0.12	-0.56 ± 0.12	-1.07 ± 0.13	0.71 ± 0.13	-0.22 ± 0.11
	July	-0.15 ± 0.18	-0.27 ± 0.19	-0.71 ± 0.21	-0.88 ± 0.21	-0.56 ± 0.22	-0.99 ± 0.21	-0.43 ± 0.19
	September	-0.89 ± 0.15	-0.91 ± 0.15	-0.57 ± 0.17	-0.63 ± 0.17	-1.10 ± 0.18	-0.69 ± 0.17	-0.24 ± 0.15
	August	-0.86 ± 0.14	-0.95 ± 0.15	-1.02 ± 0.17	-1.14 ± 0.16	-0.73 ± 0.17	-0.78 ± 0.17	0.12 ± 0.15
	Correlation coefficient with mean air temperature	R = -0.3 p = 0.68	R = -0.7 p = 0.23	R = -0.3 p = 0.68	R = -0.8 p = 0.13	R = 0.50 p = 0.45	R = 0.50 p = 0.45	R = 0.7 p = 0.23
Intercept at 15 °C (average of treatments)	November	11.21 ± 0.18	8.75 ± 0.19	11.29 ± 0.22	8.87 ± 0.22	11.48 ± 0.22	11.49 ± 0.21	0.29 ± 0.19
	October	11.25 ± 0.08	8.73 ± 0.09	11.22 ± 0.14	8.70 ± 0.14	11.28 ± 0.10	11.24 ± 0.11	0.02 ± 0.09
	July	11.49 ± 0.24	8.55 ± 0.26	11.05 ± 0.29	8.04 ± 0.29	10.92 ± 0.30	10.62 ± 0.29	-0.61 ± 0.26
	September	10.99 ± 0.13	8.33 ± 0.14	11.22 ± 0.18	8.53 ± 0.18	10.86 ± 0.16	11.14 ± 0.16	-0.15 ± 0.14
	August	10.64 ± 0.17	7.81 ± 0.18	10.65 ± 0.21	7.78 ± 0.21	10.84 ± 0.20	10.95 ± 0.20	0.19 ± 0.18
	Correlation coefficient with mean air temperature	r = -0.6 p = 0.35	r = -1 p = 0.02	r = -0.9 p = 0.08	r = -0.9 p = 0.08	r = -1 p = 0.02	r = -0.7 p = 0.23	r = -0.3 p = 0.68

Chapter 4

Early-warning indicators of critical transitions in natural aquatic ecosystems

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4.1 Abstract

Complex dynamic systems can show sudden and persistent changes in system state despite only small changes in drivers. Such critical transitions are difficult to predict as the state of the system often shows little change prior to the transition. Early-warning indicators signal the loss of system resilience and have been shown to precede critical transitions in theoretical models, paleo-climate time series, as well as in laboratory and whole lake experiments. However, the generality of early-warning indicators for detection of critical transitions in empirical time series of natural aquatic ecosystems remains largely untested. Here, we assessed four commonly used early-warning indicators on long-term datasets of five freshwater ecosystems that have experienced a critical transition and for which the relevant ecological mechanisms and drivers are now known. These case studies were categorised by three critical-transition generating mechanisms: competition, trophic cascade, and intra-guild predation. While early-warning indicators could be detected in most case studies, agreement among the four indicators was low. Nevertheless, in some cases early-warning indicators were detectable considerably ahead of the transition, suggesting that they may provide an early warning indicator for use by lake managers. At the present state of knowledge, however, early-warning indicators do not provide reliable and consistent signals of impending critical transitions. Our study strongly suggests that *a priori* knowledge of the underlying processes driving ecosystem transitions is necessary to identify the relevant state variables that are needed to successfully monitor early-warning indicators.

4.2 Introduction

Complex dynamic systems (e.g. ecosystems) can show multi-state stability and occasionally sudden transitions from one regime to another despite only slow changes in drivers (Lewontin, 1969; May, 1977; Holling, 1973; Sutherland, 1974; Scheffer et al., 2001). These critical transitions are characterised 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 stabilised by feedback loops, the thresholds for the forward and backward shifts may differ, resulting in hysteresis (Scheffer et al., 2001). A well-known example is the nutrient-driven shift between the clear, macrophyte-dominated and the turbid, phytoplankton-dominated regime in some shallow lakes (Scheffer et al., 2001, 1993). At the ecosystem level, such fundamental reorganisations affect ecological processes and hence ecosystem services and can incur large economic costs. Therefore, reliable tools to assess regime resilience are sought, ideally providing management with time to avert an impending critical transition (Biggs et al., 2009). Stabilisation through feedback loops, however, often precludes a systematic response in state variables ahead of the shift, rendering prediction of critical transitions difficult (Ditlevsen and Johnsen, 2010).

The development of early-warning indicators (EWI) derived from bifurcation theory has drawn considerable interest as a way forward, particularly for their promise of generality and detection power. These EWIs have been shown to precede critical transitions in modelled (Ditlevsen and Johnsen, 2010; Dakos et al., 2012a; Carpenter et al., 2008), experimental time series (Veraart et al., 2011; Batt et al., 2013b; Dai et al., 2012), reconstructed paleo-climate time series (Lenton et al., 2012; Dakos et al., 2008) and whole-lake experiments (Carpenter et al., 2001). Up to now, however, an assessment of the generality and the detection power of EWIs on long-term monitoring data in aquatic systems is lacking (Seekell et al., 2012).

Early-warning indicators are typically statistical metrics that quantify the loss of temporal or spatial resilience in a system and thereby provide advance warning of the potential proximity of a critical threshold (Scheffer et al., 2009). Several of these EWIs are related to critical slowing down, a characteristic property of dynamic systems close to catastrophic local bifurcations (van Nes and Scheffer, 2007). A bifurcation marks a threshold where the stability properties of the state of the system change. As the system approaches such a threshold, the return rate to equilibrium after a small perturbation slows down so that the system tends to become more similar to its own past, resulting in an increasing trend in autocorrelation at lag-1 (AR-1) (Ives, 1995). This lack of decay of the impact of past perturbations also leads to a build-up in variance, typically measured as an increasing trend in standard deviation (SD) (Carpenter and Brock, 2006). In addition to critical slowing down, the system tends to remain for longer at the basin boundary between the two alternative attractors resulting in a skewed distribution of the state variable (SK) (Guttal and Jayaprakash, 2008). A concurrent increase in SD and AR-1 in a time series also

produces higher variability in low frequency processes compared to high frequency processes in the power spectrum of a time series, which can be quantified as an increasing density ratio (DR) of variance at low to high frequencies (Biggs et al., 2009). The expected trends in AR-1, SD, skewness, and density ratio are, however, not exclusive indicators of critical transitions as false positives and false negatives can occur (Boettiger et al., 2013). Hence, prior to the application of EWIs based on critical slowing down a careful analysis is needed of whether a system is undergoing a critical transition (Dakos et al., 2014).

Regime shifts can develop from several mechanisms (Andersen et al., 2009), including: i) linear tracking of large changes in environmental conditions, ii) non-linear but continuous (reversible) responses to gradual changes in environmental conditions, or iii) non-linear discontinuous (irreversible) responses to gradual changes in environmental conditions (Andersen et al., 2009). While the first mechanism can be recognised by concurrent large changes in environmental drivers (e.g. a sudden increase in temperature), the difference between the other two examples will only become apparent when the driver is reversed. Hence, single step changes in time series cannot provide direct evidence of a critical transition (Scheffer and Carpenter, 2003). Empirical time series covering forward and backward shifts are rare. Thus, at present, the only way to identify a true critical transition is to link observed step changes to a mechanistic understanding of the driving processes that can give rise to bi-stability (Scheffer et al., 2001; Collie et al., 2004).

Lake systems have been proposed as particularly suitable ecosystems to test for critical transitions and associated EWI, also because the modular nature of lakes allows comparison across several lakes (Carpenter, 2003). In aquatic systems, a number of ecological mechanisms have been shown to generate regime shifts between alternative states. The most commonly identified mechanisms include i) competition between two or more species (May, 1977; Collie et al., 2004), ii) trophic cascades through inclusion or exclusion of top predators (Carpenter et al., 2001) or parasites (Gerla et al., 2013) resulting in overexploitation traps, and iii) intra-guild predation through resource competitors that also prey on each other (Verdy and Amarasekare, 2010; Scharfenberger et al., 2013). Natural systems are usually affected by a combination of external (environmental) and internal (trophic) processes (Brock and Carpenter, 2010). This in turn has sparked a discussion on whether the noise to signal ratios in empirical time series allow the detection of EWIs at all (Perretti and Munch, 2012). For the purpose of this paper, we selected 14 state variables of five well-documented freshwater case studies of critical transitions to test whether four commonly used EWIs (AR1, SD, SK and DR) can be detected reliably in advance of the transition. We further assessed proportional agreement among these indicators and explored whether EWI outcome depended on the mechanism, the type of state variable, the magnitude of the regime shift, and the sampling frequency. Lastly, we also tested how many years ahead of the regime shift EWIs were detectable. This study provides the first comprehensive assessment of EWIs in some of the best-documented aquatic critical transitions using time series collected in standard monitoring schemes.

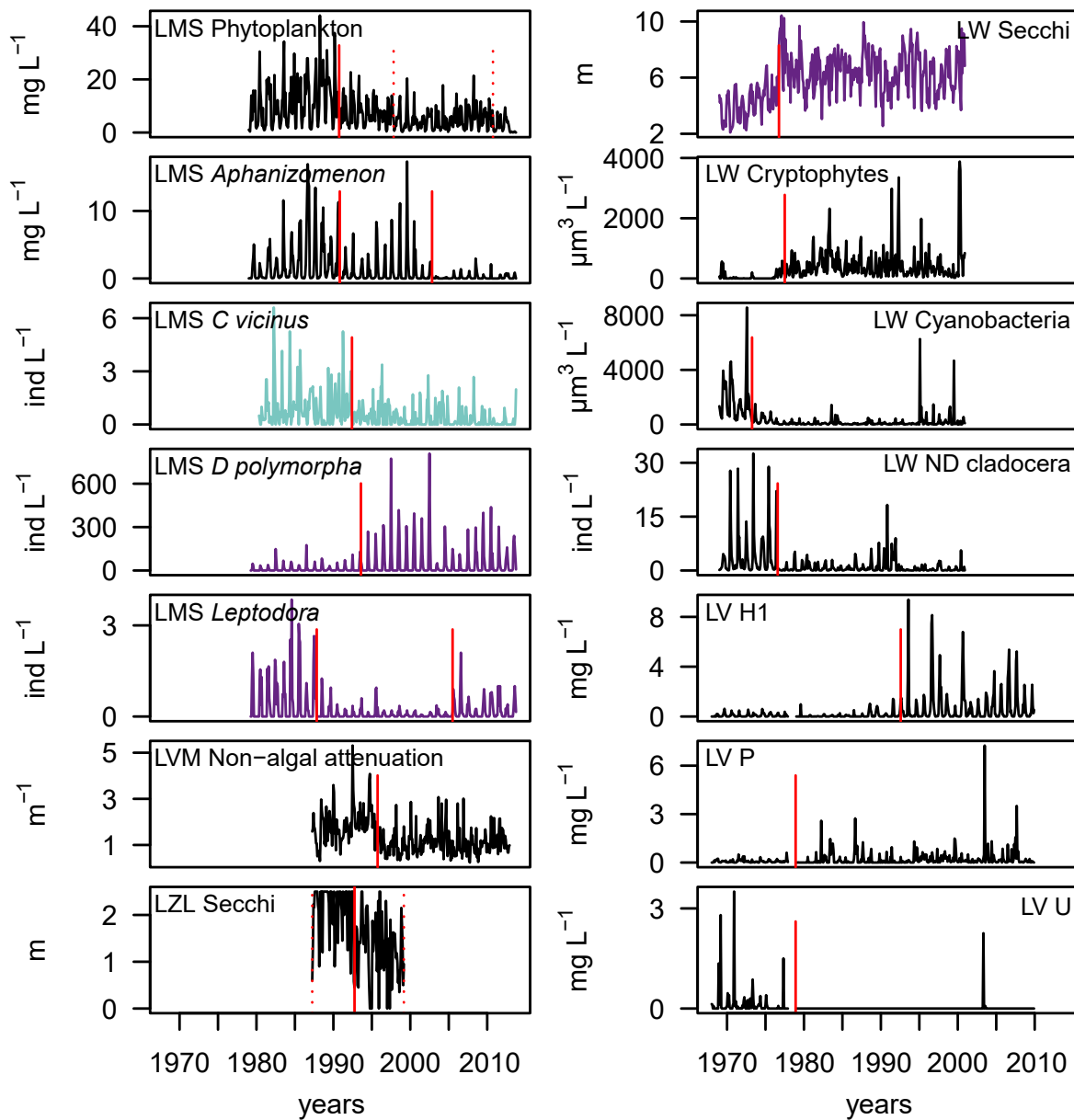


Figure 4.1: Time series of 14 selected state variables (black lines: competition, purple lines: trophic cascade, light blue line: intraguild predation) across five aquatic systems. Solid red lines indicate the timing of critical transitions (estimated by breakpoint analysis, see Methods). Dashed red lines indicate additional data-based breakpoints that were not used in this study as they were either not described in the literature (LMS phytoplankton biomass) or resulted from bio-manipulation (LZL Secchi depth)).

4.3 Results

Selection of case study ecosystems and state variables – We selected five case-study ecosystems based on expert knowledge of well-described critical transitions in aquatic ecosystems: Lake Müggelsee (Germany, LMS), Lake Veluwemeer (LVM) and Lake Zwemlust (The Netherlands, LZL), Lake Washington (United States, LW), and Lake Võrtsjärv (Estonia, LV). Based on the

literature on these case-study ecosystems, we identified relevant critical-transition generating mechanisms, which guided our choice of 14 state variables for EWI analysis. A short summary of the case study ecosystems, mechanisms, state variables and drivers is presented in Table 4.1. A more detailed description of each case study ecosystem and reasoning for the choice of 14 state variables is presented in the SI Appendix (Case studies SI.A).

Table 4.1: Summary of case study systems, relevant state variables and drivers by mechanism: trophic cascade, intraguild predation and competition.

Case Study	Shift in State Variable	Driver	Process	References
Trophic cascade				
LW	Increased water transparency	Increase in grazing pressure	Trophic cascade from longfin smelt (<i>Spirinchus thaleichthys</i>) on secondary consumer <i>Neomysis</i> on primary consumer <i>Daphnia</i> and finally on producers (phytoplankton)	Hampton et al. (2006), Edmondson (1994)
LMS	Increased <i>Dreissena polymorpha</i> larvae density	Decrease in predation pressure	Changes in dominant carnivorous zooplankton species coincided with increase in <i>D polymorpha</i> larvae	Wilhelm and Adrian (2007) , Gsell et al. (2016)
	Decreased <i>Leptodora kindtii</i> (period 1)	Increase in predation pressure	<i>Leptodora kindtii</i> are a preferred prey for fish and likely indicate changes in overall fish predation pressure	
	Increased <i>Leptodora kindtii</i> (period 2)	Decrease in predation pressure		
Intraguild predation				
LMS	<i>Cyclops vicinus</i> and <i>Cyclops kolensis</i> dominance switch	Reduction of shared food source	The inferior resource competitor <i>C vicinus</i> preys on juveniles of the smaller <i>C kolensis</i>	Scharfenberger et al. (2013)
Competition				
LMS	Decreased phytoplankton biomass	Re-oligotrophication	Reduction in nutrients decreases phytoplankton growth, improving light climate favouring macrophyte reestablishment	Kohler et al. (2005) Hilt et al. (2013)
	Decreased <i>Aphanizomenon</i> biomass (period 1)	Re-oligotrophication counteracted by spring warming	Warmer springs promote cold-adapted cyanobacteria development	Shatwell et al. (2008)
	Decreased <i>Aphanizomenon</i> biomass (period 2)	Re-oligotrophication	Reduction in nutrients decreases phytoplankton growth, improving light climate favouring macrophyte reestablishment	Kohler et al. (2005)
LW	Decreased non- <i>Daphnia</i> cladoceran density	Reduction in predation pressure on <i>Daphnia</i>	Indirect effect of trophic cascade through increasing resource competition by <i>Daphnia</i>	Hampton et al. (2006), Edmondson (1994)
	Increased cryptophytes biovolume	Re-oligotrophication	Reduction in nutrients decreases cyanobacteria competitive ability and release other phytoplankton from competition	
	Decreased cyanophyte biovolume	Re-oligotrophication		
LZL	Decreased water transparency	Eutrophication, epiphyte shading and herbivory	Competition between submerged vegetation and phytoplankton under eutrophication and herbivory on macrophytes	Van De Bund and Van Donk (2002), Vandonk and Gulati (1995)
LVM	Deceased non-algal attenuation	Re-oligotrophication, reduction in benthivorous fish	Recovery of submerged vegetation cover and subsequent stabilisation of sediments	Ibelings et al. (2007)
LV	Increased functional group P Decreased functional group U	Eutrophication	Competition between functional groups P (eutrophic epilimnion species) and U (summer epilimnion species)	Nöges et al. (2007) Nöges et al. (2010)
	Increased functional group H1	Re-oligotrophication	Competition between non-nitrogen fixing and di-nitrogen fixing species (functional group H1)	

Breakpoint detection and seasonal adjustment – We used three complementary methods (piece-wise linear regression, Pettit-test and STARs, see Methods) to robustly assess the timing of breakpoints (i.e. large, persistent step change) in the time series of each state variable. All 14 state variables showed one (n=12) or two (n=2) breakpoints (Table 4.2 and Figure 4.1), resulting

in 16 pre-breakpoint time series (i.e. from start to step change) for further analysis. In all state variables, the timing of the breakpoints confirmed the timing of critical transitions as reported in the literature (Table 4.2). Information on the data structure including time period covered by each time series, sampling interval, number of data points and the percentage of missing values in the pre-breakpoint time series are summarised in the SI Appendix (Table S2). Each state variable showed significant differences in the pre- to post-breakpoint means (Table 4.2), the step magnitude at the breakpoint ranged from 0.24*standard deviation to 1.59*standard deviation (Table 4.2). Each pre-breakpoint time series was detrended and seasonally adjusted using a Gaussian smoother with a bandwidth corresponding to 12 (monthly) or 26 (bi-weekly datasets) data points. Testing for remaining linear trends and seasonal ARIMA signals in the residual time series showed in some time series a remaining, but much reduced seasonal signal (SI Appendix, Table S2).

Table 4.2: Overview for all 14 state variables (16 time-series) listing the direction of the shift, timing of the break-point (year-month), step height (scaled to standard deviation), the two-sample Welch test significance, and for each EWI the sign of the median of the trend distribution (across all rolling window-sizes). Bold t (Welch) values are significant at the $\alpha = 0.05$ level. The tested EWS are autocorrelation at lag-1 (AR-1), standard deviation (SD), skewness (SK), and density ratio (DR). Median trends that corresponded with the theoretical expectation were coded with '+', trends that showed the opposite to the expectation with '-'. Brackets denote AR-1 trends crossing zero on the scale.

Case Study	State Variable	Direction of Shift	Break-Point (Year-Month)	Step (scaled to SD)	t (Welch)	AR-1	SD	SK	DR
LMS	Phytoplankton mg L ⁻¹	Decrease	1990-5	0.803	9.92	+	+	+	+
	<i>Aphanizomenon</i> mg L ⁻¹ P1	Decrease	1990-11	0.413	4.47	+	+	-	+
	<i>Aphanizomenon</i> mg L ⁻¹ P2	Decrease	2002-12	0.317	5.92	-	+	+	-
	<i>Cyclops vicinus</i> ind L ⁻¹	Decrease	1992-6	0.629	7.86	+	-	+	+
	<i>Dreissena polymorpha</i> larvae ind L ⁻¹	Increase	1993-9	0.388	-6.79	+	+	-	+
	<i>Leptodora kindtii</i> ind L ⁻¹ P1	Decrease	1987-11	0.776	6.58	-	+	-	-
	<i>Leptodora kindtii</i> ind L ⁻¹ P2	Increase	2005-7	0.239	-5.21	(+)	-	-	+
LW	Secchi depth m	Increase	1976-10	1.29	-21.94	+	+	-	+
	Cryptophyceae 100 μ m ³ L ⁻¹	Increase	1977-7	0.627	-13.16	(+)	-	-	+
	Cyanophyceae 100 μ m ³ L ⁻¹	Decrease	1973-4	1.587	9.33	-	-	-	-
	Non- <i>Daphnia</i> cladocerans ind L-1	Decrease	1976-8	0.783	6.09	-	+	+	-
LV	H1 g m ⁻³	Increase	1992-8	0.569	-6.75	(+)	+	-	+
	P g m ⁻³	Increase	1977-12	0.281	-4.61	(+)	-	+	+
	U g m ⁻³	Decrease	1977-12	0.532	3.17	(+)	-	+	+
LVM	Non algal attenuation m ⁻¹	Decrease	1995-9	1.058	9.05	-	-	-	-
LZL	Secchi depth m	Decrease	1992-9	1.098	7.97	(-)	+	+	-
Proportion of EWI trend distribution medians corresponding to the theoretical trend expectation						10/16	9/16	7/16	10/16

Early-warning indicator analysis – In 14 out of 16 analysed time series, a loss of resilience before the breakpoint was signalled by at least one of the four EWI metrics (coded '+' in Table 4.2, detailed figures in SI Appendix SI.C) based on the median of the trend distribution across yearly increments of rolling time-windows (see Methods). We found rising AR-1 and DR trends in 10 cases each (63 %), rising SD trends in 9 cases (56 %), and theoretically expected SK trends in 7 cases (44 %) (Figure 4.1, Table 4.2). In some cases, AR-1 trends were increasing

from negative values to positive ones (crossing 0 in AR-1 trend evolutions in figures in SI Appendix [SI.C](#)). Here we counted these trends as positive signals (coded ‘(+)’ in Table [4.2](#)). In several cases, indicators showed trends opposite to the theoretical expectation (coded ‘-’ in Table [4.2](#)). Only in one state variable (phytoplankton biomass in Lake Müggelsee, LMS) all EWIs showed the theoretically expected trends, while in two state variables (non-algal attenuation in Lake Veluwemeer (LVM) and cyanobacteria biomass in Lake Washington (LW) all EWIs failed. The proportional agreement between AR-1 and SD trends was low (5 cases, 31 %), but higher between AR-1 and DR trends (10 cases, 63 %). Logistic regressions showed no significant relationship between EWI outcome and mechanism (competition, IGP or trophic cascade), state variable category (species, group, ecosystem), step change magnitude, length of pre-breakpoint time series nor sampling interval (bi-weekly or monthly).

Sensitivity and significance of EWI trends – We estimated the sensitivity of EWI trends to the number of years covered by the rolling (data-) window approach by repeating the EWI analysis with yearly increments of data covered by the rolling window. Sensitivity was therefore shown as the distribution of trends (boxplots in Figure [4.1](#)) and expressed as the proportion of trends that did not differ qualitatively (i.e. in sign) from the median of the respective trend distribution (white bars in Figure [4.1](#)). Hence, a high proportion denotes low sensitivity to rolling-window size. A large majority of trends were not sensitive to rolling window size. Out of all 64 EWI trends (16 time series by four EWIs) only three highly significant trends were observed (using a cut-off of >50% significant differences between data-based and surrogate-based trends across all rolling-window sizes, see Methods). In 29 EWI trends fewer than 50% significant differences, and in 32 cases no significant differences between data-based and surrogate-based trends were observed (grey bars in Figure [4.1](#)).

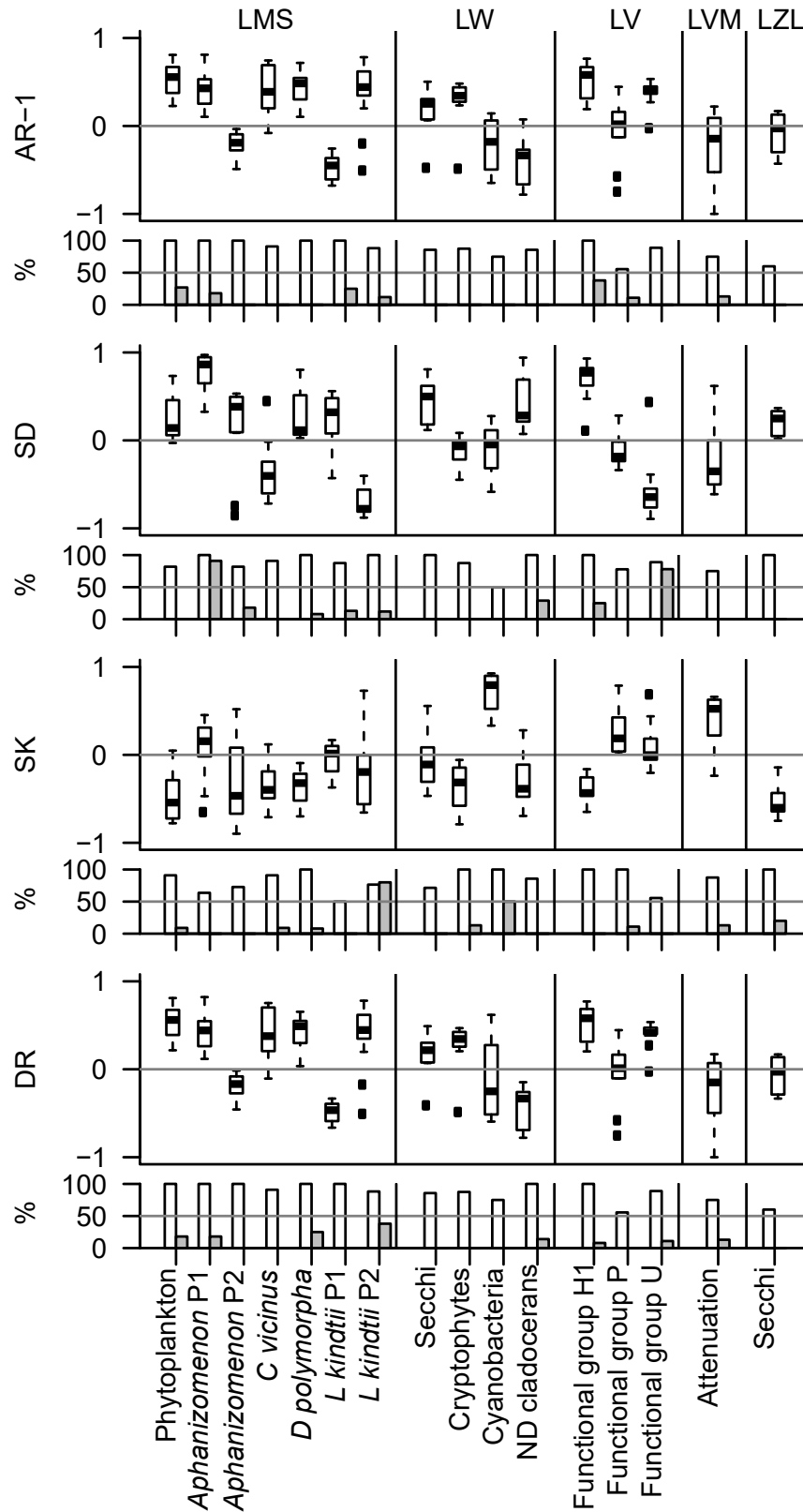


Figure 4.1: Trend distributions per Early-Warning Indicator (panel) and per analysed time series (boxplots) with the associated proportional sensitivity (white bar plot) and significance (grey bar plot).

Early warning indicators in stepwise shortened time series – To assess whether EWI trends could have been detected based on incomplete data that would have been available one or several years before the actual transition took place, we repeated the EWI analysis on stepwise shortened (yearly increment) time series of each state variable. In 14 (out of 16) time series, at least one of the EWIs would have indicated a loss in ecosystem resilience 1 to >10 years before the critical transition. In three instances EWIs only occurred in the year before the shift (Table 4.3), while in 17 instances the median of the EWI trend distribution remained positive (i.e. showing the expected sign) up to the minimum time series period that was analysed (three years).

Table 4.3: Early detection of EWIs expressed in number of years before the actual shift that each EWI would already have been detectable. Numbers indicate the maximum number of years deducted from the full pre-breakpoint time series before the theoretically expected trend disappeared (i.e. the median of the trend distribution changed sign). EWI trends were coded with ‘NA’ if they showed the opposite to the theoretically expected trends based on the full pre-breakpoint time series. Brackets denote AR-1 trends crossing zero on the scale.

Case Study	State Variable	AR-1	SD	SK	DR
LMS	Phytoplankton	3	6	4	3
	<i>Aphanizomenon</i> Period 1	>7	>7	NA	>7
	<i>Aphanizomenon</i> Period 2	NA	>7	>7	NA
	<i>Cyclops vicinus</i>	>7	NA	3	>7
	<i>Dreissena polymorpha</i> larvae	2	>10	NA	6
	<i>Leptodora kindtii</i> Period 1	NA	>4	NA	NA
	<i>Leptodora kindtii</i> Period 2	(>10)	NA	NA	9
LW	Secchi depth	0	1	NA	0
	Cryptophyceae	(>3)	NA	NA	>3
	Cyanophyceae	NA	NA	NA	NA
	Non- <i>Daphnia</i> cladocerans	NA	>4	3	NA
LV	H1	(3)	6	NA	3
	P	(1)	NA	>5	1
	U	(>5)	NA	1	>5
LVM	Non algal light attenuation	NA	NA	NA	NA
LZL	Secchi depth	(NA)	0	>3	NA

4.4 Discussion

In this study we assessed the detectability of four commonly used and easily calculated EWIs (AR-1, SD, SK and DR) in empirical time series of freshwater ecosystems that had experienced a critical transition as judged by expert knowledge. By combining high quality empirical time series with ecological theory and standardised analysis methods we show that EWIs can precede critical transitions in natural aquatic ecosystems despite potential shortcomings of empirical datasets such as observation error, sparse sampling or low signal to noise ratio (Dakos et al., 2012a; Boettiger et al., 2013; Perretti and Munch, 2012). However, in a large proportion of cases the EWIs failed, and the cases with positive EWIs generally showed low or no significance. In addition, we found also low agreement among signals. Moreover, no relationship between EWI outcome and potential predictors (mechanism, system level, magnitude of step or sampling

interval) was observed, although these results may suffer from the small dataset size. Nonetheless, in most of the records in which we positively detected EWIs, informative trends in EWIs could also have been detected a few years ahead of the actual shift.

Use of ecological understanding in the choice of state variables – The choice of case studies and state variables in our study was based on expert knowledge of ecosystems that experienced critical transitions. Such an a priori choice of state variables for EWI analysis implicitly excluded assumptions based on other transition types such as responses to step changes in the driver (Boettiger and Hastings, 2012). Moreover, even if ecosystems fall into the framework of critical transitions, widespread and strong perturbations may synchronise the timing of shifts in multiple unstable ecosystems (Reid et al., 2016) adding to the difficulty in choosing relevant variables and interpreting EWIs. To substantiate our choice of ecosystems and relevant state variables, we therefore relied on linking knowledge of ecosystem-specific ecology with regime shift theory (Collie et al., 2004). Additionally, the case studies were chosen based on the availability of long time-series of monitoring data of relevant state variables at sufficiently high temporal resolution and with few gaps (Spears et al., 2016). As mechanisms generating critical transitions can operate at all ecosystem levels, relevant state variables range from discrete variables such as species-specific biomass (e.g. Scharfenberger et al. (2013)) to aggregated variables such as Secchi depth, turbidity or metabolism (e.g. Batt et al. (2013b)). Despite our conscientious choice of ecosystems and state variables we still found contradicting patterns in EWIs.

Detection and agreement among indicators – Not all EWI metrics were equally reliable in detecting impending transitions (Table 4.2). In 44 % of the total of 64 cases the EWI failed, and agreement among EWI signals was low. Agreement between AR-1 and SD trends has been postulated as a minimum requirement to signal the approach of a tipping point (Ditlevsen and Johnsen, 2010), and these two signals are expected to be easy and straightforward to detect (Dakos et al., 2014). In our dataset, these two signals were consistent in 5 out of 16 time series examined. Critical transitions with increasing AR-1 and decreasing SD have also been observed in other studies (Livina et al., 2012). Potentially, such inconsistency between indicators may occur in ecosystems that are subject to multiple concurrent regime-shift generating processes that may or may not interact via shared state variables and that may react differently to drivers and additional noise (Brock and Carpenter, 2010). If such connected regime-shift processes work towards muffling variance in the measured state variable, the variance-based EWI signal may be suppressed (Brock and Carpenter, 2010). Transitions from cycles to stable points can also generate decreasing variation (38) and may explain decreasing SD trends in the Lake Müggelsee Cyclops vicinus abundances or the Lake Vörtsjärv functional group U biomass. It remains unclear why in some cases the AR-1 trends were negative (e.g. Lake Washington non-Daphnia cladocerans). Negative autocorrelation may originate from a too large sampling interval in cyclic variables (e.g. population cycles) resulting in the sampling of too few points within each cycle. Detection of EWIs has often been related to the availability of high sampling frequency data

(Carpenter et al., 2011), however, a study on subsampling of data series showed that EWIs could still be detected robustly in infrequently sampled data as long as the length of the data series was sufficient (Clements et al., 2015). Our study was based on data sampled at (or averaged to) biweekly and monthly intervals. Monthly intervals are rather long compared to the generation or reaction times of the state variables tested in our study (e.g. phytoplankton generation times are in the order of days, therefore monthly averages of biomass aggregate multiple generations of phytoplankton). Nevertheless, we could not detect a relationship between sampling interval and the proportion of failing EWIs in our dataset.

Early detection based on incomplete time series – In our analysis of empirical time series, we also showed that informative changes in some of the EWI metrics were already detectable several years preceding the actual shift, although there were large differences in detection windows by state variables. These differences may partly be explained by the fact that the actual shift is usually triggered by external stochastic perturbations which in turn are often independent of the drivers of ecosystem stability loss (Scheffer et al., 2012). However, in many cases EWIs indicated sustained instability over the period tested in our study which may be attributable either to a too short time span available for testing (e.g. Lake Zwemlust) or to unrecognised interacting processes that promoted prolonged instability in these ecosystems.

Conclusion – Despite our informed and conscientious choice of case study ecosystems and state variables, we found relatively low detectability of EWIs prior to the actual shift, and when EWIs were detected, the agreement among EWIs was low. Our findings agree with results from an assessment of the detectability and agreement of EWIs preceding non-linear transitions by Burthe et al. (Burthe et al., 2015). Although it is encouraging that we could detect EWIs in some of our empirical aquatic time series using data derived from commonly used monitoring schemes that were not designed for this purpose, the lack of detection reliability and agreement between signals limits the current application of EWIs to well-understood ecosystems only (Spears et al., 2016). However, harnessing EWIs as metrics of resilience loss in such well-understood ecosystems may help in planning for the unpredictable and could be part of strategic foresight programs for management and conservation (Cook et al., 2014). Using existing frameworks (e.g. alternative stable state theory) and in-depth ecosystem knowledge to infer the feedback loops involved in critical transitions, lake managers may be able to make assumptions on the underlying mechanisms generating transitions in well-understood ecosystem types such as shallow lakes (Scheffer et al., 1993), whereby the assumptions underlying critical transition dynamics need to be taken into account. One way forward from here may be to harness additional information on ecosystem states and their resilience using derived estimates that represent critical transition generating processes more directly such as trophic or competition interaction strengths either from specific and well-parameterised ecosystem models such as PCLake (Kuiper et al., 2015) or from detailed field data (Francis et al., 2014) as state variables. These data can serve for testing patterns of EWIs and hopefully allow us to increase our search patterns in these EWIs

instead of relying on a generic increase in variance without understanding the inherent variability in ecosystems. Additionally, high frequency measurements of biotic and abiotic variables in aquatic ecosystems are becoming increasingly available and may alleviate the restrictions on EWI detection due to sparse sampling (Spears et al., 2016). In the meantime, the reliability of EWI's for predicting abrupt shifts in ecosystem state should be treated with caution.

4.5 Methods

Data preparation and breakpoint detection – The data preparation and statistical analysis protocol was identical for all 16 state variable time series. Each time series was analysed at the highest temporal resolution available or at a lower resolution that resulted in less missing data (see SI Appendix Table S2 S2). Lakes Müggelsee and Washington were analysed at biweekly intervals, all other time series at monthly intervals. As our time series methods require continuous and equidistant data, we imputed missing values up to a maximum of four consecutive time steps using a Kalman filter (Durbin and Koopman, 2012). Time series with longer gaps were shortened to start or end at the gap (e.g. Lake Vörtsjärv). Each time series was standardized by mean centering and standard deviation scaling for convenient comparison of step change magnitudes. The timing of the step was determined by breakpoint analysis as step changes in the respective state variables may differ from the timing of whole-system step changes reported in the literature. Robust estimates of the timing of step change were achieved by employing three complementary breakpoint estimation methods: a) additive decomposition of time series in seasonal, trend and residual components and subsequent iterative fitting of piecewise linear season and trend models (Verbesselt et al., 2010a) using the R package “bfast” (Verbesselt et al., 2010b), b) testing for step changes in the average using the Pettitt test (Pettitt, 1979) and c) STARS, a combination of a sequential partial CUSUM method and a t-test (Rodionov, 2004). If at least two methods showed similar timing for a step change (± 12 months), the standardized original time series was split at that breakpoint. Differences between pre- and post-breakpoint means of the time series were tested with a Welch two sample t-test (Welch, 1947) (Table 4.2).

Due to a one-year gap in the time series, breakpoint timing of two state variables (LV functional groups P and U) was assessed differently: here we assessed whether large changes in the biomasses of the functional groups occurred during the gap indicating a potential shift. The pre-1978 and post-1978 time series showed significantly different means for functional group U (Welch two-sample t-test, $t=3.52$, $df=128$, $p<0.001$, difference in means = $0.29 \times \text{standard deviation}$) and functional group P (Welch two-sample t-test, $t=-4.67$, $df=439$, $p<0.001$, difference in means = $0.55 \times \text{standard deviation}$) suggesting that the shift in functional groups U and P occurred during the year 1978 (Table 4.2). As both of these time series showed no further breakpoints in the years after 1978, we conservatively assumed 1977 to be the year of the regime shift.

Early-warning indicator analysis – The pre-breakpoint time series was seasonally adjusted using a Gaussian smoother with a kernel bandwidth based on the number of data points per year (i.e. 12 for monthly and 26 for bi-weekly datasets). A bandwidth of one year was chosen to account for yearly recurring patterns while retaining informative low and high frequency variability other than long-term trend and season (Dakos et al., 2008). The residuals were then passed on to analysis of EWIs AR-1, SD, SK, and DR with subsequent sensitivity and significance testing using the R package “earlywarnings” (Dakos et al., 2012a).

Sensitivity and significance testing – The slope of the trends in the resulting temporal evolution of EWIs from the rolling-window approach were quantified by the non-parametric Mann-Kendall trend test, which tests for monotonic trends based on the Kendall τ rank correlation coefficient (Dakos et al., 2012a). As the size of the rolling time-window can affect the slope of EWI trends (Livina et al., 2012), a sensitivity analysis was performed estimating the distribution of trends and proportion of trends that did not differ qualitatively (i.e. in sign) from the median of the trend distribution using yearly increments of the residuals time series covered by the rolling time-window (two to n-two years, function “sensitivity_ews” (Dakos et al., 2012a), where n was the number of years in each pre-breakpoint time series). Significance testing was conducted by comparing the data-based EWI trend against a bootstrapped distribution of 200 surrogate time series-based EWI trends. The surrogate time series were generated based on an ARMA(p,q) model fitted on the residuals time series. The bootstrapped distribution of trends depicts the probability that a particular trend could occur by chance in time series of that ARMA structure (function “surrogates_ews” in R-package “earlywarnings” (Dakos et al., 2012a)). The data-based EWI trend was deemed significant if it fell on one of the 5% tails of the surrogate-based trend distribution ($\alpha=0.1$). This was repeated across all time-window sizes and a proportion of the >50% significant differences over all window sizes was considered as a highly significant EWI signal.

Relationship of EWIs with state variable categories and agreement among signals – The relationship between the occurrence of each EWI and predictor variables mechanism (competition, IGP or trophic cascade), state variable category (species, group, ecosystem), step change height, length of pre-breakpoint time series and sampling interval (bi-weekly or monthly) was tested with logistic regressions (identity link for continuous predictors (step change height and time series length); logit link for categorical predictors) with Bonferroni corrected post hoc testing. The median of the trend distribution across all time-window sizes was used to assess agreement among EWIs.

Early detection of early-warning indicators – To assess how timely EWIs could have detected the approaching critical transition, we quantified EWI trends on stepwise shortened pre-breakpoint time series, starting with the full time period and continuing with stepwise reduction of the time series by cutting off the last year of data. The minimum time series length was set to three

years to allow meaningful Kendall τ estimation. How many years before the shift an EWI could already have been detected was set by assessing the maximum number of stepwise reductions before the theoretically expected EWI trend disappeared (i.e. when the median of the Kendall τ trend distribution turned negative for AR-1, SD and density ratio or changed sign for skewness). All data analyses and graphing were conducted using the R language environment for statistical computing ([R Core Team, 2012](#)) and associated library extensions.

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Chapter 4 – SI Appendix

SI.A Case study descriptions

Lake Müggelsee

Re-oligotrophication driven change in competition, food availability driven change in intraguild predation and trophic cascade driven change in predation pressure

The Müggelsee dataset spans a total of 34 years, starting in January 1979. Müggelsee is a shallow (mean depth 4.9 m, max depth 8m), polymictic and eutrophic lake situated southeast of Berlin (Germany) with a surface area of ~750 ha and a retention time of about 6-8 weeks (Kohler et al., 2005; Hilt et al., 2013). A detailed description of the regular sampling procedure and sample processing is provided in Gerten and Adrian (Gerten and Adrian, 2000). Kohler et al. (2005) have described three distinct eutrophication periods in the lake: a hyper-eutrophic period (1979-1990) characterised by turbidity and loss of submerged vegetation; a transient period (1991-1996) characterised by internal loading of phosphorus, a reduction in phytoplankton biomass, reappearance of spring clear-water phases, and slow recovery of submerged vegetation (Hilt et al., 2013); and a eutrophic period (1997-2003) characterised by slowly decreasing total nitrogen (TN). However, the lake has not fully shifted to a clear state, probably due to interfering processes such as a) the reduction in TN leading to a competitive advantage for N-fixing cyanobacteria in summer conditions; b) climate warming leading to increased internal loading as well as to an increase in stratification events that promote cyanobacterial blooms; c) constant grazing pressure by waterfowl and fish inhibiting recovery of macrophytes; or d) potentially increasing cyprinid fish population due to reduced commercial interest after the German reunification, leading to increased predation pressure on zooplankton in the period 1990 to probably 2005 (Kohler et al., 2005; Hilt et al., 2013).

Three mechanisms leading to regime shifts at different ecosystem levels appear important in Müggelsee, the eutrophication / re-oligotrophication driven reduction in phytoplankton biomass; a food-availability driven shift in an intraguild predation system of two copepod species; and a predation pressure driven shift in *Dreissena polymorpha* larvae and in *Leptodora kindtii* abundances. Five variables were selected for early-warning signal (EWI) analysis, two pertaining to the re-oligotrophication driven shift in phytoplankton biomass: total phytoplankton biomass and *Aphanizomenon* biomass (Kohler et al., 2005), (Wagner and Adrian, 2009b); one time series pertaining to the food-availability driven shift in the *Cyclops vicinus* and *Cyclops kolensis* intraguild predation system (Scharfenberger et al., 2013) and two time series of shifts in *Leptodora kindtii* and in *Dreissena polymorpha* larvae which may have been caused by changes in fish predation pressure on carnivorous zooplankton cascading on to *Dreissena* larvae (Özkundakci et al., 2016) although the rise of *Dreissena* larvae has also been linked to earlier spawning under warming

(Adrian et al., 2006; Wilhelm and Adrian, 2007). Testing the *Cyclops kolensis* abundance time series was not possible as the pre-breakpoint time series was severely censored by a detection limit. Overall the dataset had few missing values except for the first period of *L. kindtii*. We chose to include this time series nonetheless as the missing values occurred during winter periods, a period in which *L. kindtii* are known to not occur in the pelagic. For a summary on number of data points and percentage of missing data see Table S2.

Lake Zwemlust

Eutrophication driven change in competition

The full Zwemlust dataset encompasses 16 years, starting in May 1986 and ending in August 2001. Zwemlust is a small (~1.5 ha), shallow (mean depth 1.5m), eutrophic and polymictic lake situated close to the city of Utrecht (The Netherlands). Zwemlust has no surface in- or outflow but receives high external phosphorus (P) and nitrogen (N) loadings by seepage from the nearby River Vecht (Vandonk and Gulati, 1995) leading to persistent phytoplankton blooms and low water transparency prior to 1987. The lake was restored twice by biomanipulation (March 1987 and April 1999). These two dates also represent the boundaries of the data analysed here, see also Table S2. The first biomanipulation in 1987 included draining the lake, removal of planktivorous fish, stocking of piscivorous fish, creation of zooplankton shelter and planting of macrophytes. These manipulations were successful in shifting the lake into a macrophyte dominated, clear state. However, Zwemlust experienced a return of low water transparency periods in summer and a loss of submerged vegetation in 1992. In 1999, the second, less severe biomanipulation successfully shifted the system back to a clear state (Van De Bund and Van Donk, 2002). The loss of system resilience was explained by a combination of selective herbivory by coots (*Fulica atra*) promoting a macrophyte succession to species less resistant to epiphyte shading (Van De Bund and Van Donk, 2002) in combination with epiphyte growth due to continuing high phosphorus availability (Vandonk and Gulati, 1995). Secchi depth was selected to test this eutrophication driven regime shift for EWIs.

Lake Veluwemeer

Eutrophication and light climate driven change in competition

The Veluwemeer dataset spans almost 26 years from April 1987 to December 2012 (Rijkswaterstaat, 2014). Veluwemeer is a shallow (mean depth of 1.5 m), polymictic lake with a surface area of 3 050 ha situated in the centre of the Netherlands. The lake is directly connected to Lakes Wolderwijd and Drontermeer. Ibelings et al. (2007) identified four distinct phases: i) Initial clear state (1957-1970) characterised by increasing eutrophication by waste water effluent and run off from adjacent agricultural lands, widespread loss of macrophyte cover and a fish community change to bream dominance. Increased turbidity was caused by cyanobacteria blooms (mainly *Planktothrix agardhii*) and wave re-suspension of particles. ii) Turbid state (1971-1985) characterised by reduction of external P loading by implementing waste water

treatment plants and winter flushing of the lake with calcium rich polder water, resulting in a reduction in chlorophyll but not in turbidity due to resuspension of sediment particles. iii) Transient period (1986-1995) characterised by the co-existence of clear (above *Chara* stands) and turbid (above deeper lake areas) patches after a series of severe winters helped break the dominance of *Planktothrix* sp. blooms. From 1994 onwards, *Chara* stands expanded rapidly in parallel to a reduction in the benthivorous fish population and the return of zebra mussels into the lake. iv) Clear state (1996-2012) stabilised by a high *Chara* coverage, high densities of zebra mussels and a fish community composition change to planktivorous species dominance resulting in a stabilisation of the sediment and a reduction in non-algal light attenuation (i.e. the non-chlorophyll related turbidity, see (Ibelings et al., 2007)). The clear state seems to be resilient as it has recovered from perturbations such as dredging and record precipitation. Non-algal light attenuation was selected to test this eutrophication driven regime shift for EWIs.

Lake Washington

Re-oligotrophication driven change in competition and release from overexploitation trap

The dataset for Lake Washington analysed here spans 27 years from January 1969 to December 2000. Lake Washington is a large (~8 800 ha) and deep (maximum depth ~65 m) lake within the city of Seattle, WA, see Table S2 . Lake Washington experienced modest eutrophication from the 1940s to 1968 by sewage water inflow. Secchi depth was reduced, and *Oscillatoria* blooms were common (Hampton et al., 2006). Since 1968 wastewater is diverted from the lake which greatly reduced the input of external P to the lake and had an immediate effect on the phytoplankton biomass production and Secchi depth. By 1972 the lake was considered recovered (Edmondson, 1994). In 1976, Secchi depth improved further, which was attributed to the establishment of *Daphnia* and increased grazing pressure on the summer phytoplankton bloom (Edmondson and Litt, 1982). The sudden establishment of *Daphnia* in the lake was attributed to a trophic cascade releasing *Daphnia* from an overexploitation trap and a P-reduction related decrease in low food quality *Oscillatoria* blooms. The trophic cascade was probably initiated by an unintentional improvement of spawning grounds for longfin smelt (*Spirinchus thaleichthys*) during flood-control measures on the lake's main tributary (Edmondson and Abella). These fish started preying heavily on *Neomysis*, releasing *Daphnia* from predation pressure (Edmondson, 1994; Murtaugh, 1989). The increase of more palatable phytoplankton groups such as cryptophytes co-occurred with the disappearance of the poor food quality species *Oscillatoria* (Infante and Abella, 1985) from the lake in 1976 (Edmondson, 1994), which may have resulted from changed competitive ability of these two groups under nutrient ratio changes in the lake in the wake of the waste water diversion since 1968.

The period of strong nutrient reduction due to improved sewage management from 1963 -1968 was excluded from the analysis as a biotic response to such a strong external change would not fall into the category of critical transitions (i.e., it is a large response primarily to large

changes in an external forcing variable). Hence the analysis focussed on the biotic response of the cladoceran competitors *Daphnia* and non-*Daphnia* cladocerans to the trophic cascade and on the response of the phytoplankton competitors Cryptophytae and *Oscillatoria* in the period after sewage diversion. The assumed loss of *Neomysis* due to the trophic cascade could not be observed as data on night-time pelagic *Neomysis* abundances in the lake were not available. Moreover, the time series of *Daphnia* was not analysed as pre-shift *Daphnia* abundances were mostly below detection limit.

Lake Võrtsjärv

Competition under environmental change

Lake Võrtsjärv is a large (~27 000 ha), shallow (mean depth ~2.8 m), eutrophic and polymictic lake situated in central Estonia. The lake is connected to six inflows and one outflow to Lake Peipsi. The lake experienced an increase in water levels from 1964 up to the early 1990s, and a decrease in water levels after the early 1990s; however, large fluctuations in water levels have occurred throughout. High water levels in combination with increased colouring of the water by humic matter decreased the area available for the colonisation by submerged macrophytes. The reconstructed eutrophication history of the lake showed an increase in loading since the 1950s with peak loadings during the 1980s (Nõges et al., 2007) exposing the lake to increasing eutrophication. Phytoplankton biomass increased with eutrophication and peaked in the 1970s (Nõges et al., 2010). In 1977/78 a sudden increase in water level brought about a complete and permanent reconfiguration of the phytoplankton community composition and a marked decrease in summer biomasses. Summer epilimnion species (functional group U) such as *Uroglena* sp (Reynolds et al., 2002) declined suddenly and eutrophic epilimnion species (functional group P) such as *Aulacoseira granulata* increased abruptly. Moreover, the turbid mixed layer species (functional group S1) changed from a *Planktolyngbya limnetica* and *Aulacoseira ambigua* dominated group to a dominance of more shade resistant but also shade generating phytoplankton species such as *Limnotherix* sp. (Nõges et al., 2010). A sudden increase in 1993 of di-nitrogen fixing species such as *Aphanizomenon skujae* (functional group H1) was attributed to declining N:P ratios particularly at low water levels which promoted increased denitrification and increased phosphorus release from the sediment (Nõges et al., 2010).

To reflect the functional changes in the plankton community time series functional groups (Reynold groups) were selected for further analysis, in particular the groups U (summer epilimnion species such as *Uroglena*, which tolerate low nutrients but are sensitive to CO₂ limitation), P (eutrophic epilimnion species such as *Fragilaria crotonensis* or *Aulacoseira granulata*, which tolerate mild light and C limitation but are sensitive to stratification and Si deficiency) and H1 (dinitrogen-fixing *Nostocaceae* such as *Aphanizomenon* or *Anabaena flos-aquae*, which tolerate low nitrogen and carbon but are sensitive to poor light, low phosphorus and mixing) (Reynolds et al., 2002).

SI.B Table

Table S2: Summary of overall period covered, aggregation interval, number of pre-breakpoint time series data points, percentage of missing data with comments and remaining seasonal ARIMA structure after seasonal adjustment by Gaussian smoother.

Case Study System	State Variable	Overall Time Period	Interval	# Data Points	% Missing Data	Comments on missing data	Seasonal ARIMA AR, I, MA
LMS	Phytoplankton	1979 - 1990	bi-weekly	295	2.4		1,0,0
	<i>Aphanizomenon</i> Period 1	1979 - 1990	bi-weekly	308	2.7		0,0,2
	<i>Aphanizomenon</i> Period 2	1990 - 2002	bi-weekly	313	2.9		1,0,2
	<i>Cyclops vicinus</i>	1980 - 1992	bi-weekly	315	16.2	Winter bi-weekly 1-6	0,0,0
	<i>Dreissena polymorpha</i> larvae	1979 - 1993	bi-weekly	374	15.5	Winter bi-weekly 1-6	0,0,0
	<i>Leptodora kindtii</i> Period 1	1979 - 1987	bi-weekly	222	24.4	Winter bi-weekly 1-6	1,0,1
	<i>Leptodora kindtii</i> Period2	1987 - 2005	bi-weekly	461	3.3		2,0,0
LW	Secchi depth	1969 - 1976	bi-weekly	202	1.5		0,0,2
	Cryptophyceae	1969 - 1977	bi-weekly	222	4.9		1,0,1
	Cyanophyceae	1969 - 1973	bi-weekly	110	9.1		1,0,1
	Non- <i>Daphnia</i> cladocerans	1969 - 1976	bi-weekly	198	6.1		1,0,1
LV	H1	1979 - 1992	monthly	163	11.6	Mostly winter month	1,0,0
	P	1968 - 1977	monthly	120	0.8		2,0,3
	U	1968 - 1977	monthly	120	0.8		1,0,1
LVM	Non-algal light attenuation	1987 - 1995	monthly	102	8.8		0,0,1
LZL	Secchi depth	1987 - 1992	monthly	66	4.5		0,0,0

SI.C Overview figures of early-warning indicators per time series

S3.1 Lake Müggelsee phytoplankton biomass

S3.2 Lake Müggelsee *Aphanizomenon* biomass period 1

S3.3 Lake Müggelsee *Aphanizomenon* biomass period 2

S3.4 Lake Müggelsee *Cyclops vicinus* density

S3.5 Lake Müggelsee *Dreissena polymorpha* larvae density

S3.6 Lake Müggelsee *Leptodora kindtii* density period 1

S3.7 Lake Müggelsee *Leptodora kindtii* density period 2

S3.8 Lake Washington Secchi depth

S3.9 Lake Washington Cryptophyceae bio volume

S3.10 Lake Washington Cyanophyceae bio volume

S3.11 Lake Washington non-*Daphnia* cladocerans bio volume

S3.12 Lake Vörtsjärv functional group H1 biomass

S3.13 Lake Vörtsjärv functional group P biomass

S3.14 Lake Vörtsjärv functional group U biomass

S3.15 Lake Veluwemeer non-algal light attenuation

S3.16 Lake Zwemlust Secchi depth

Here we present two figures per time series showing early-warning indicators, sensitivity to data-window size and significance for the full pre-breakpoint time series (A figures) and sensitivity and significance across stepwise shortened pre-breakpoint time series (B figures).

Upper figure: Early-warning indicator trends, sensitivity and significance of the full pre-breakpoint time series

top panel: standardized pre-breakpoint time series (black) and Gaussian smoother (red)

second panel: residual time series (standardized pre-breakpoint time series - Gaussian smoother)

third panel: The evolution of an autoregressive coefficient of a first order AR model (AR-1) fitted on the data under a 50% rolling data-window. Theory expects that AR-1 increases in the approach to a breakpoint. Bold font y-axis labels point out cases where AR-1 is negative. To the right are two box plots:

Right boxplot: Sensitivity: distribution of trends across all data-window sizes (yearly increments) of the original standardized pre-breakpoint time series “org”. Significance: distribution of trends based on 200 surrogate time series with the same ARMA structure “sg”. Mean differences and range of trend distributions show how much data-based trends differ from surrogate-based trends.

fourth panel: The evolution of standard deviation (SD) estimated on the data under a 50% rolling data-window. Theory expects that SD increases in the approach to a breakpoint. Boxplot as in AR-1.

fifth panel: The evolution of skewness (SK) estimated on the data under a 50% rolling data-window. Theory expects that SK shows a trend in the direction of the change at the breakpoint (i.e increase or decrease) in the approach to that breakpoint. Boxplot as in AR-1.

bottom panel: The evolution of density ratio (DR) of the power spectrum of the data estimated as the ratio of low frequencies over high frequencies within a 50% rolling data-window. Theory expects that DR increases in the approach to a breakpoint. Boxplot as in AR-1.

Lower figure: Early-warning indicator trends, sensitivity and significance of stepwise shortened time series

Top left panel: distribution of AR-1 trends (based on all data-window sizes) of the original standardized pre-breakpoint time series (grey) and of 200 surrogate time series with the same

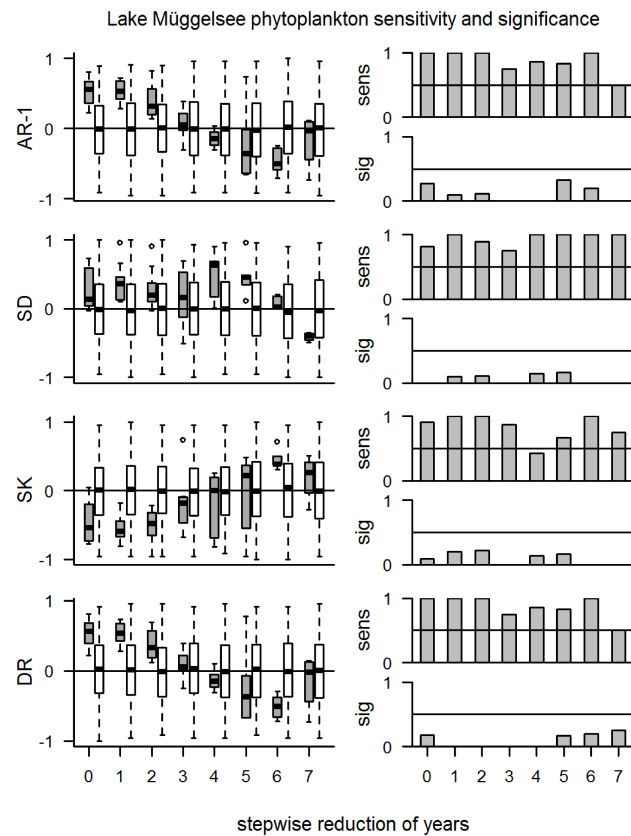
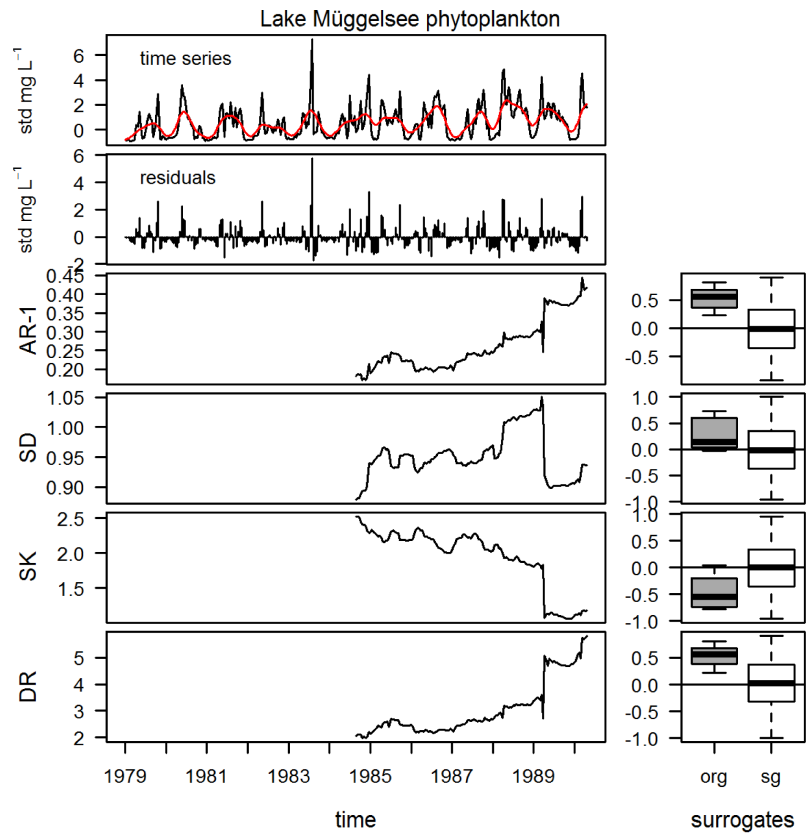
ARMA structure (white) across stepwise shortened pre-breakpoint time series (yearly increments, see x-axis). The proportion of trends with a slope significantly different from zero across all data-window sizes is shown in the bar-plot “sens”, the proportion of significant differences ($\alpha=0.1$) between original and surrogate time series trends is shown in bar-plot “sig”.

Second left panel: distribution of SD trends (based on all data-window sizes) of the original standardized pre-breakpoint time series (grey) and of 200 surrogate time series with the same ARMA structure (white) across stepwise shortened pre-breakpoint time series (yearly increments). The associated bar-plots show sensitivity and significances.

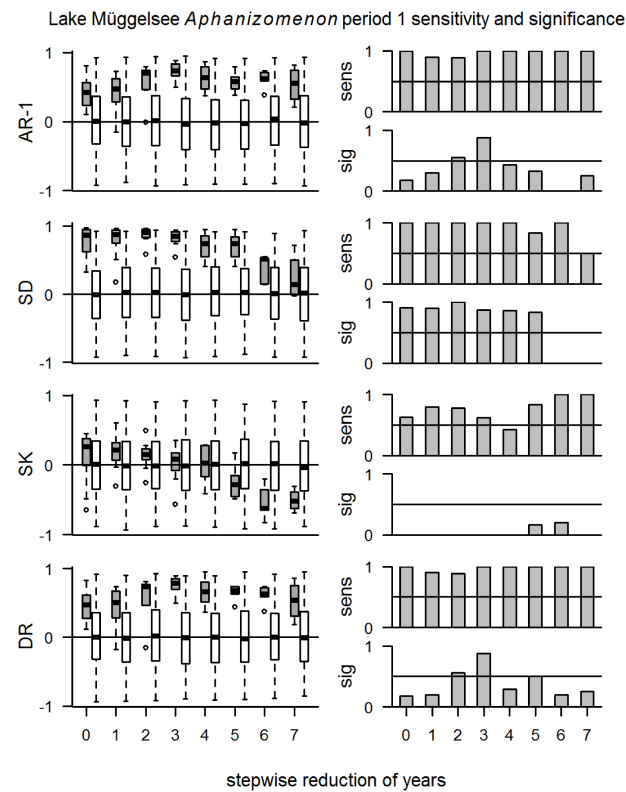
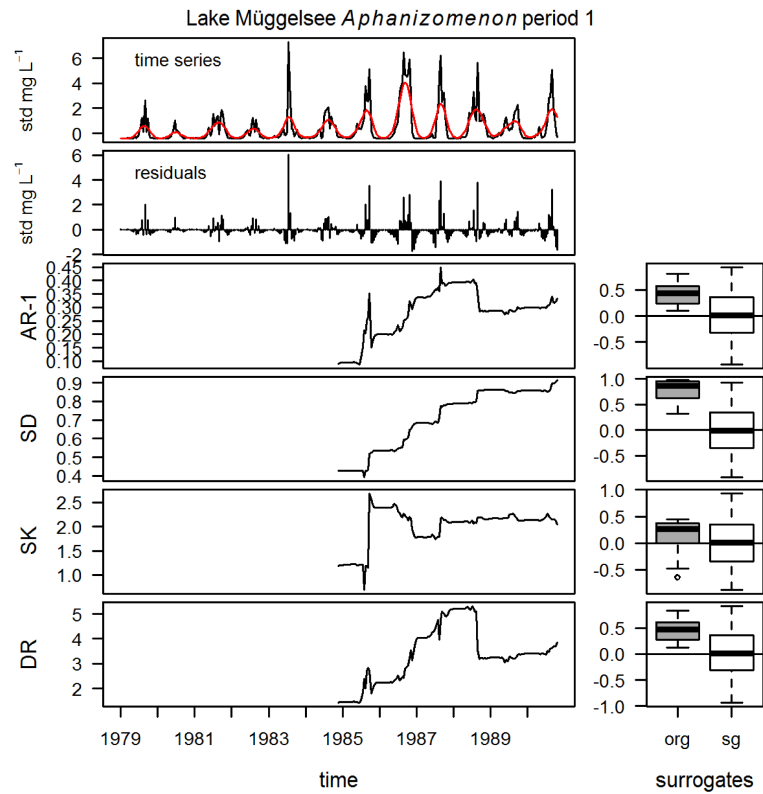
Third left panel: distribution of SK trends across all data-window sizes of the original standardized pre-breakpoint time series (grey) and of 200 surrogate time series with the same ARMA structure (white) across stepwise shortened pre-breakpoint time series (yearly increments). The associated bar-plots show sensitivity and significances.

Bottom left panel: distribution of DR trends across all data-window sizes of the original standardized pre-breakpoint time series (grey) and of 200 surrogate time series with the same ARMA structure (white) across stepwise shortened pre-breakpoint time series (yearly increments). The associated bar-plots show sensitivity and significances.

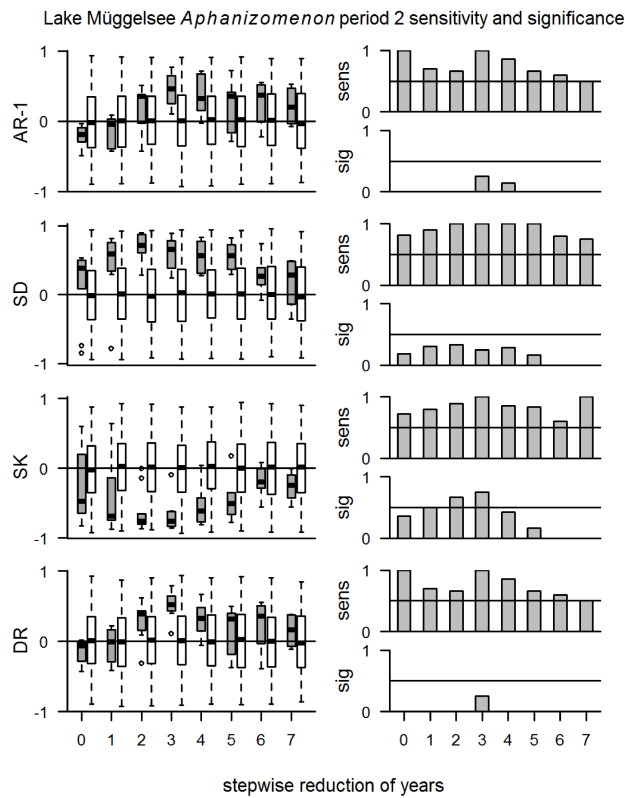
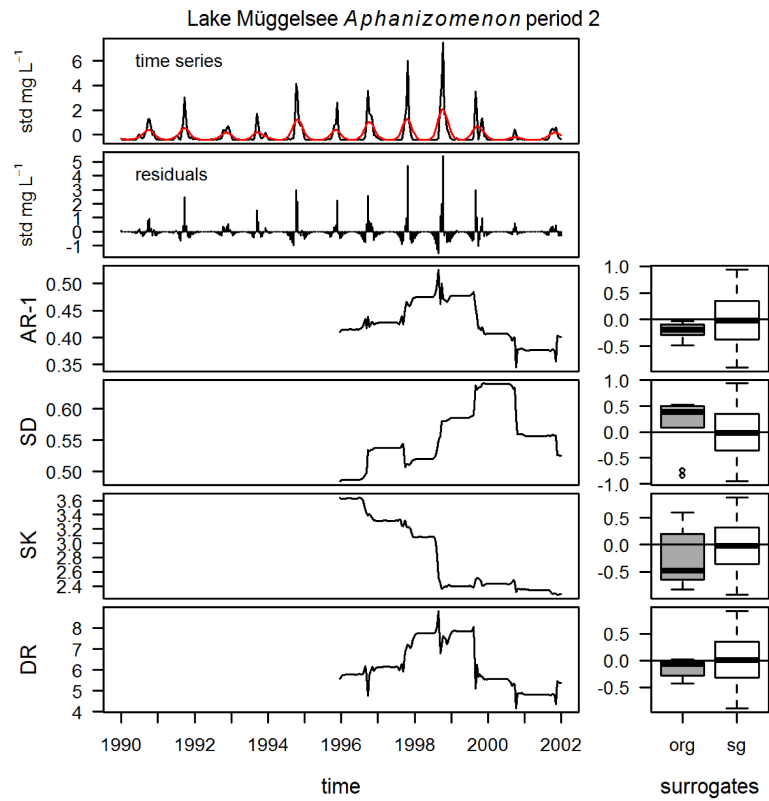
S3.1 Lake Müggelsee: Phytoplankton biomass



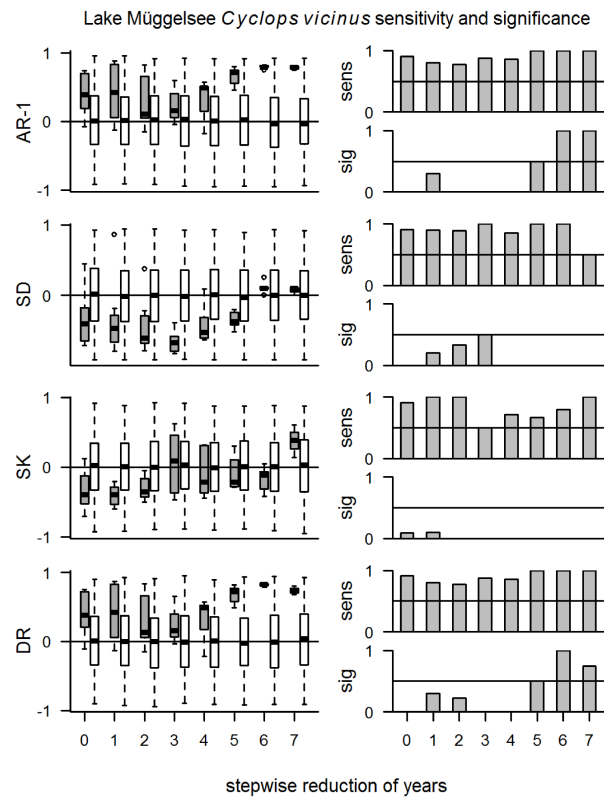
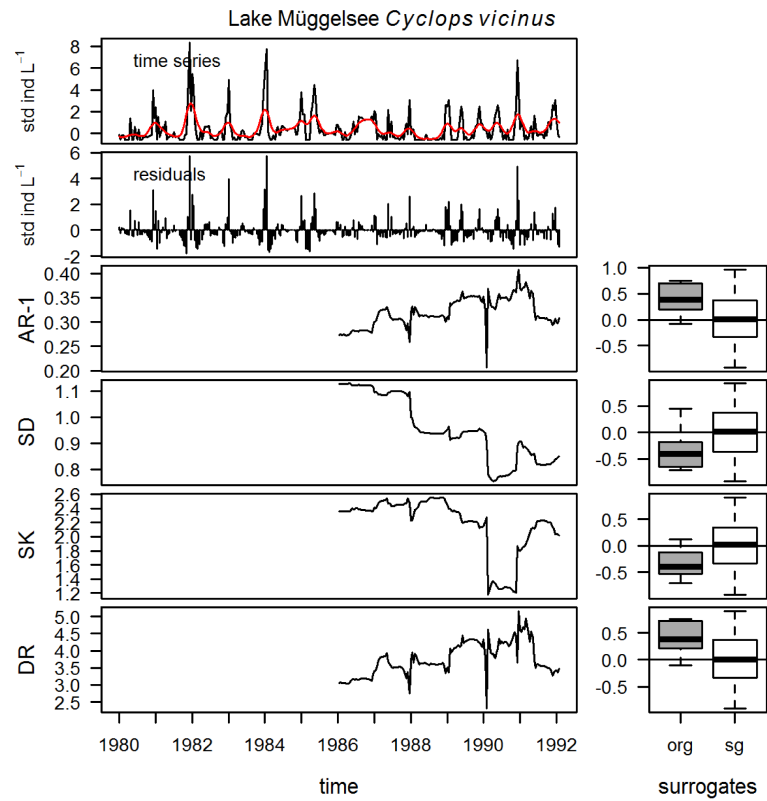
S3.2 Lake Müggelsee: *Aphanizomenon* biomass period 1



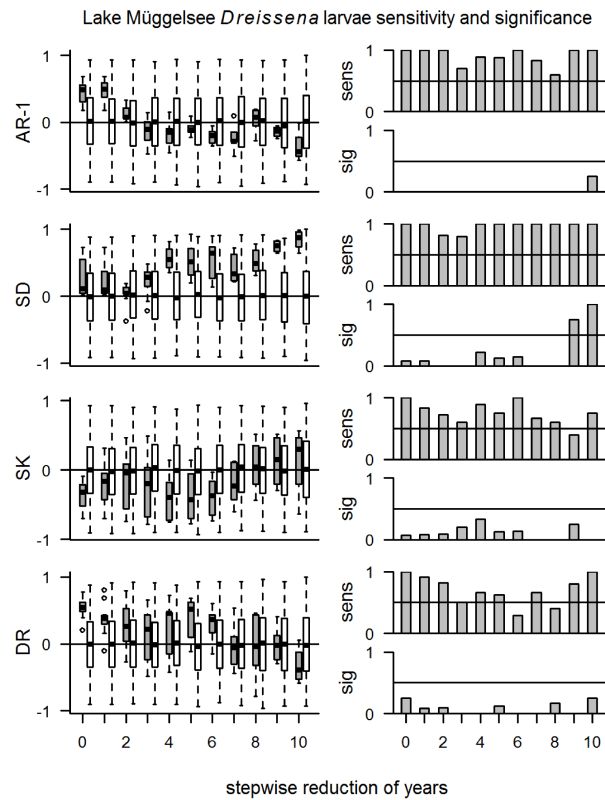
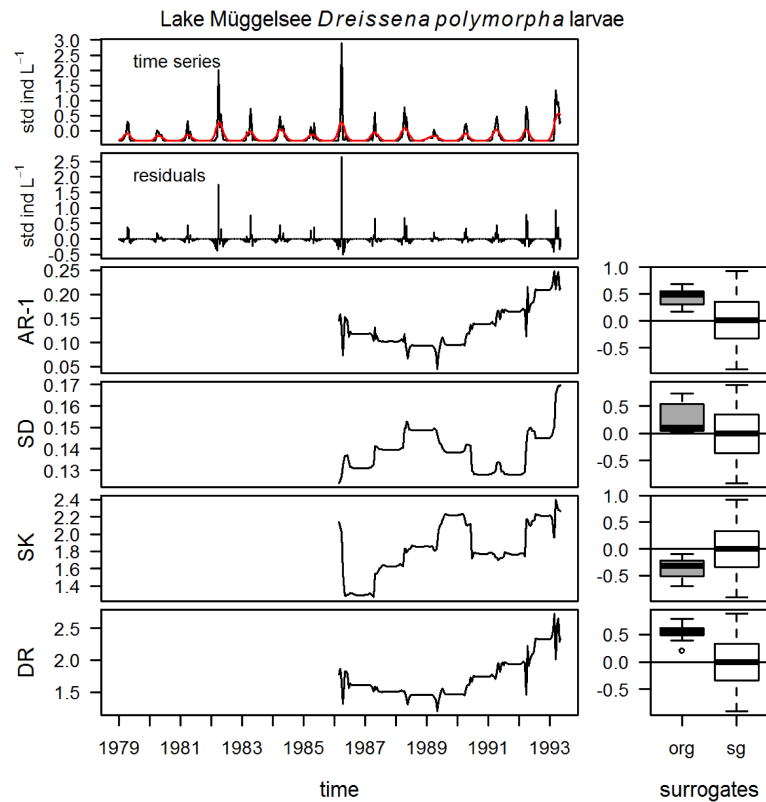
S3.3 Lake Müggelsee: *Aphanizomenon* biomass period 2



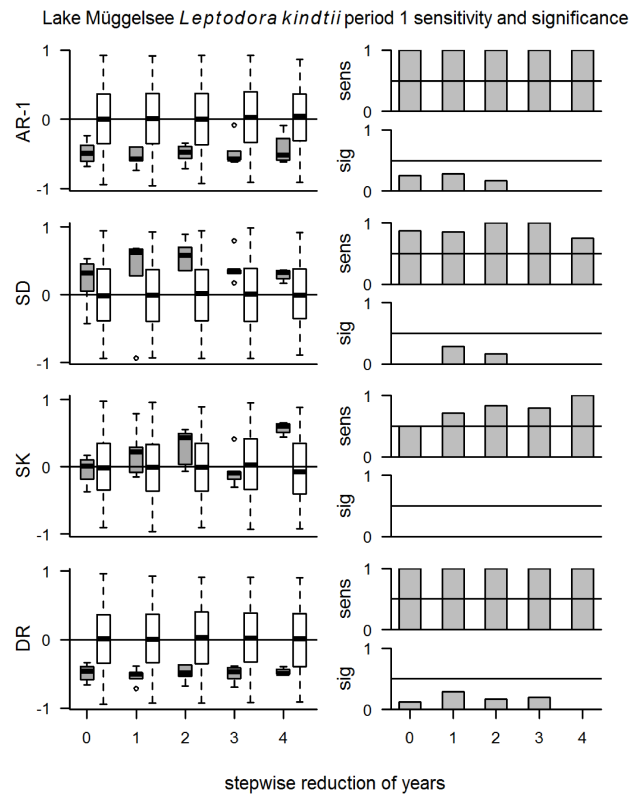
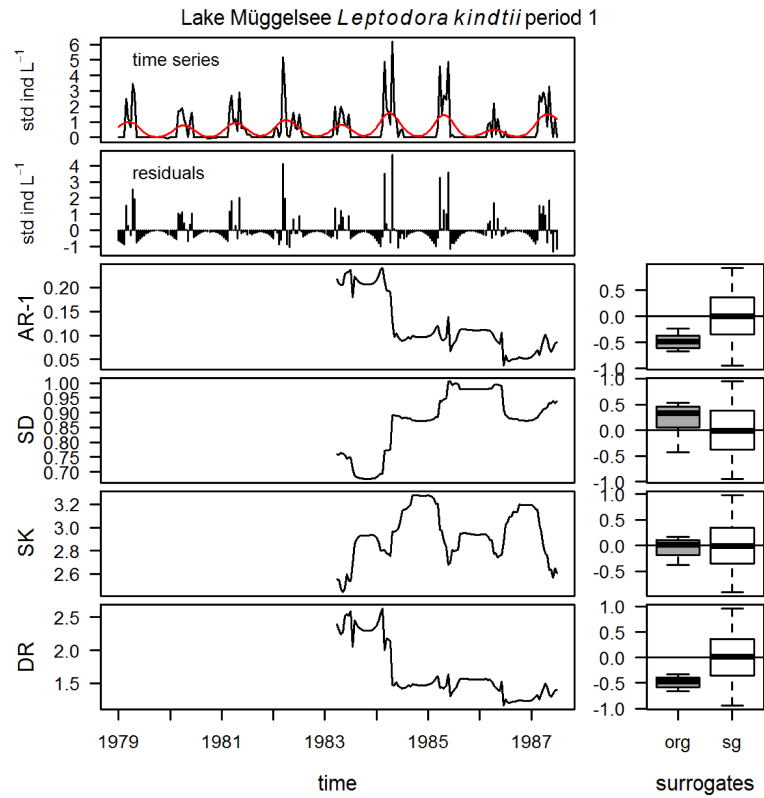
S3.4 Lake Müggelsee: *Cyclops vicinus* density



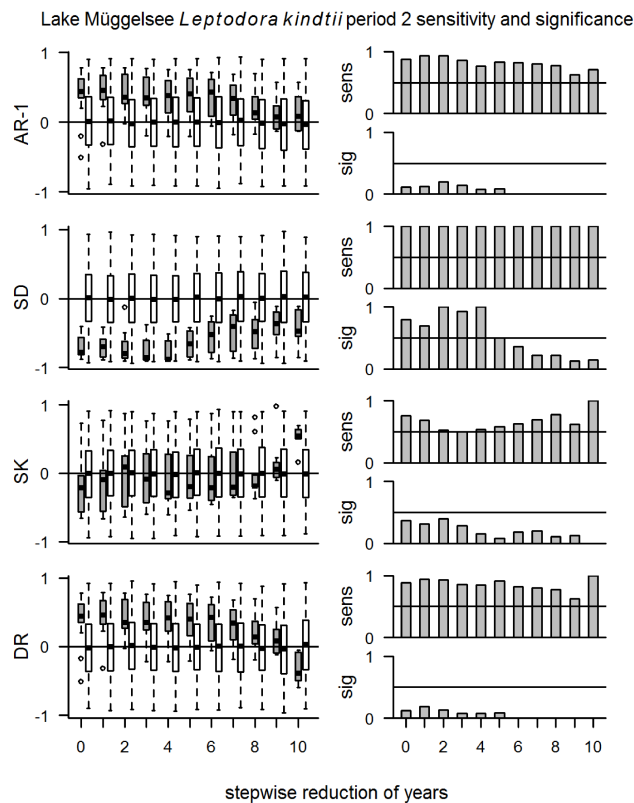
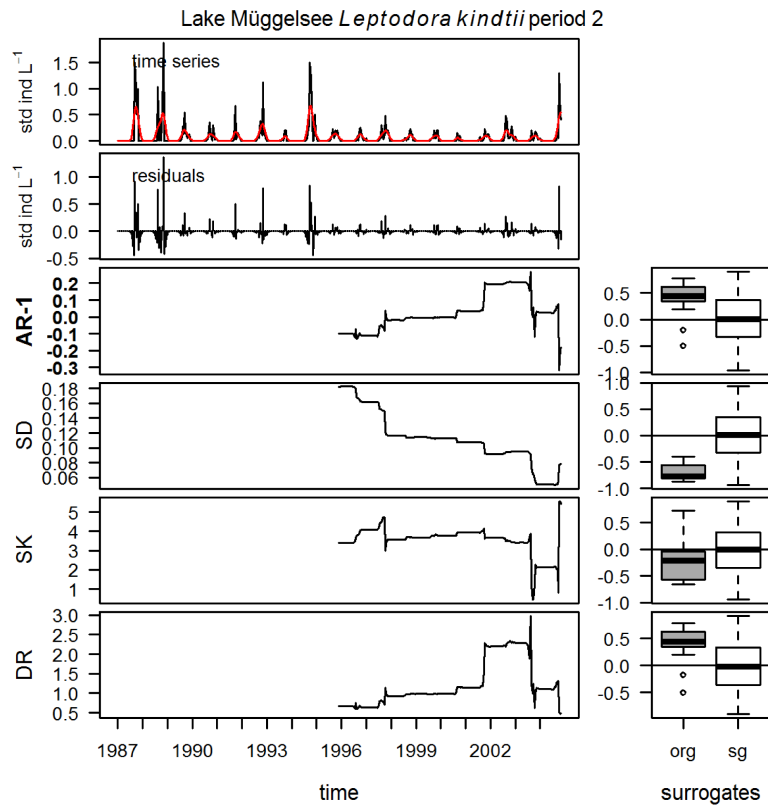
S3.5 Lake Müggelsee: *Dreissena polymorpha* larvae density



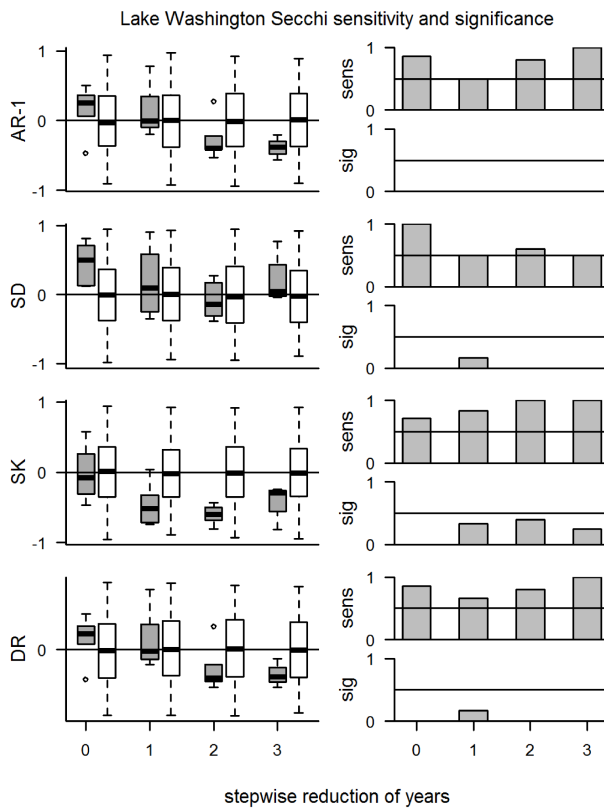
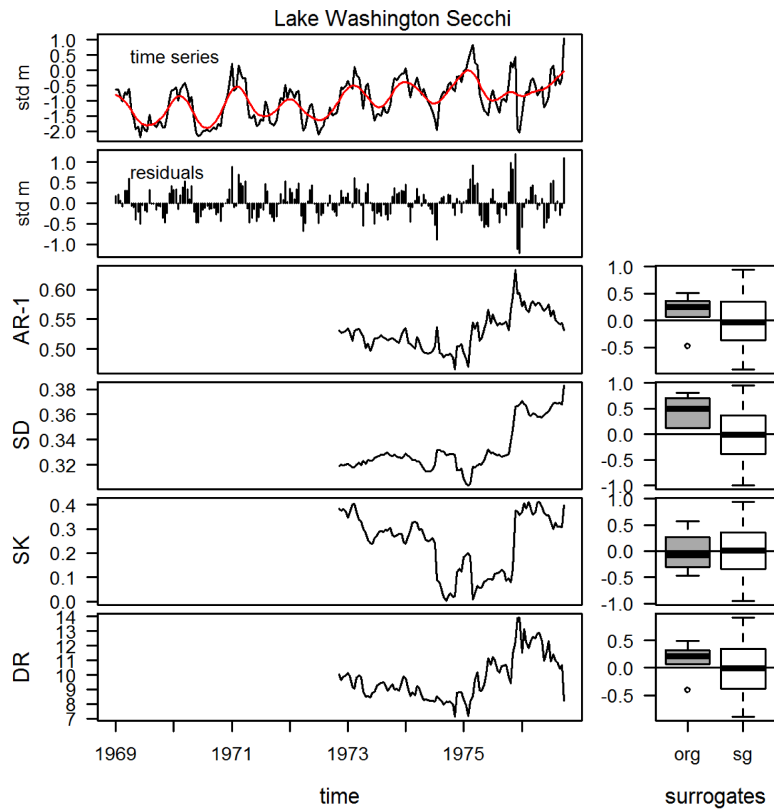
S3.6 Lake Müggelsee: *Leptodora kindtii* density period 1



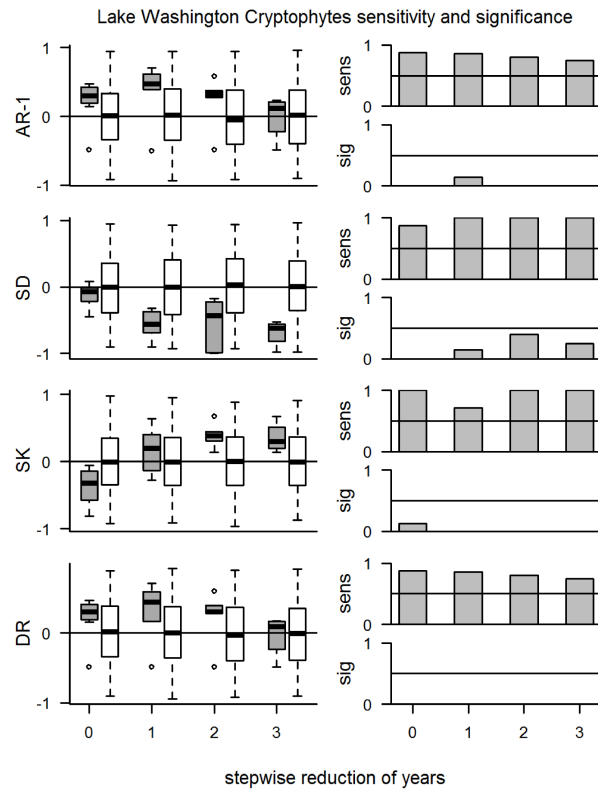
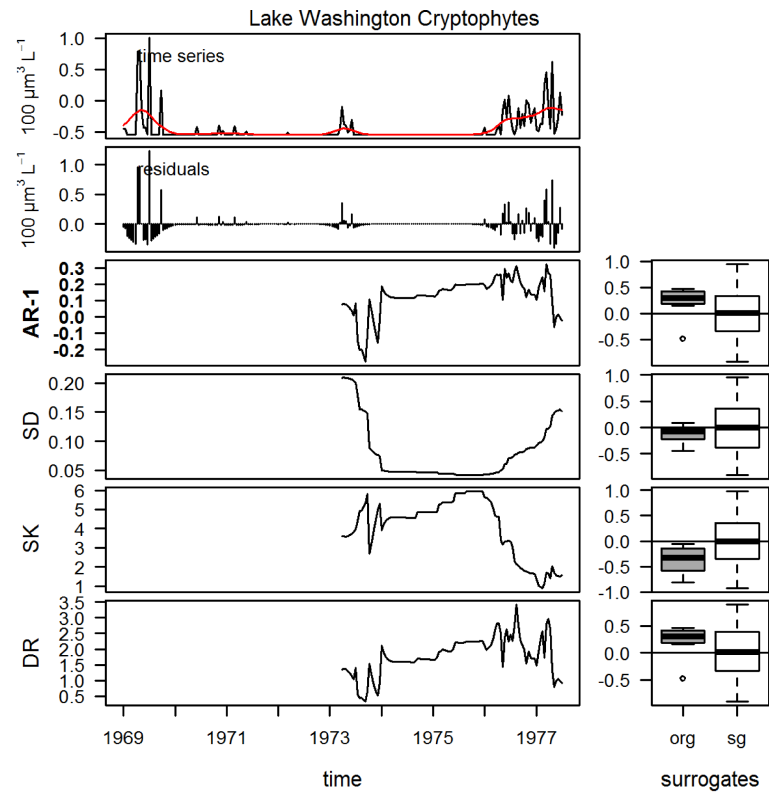
S3.7 Lake Müggelsee: *Leptodora kindtii* density period 2



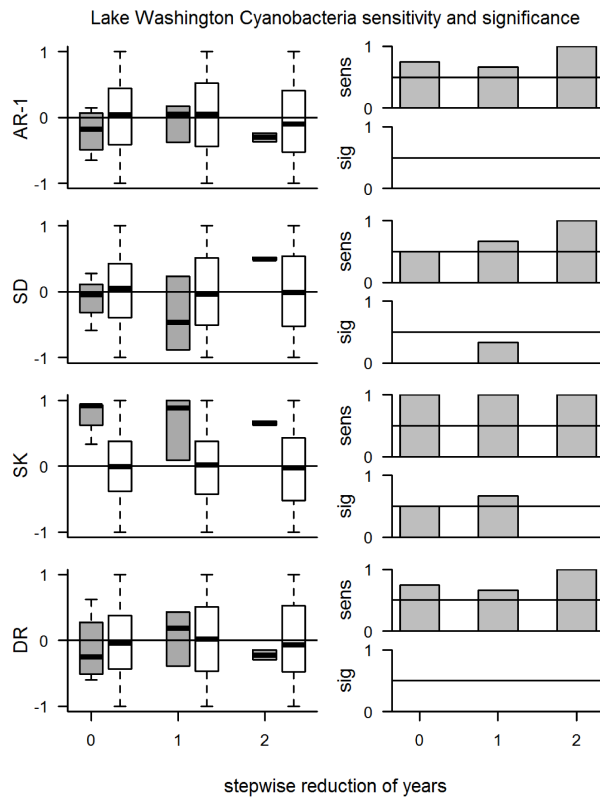
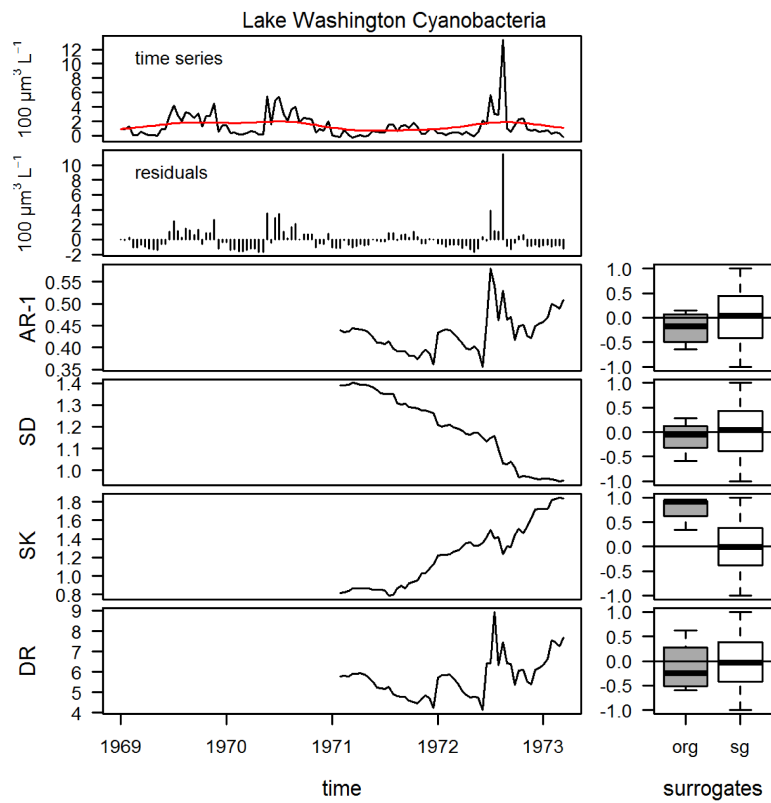
S3.8 Lake Washington: Secchi depth



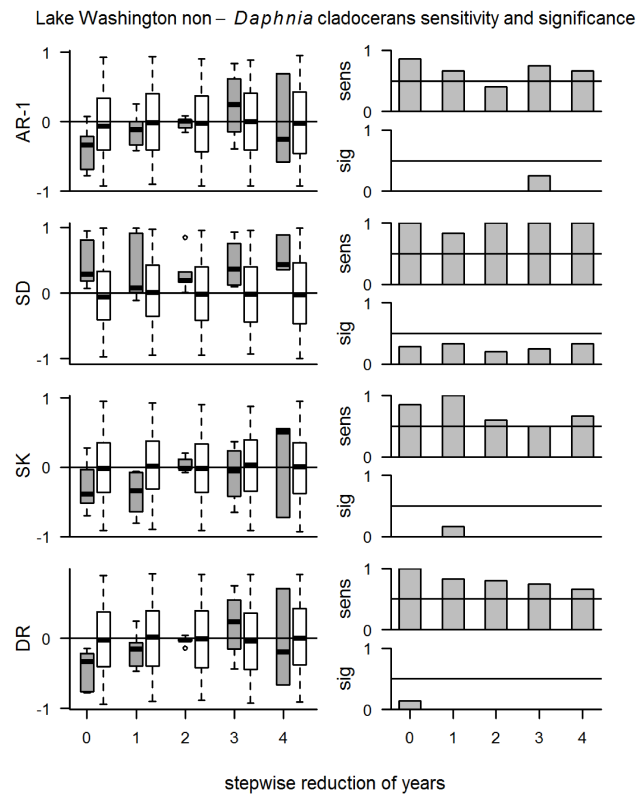
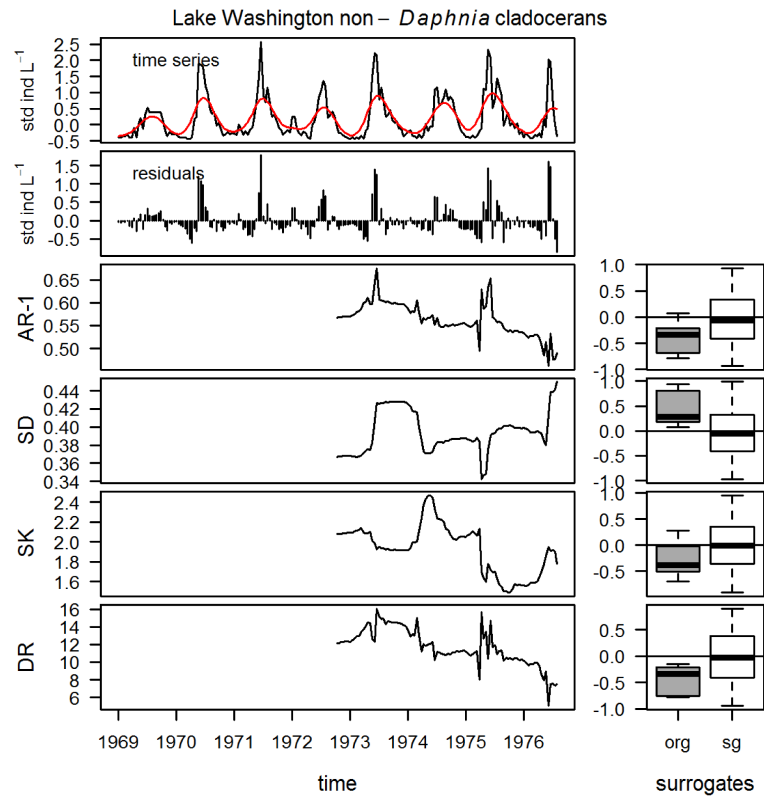
S3.9 Lake Washington: Cryptophytes bio volume



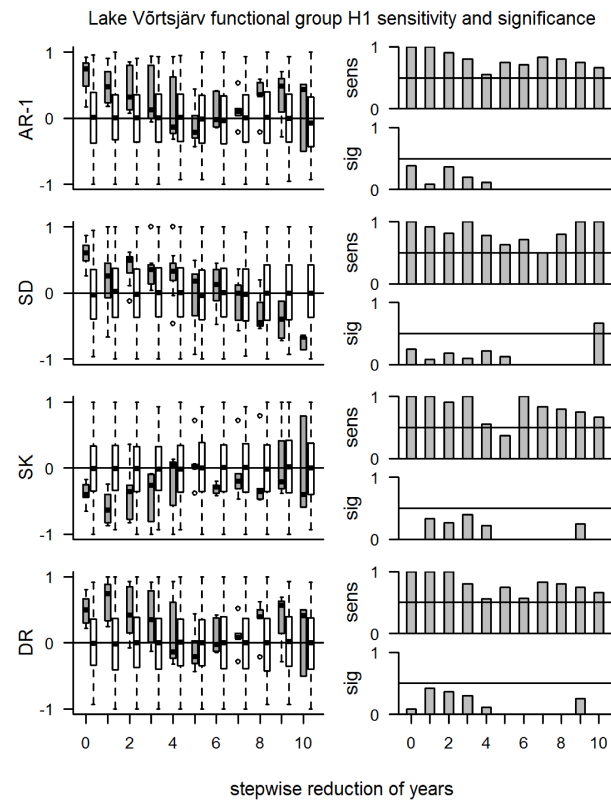
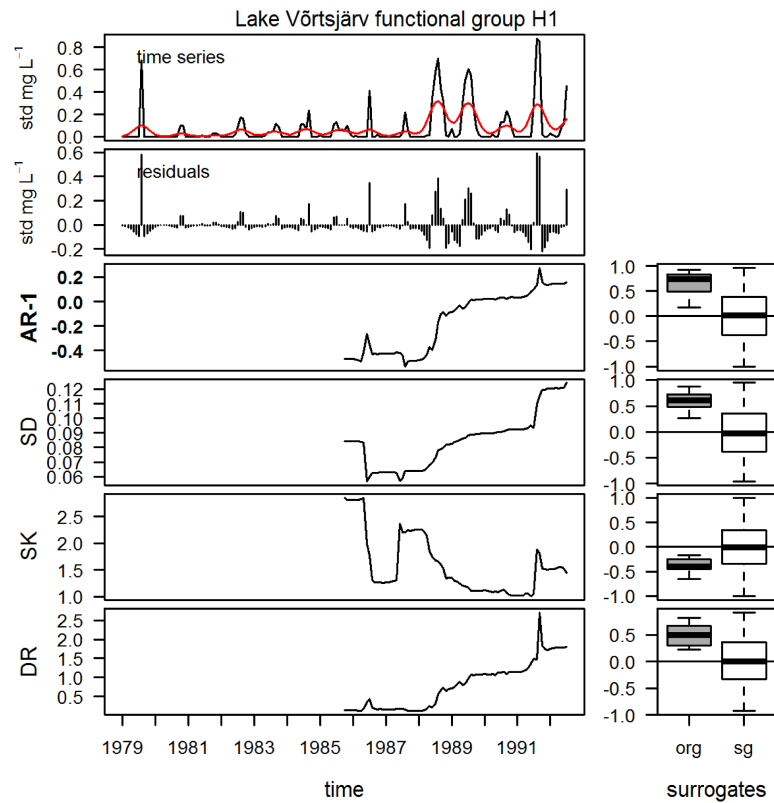
S3.10 Lake Washington: Cyanobacteria bio volume



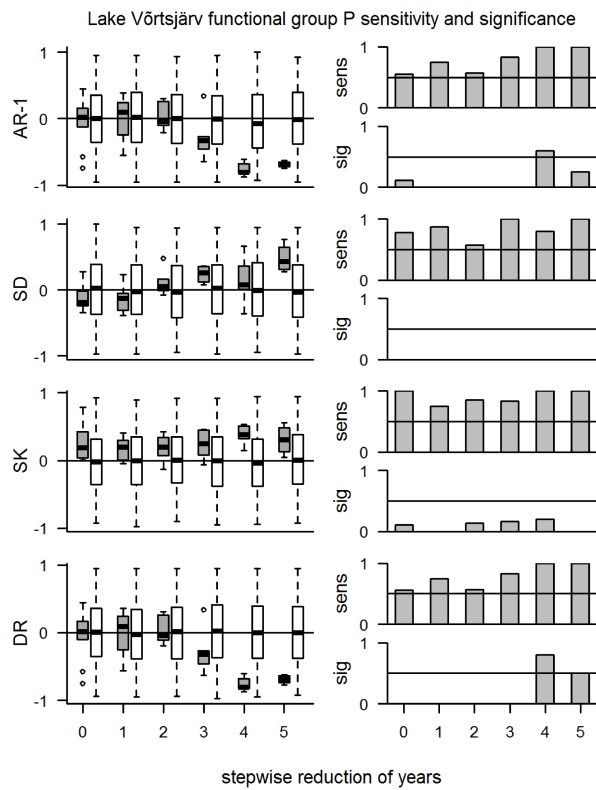
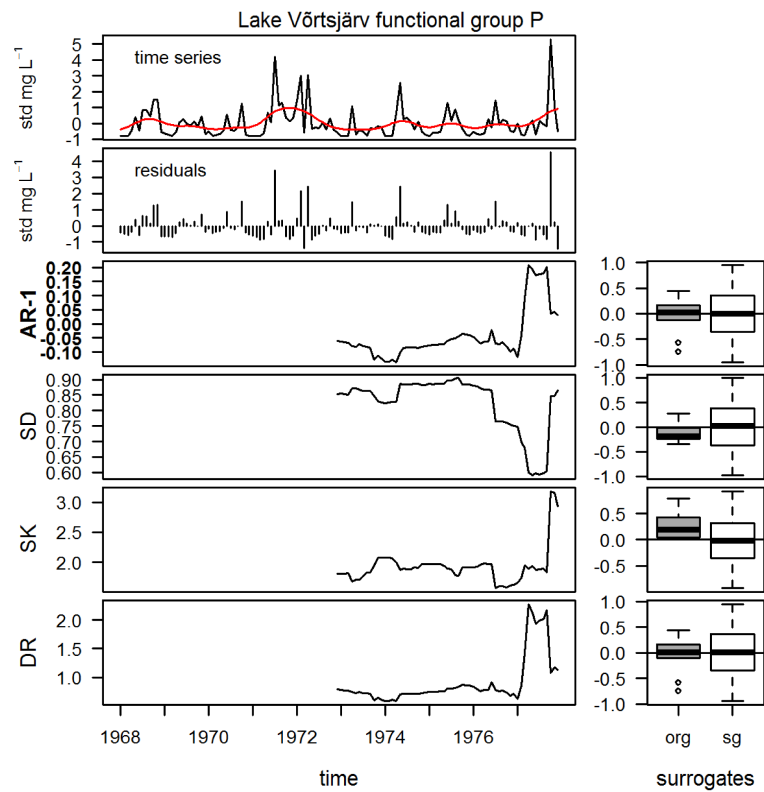
S3.11 Lake Washington: Non-*Daphnia* Cladocera density



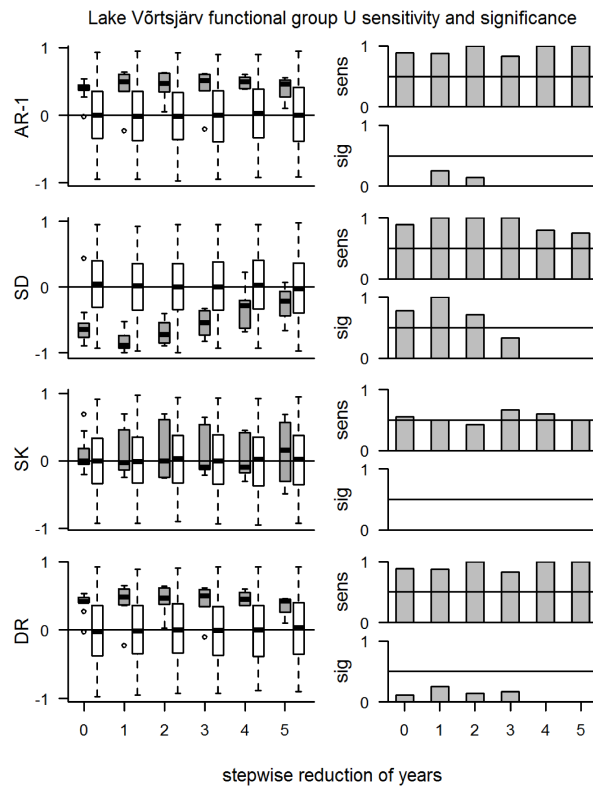
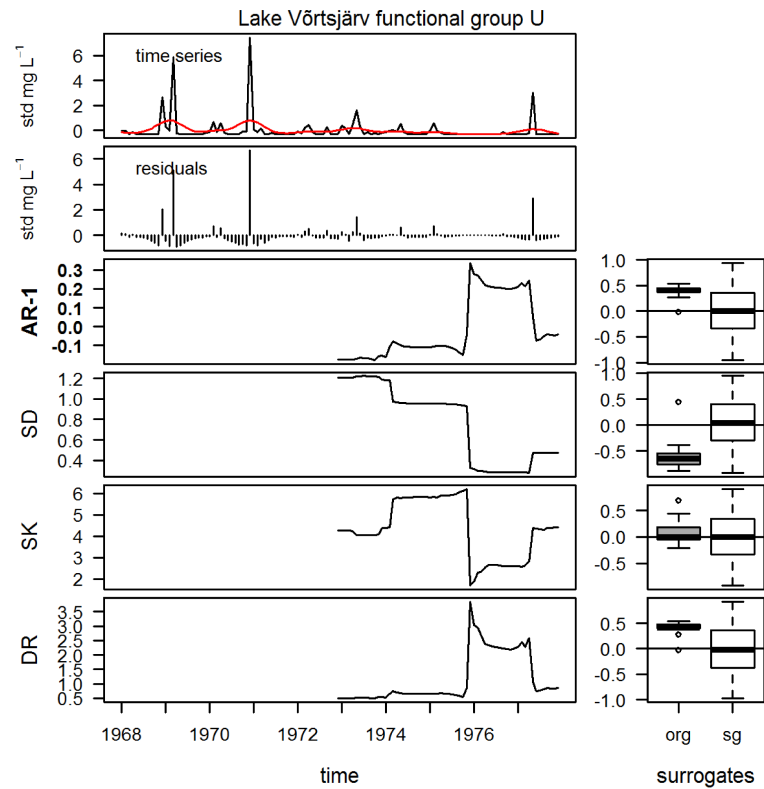
S3.12 Lake Vörtsjärv: Functional group H1 biomass



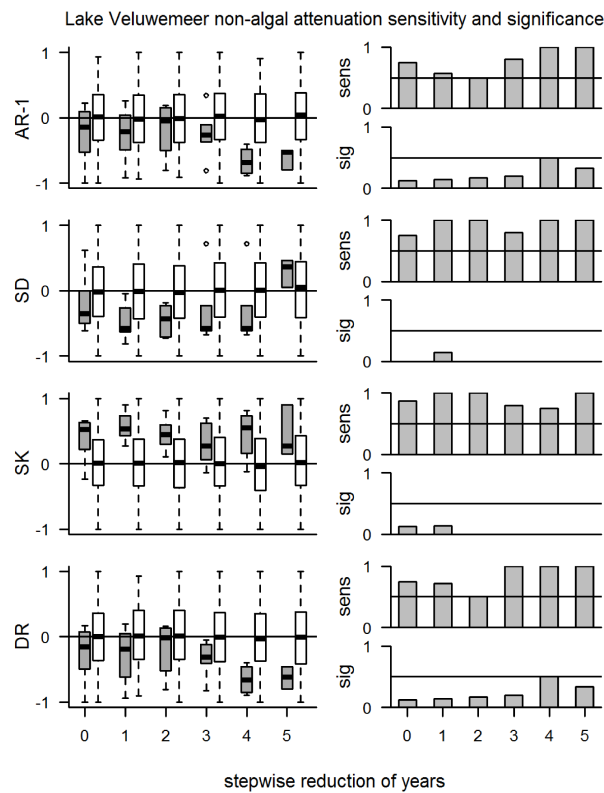
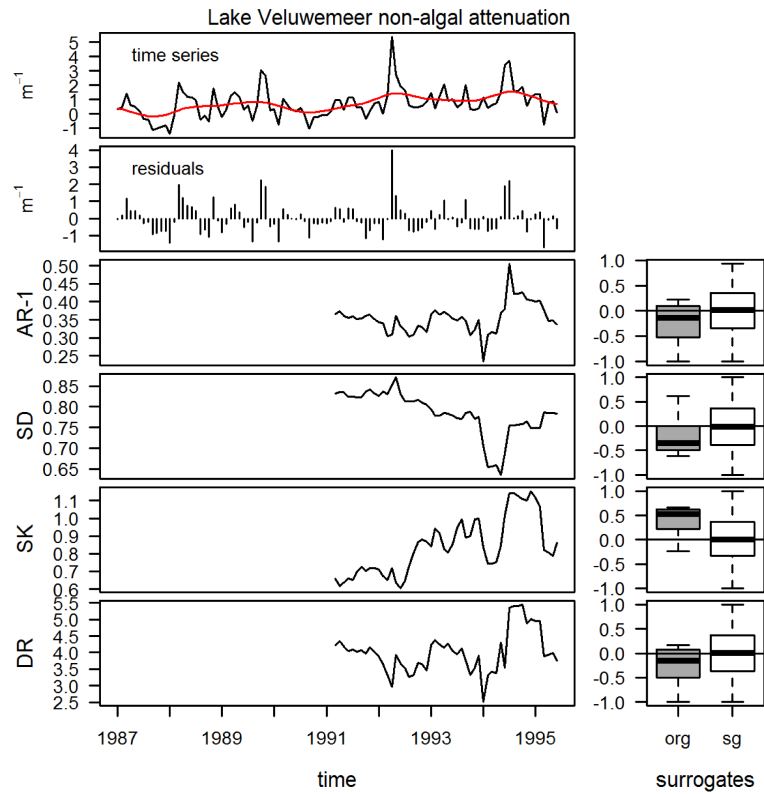
S3.13 Lake Vörtsjärv: Functional group P biomass



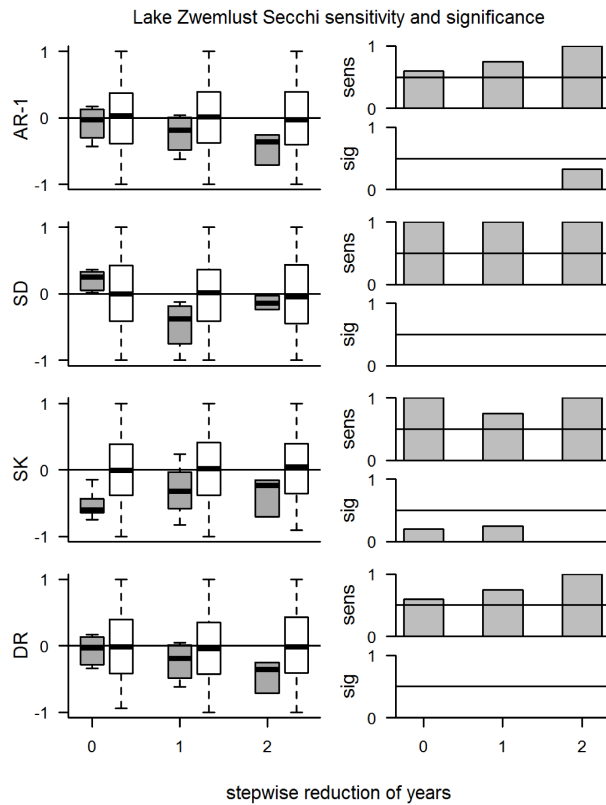
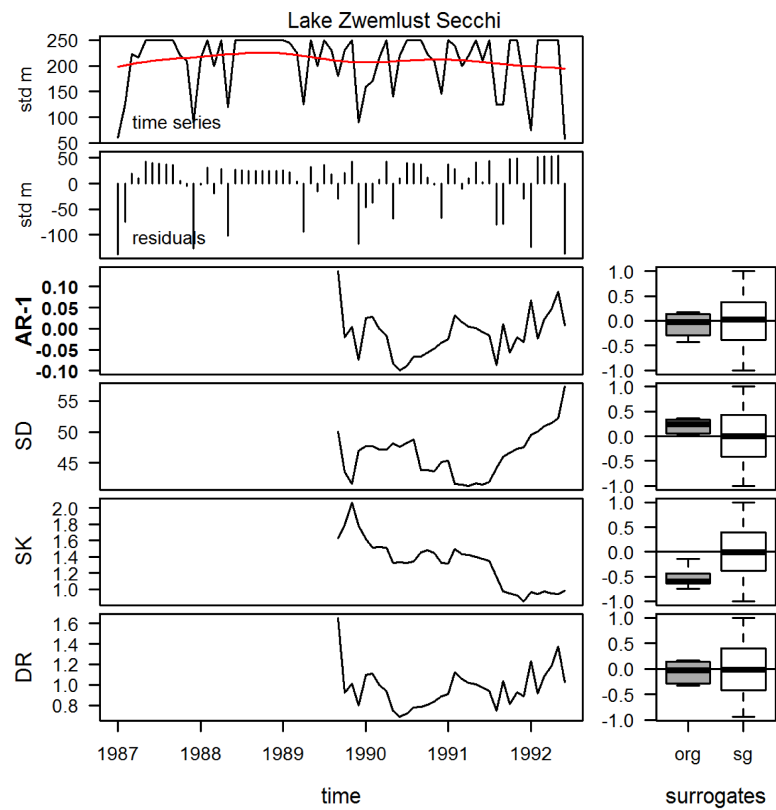
S3.14 Lake Vörtsjärv: Functional group U biomass



S3.15 Lake Veluwemeer: Non-algal attenuation



S3.16 Lake Zwemlust: Secchi depth



Chapter 5

General Discussion

The aim of this thesis was to contribute to a better understanding of threshold-driven changes in shallow lake ecosystems and to assess the potential of generic early warning indicators (EWIs) to predict discontinuous regime-shifts in lakes based on observational data from standard monitoring schemes. Shallow lakes are the most numerous lake type and many of them are under pressure of simultaneous changes in multiple driving forces, including eutrophication and climate related parameters like temperature, water level change and likelihood of extreme events. Changes in those key driving forces increase the likelihood that thresholds might be crossed, thereby triggering fundamental changes in the functioning of lakes and impair important ecosystem services like carbon sequestration. This makes it important to better understand the relevance of threshold-driven changes in shallow lake ecosystems and to identify the underlying mechanisms and drivers. In an analysis of an abrupt dominance shift between two coexisting copepod species in Müggelsee we successfully linked data-driven analysis with intraguild predation (IGP) theory and identified a smooth as well as an abrupt regime-shift. We illustrated how observational data on plankton communities match predictions derived from theoretical models from population ecology. While Müggelsee experienced concurring changes in trophic state and a long-term increase in average water temperatures, we identified trophic state as the principle driver (**Chapter II**). In a second case study we analysed the functional threshold between GPP and ER in regard to three overlapping forces, namely water temperature, trophic state and water level. The temperature gradient was established in a pan-European space-for-time mesocosm experiment under two different nutrient and water levels. The observed differential increases of GPP and ER with increasing temperature closely matched predictions from Metabolic Theory of Ecology. Yet, the functional temperature thresholds over the temperature gradient at which GPP and ER switched dominance were higher for eutrophic than for mesotrophic systems, while water level had only a minor influence. This study pointed to the need to incorporate resources into the MTE (**Chapter III**). Finally, we tested the usefulness and reliability of EWIs to signal an impending discontinuous regime shift based on standard monitoring data. Contrary to expectation, even though many EWIs were positively detected, there was little consistency between the four tested EWIs. The reason for those inconsistencies is far from obvious and no general agreement exists how to handle them best. Thus, with this study an important step was taken, to reveal current shortcomings of generic EWIs. Intuitive expectations of the concept as a universal tool to indicate impending regime shifts in ecosystems are not met (**Chapter IV**).

Common to all the presented case studies was that data driven analysis was closely linked with current ecological theory. The explicit bridge between data and theory facilitated both

interpretation of results but also informed theory.

5.1 Combining theory with empirical data facilitates improved understanding on threshold-driven changes

The aim of theoretical approaches is the formulation of general patterns of causal relationships, while empirical data mirror the high individuality and stochastic nature of biological systems. A close link between theory and data is needed to improve our causal understanding and ability to predict global change effects on ecosystems, but also to understand which processes are of importance under which circumstances, as well as the inherent uncertainties of our predictions. In the following I will discuss how the main findings presented in this thesis contributed to improving the understanding of the relevance of threshold-driven changes in lake ecosystems, the processes and mechanisms behind them and their timely prediction. I will highlight how the three performed studies in which we linked data driven analysis with current ecological theory facilitated this aim, but also identify how some of the analyses could be carried on to further improve our understanding.

5.1.1 Augmenting data driven regime shift analysis with mechanistic understanding from process based models

The expectation that anthropogenic forced changes in many key driving parameter of ecosystems increases the probability that thresholds of non-linear systems might be crossed, highlights the need to better understand threshold driven changes with particular acuteness. Despite this impending risk and well documented examples of regime shifts in several ecosystems (Scheffer, 2009) and in particular in shallow lakes (Scheffer, 2004), the actual relevance of theoretical predicted threshold driven shifts in complex ecosystems is still an active field of research (Reid et al., 2005). There still seems to exist a discrepancy between the general popular recognition of regime shifts and the general confidence that they are indeed of wide spread relevance in complex natural systems where multiple processes are at work at the same time and external environmental heterogeneity can weaken discontinuous regime shifts and reduce hysteresis (van Nes and Scheffer, 2005). A reason for the discrepancy might lay in the fact that while there are sound theoretical (Scheffer, 2009) and empirical (Wagner and Adrian, 2009b; Reid et al., 2016) grounds for threshold-driven changes, the actual linkage of these observations to abrupt or discontinuous regime shifts, i.e. the exclusion of a smooth regime shift, the differentiation between abrupt and discontinuous regime shifts, is challenging (Andersen et al., 2009; Scheffer, 2009; Bestelmeyer et al., 2011). Furthermore, individual processes behind many sudden shifts found in time series are still poorly understood, since the establishment of causality from long-term observational data is only approximately possible.

In the *C. vicinus* – *C. kolensis* case study in **chapter II**, I showed that the close linkage between

process based models and data proved successful in the identification of the nature of long-term response pattern towards environmental change, as well as the identification of threshold values for the major driving forces. While data driven breakpoint-, trend- and correlation based analysis helped to identify and distinguish a smooth and an abrupt regime shift, the link to intraguild predation (IGP) food web theory enabled me to identify the mechanisms behind this dynamics and thus to come as close to a causal understanding as likely possible based on observational data. At the same time, I demonstrated that results from mechanistic models are of relevance in community dynamics in complex ecosystems, despite the fact that they are often criticised as oversimplifications. A link and the collection of compelling evidence does not provide proof. Yet, increasing certainty can further be gained by repeated identification of IGP dynamics in lakes. Repeated identification of such dynamics could also facilitate a further differentiation between an abrupt or discontinuous regime shift for *C. kolensis*, which was not possible with a single observation. While the *C. kolensis* – *C. vicinus* example is a rather lake specific case study in itself, it nicely documents that further confirmation and general recognition of the importance of the dynamics of IGP food webs would be an interesting enrichment of food web research where simple linear food webs are often in the focus.

5.1.2 Testing predictions from dynamic theory with long-term observational data

When investigating the dominance shift between *C. kolensis* and *C. vicinus* results from dynamic models were used to identify a plausible mechanisms leading to a singular abrupt reorganisation observed in the long-term observational data. In the study of EWIs a set of long-term observational data was used to systematically assess the occurrence of EWIs, which are predicted by dynamic system theory to exist prior to a discontinuous regime shift, due to critical slowing down. The existence and detectability of EWIs has been successfully proven in modelled, experimental and reconstructed paleo-climate time series. An important next step towards a much desired generic tool for management to detect impending discontinuous regime shifts in good time, is a systematic assessment of their reliability for long-term observational data. The reliability of EWIs for data from natural system is not at all obvious. In natural ecosystems many processes simultaneously shape the measured parameter, such that the signal from the process of interest might be heavily intermingled with noise. For instance, based on model simulations, it has been shown that the interacting effects of co-occurring regime shift generating mechanisms with shared state variables can lead to either magnified or muffled variance in the shared state variable (Brock and Carpenter, 2010). Furthermore, standard monitoring schemes often have only monthly or even lower temporal resolutions, including sampling gaps, which seriously under samples many of the faster processes particular on plankton level (Aguilera et al., 2016). Clements et al. (2015) found that only sufficient length of a time series could make up for the detrimental effect of low sampling frequency to detect EWIs based on simulated and experimental data. Despite these obstacles only 2 out of our 16 tested variables showed none of the tested EWIs in our analysis.

Additionally and important for management, EWIs could be detected considerable time periods before the regime shift. Yet, consistency between the four tested indicators was generally low and even the most reliable EWIs, lag-1 autocorrelation and density ratio, could detect only 10 out of 16 regime shifts. Lag-1 autocorrelation and density ratio have been reported as the most robust indicators before (Dakos et al., 2012b; Clements et al., 2015). In our assessment lag-1 autocorrelation and density ratio were not only the most reliable, but also with 100% accordance the most consistent ones. Even so this is encouraging, only four of the detected EWIs withstood rigorous significance testing. This is the case despite the fact that lakes, as 'closed' systems with fast process time scales, are deemed particular suitable system to detect EWIs. Moreover, we conducted hindsided detection, i.e. we tested for EWIs on sudden changes, where in-depths prior analysis gave a fair certainty that, these were indeed cases of discontinuous regime shifts. However, like in the case study on the dominance shift between *C. vicinus* and *C. kolensis* there is no way for an ultimate proof and we can not exclude the possibility that some failure to detect EWIs might stem from a misclassification of the sudden shift as discontinuous regime shift. However, failure to detect EWIs and inconsistency between them could have several other possible reasons. Tests on the robustness of variance and autocorrelation, for instance, showed that while increasing autocorrelation could always be detected, variance sometimes decreased close to bifurcation points, because critical slowing down can make the system too slow to follow high frequency fluctuation in the environment (Dakos et al., 2012b). Boerlijst et al. (2013) proved that while critical slowing down is a universal property of fold bifurcation, EWIs still do not manifest necessarily in all state variables of a dynamic system (false negatives). Finally, Boettiger et al. (2013) pointed to the fact that EWIs are neither exclusive for discontinuous regime shifts (false positives), in the sense of alternative stable states with hysteresis, nor do they capture all possibilities of rapid and fundamental changes in ecosystem. Other bifurcation types like Hopf- and transcritical bifurcations experience critical slowing down (Kéfi et al., 2013; Batt et al., 2013a; Dakos et al., 2014) and thus show EWIs. Even smooth as well as abrupt regime shifts, which do not have bifurcation points, have been demonstrated to show increased standard deviation and AR(1) (Kéfi et al., 2013). On the other hand regime shifts caused by chaotic crisis, noise-induced transitions, or Maxwell point transitions in heterogeneous spatial systems are not expected to be preceded by EWIs (Boettiger et al., 2013; Dakos et al., 2014). One must conclude that based on current knowledge EWIs can only be applied if expert knowledge of the system is extensive, and as such the concept is far from being a ready to use tool for ecosystem management. By disclosing those critical shortcomings of the current concept of EWIs we give important impulses for future research into EWIs. Suggested ways forward could be the combination of spatial and temporal indicators (Dakos et al. 2010), indicators from food web stability (Kuiper et al., 2015) or model-based approaches (Boettiger and Hastings, 2012; Dakos et al., 2014). For the potential shortcomings in data resolution the increasing availability of high frequency data from automated measuring stations, particular for lakes, but also remote sensing, could be an important step forward (Batt et al., 2013b).

However, one could also raise the question if the often strong focus on discontinuous regime shifts, i.e. fold bifurcations with alternative stable states and hysteresis is too short sighted. It is unarguable that hysteresis can add additional difficulties for ecosystem restoration. In the context of global change, however, every reversal in a driver, not just the ones burdened with hysteresis, can be challenging, due to the enormous international coordination needed to take effective counter measures. A sad example for such tremendous difficulties is the problem to agree upon and organize international efforts to reduce greenhouse gas emission to stabilise climate. An example where a reversal of already occurred changes is likely impossible are pollutants with very long half-value times like micro plastics. In this light, any type of abrupt shift causing major changes in the environment could be declared as serious. Following the precautionary principle could at all be the better choice from a management perspective, than hoping for a last minute alarm clock.

5.1.3 Testing hypotheses derived from theory with data from large scale mesocosm experiments

In a very promising example to gain understanding by linking theory with empirical data we used MTE in combination with findings from empirical (del Giorgio and Peters, 1994; Hanson et al., 2003; Duarte et al., 2004; Staehr et al., 2010b; Solomon et al., 2013) and theoretical studies (Dakos et al., 2012b; Cross et al., 2015) about temperature, nutrient and light effects on metabolism to formulate hypothesis followed by subsequent testing in our space-for-time pan-European mesocosm experiment. Here we encountered the strength of experimental tests under controlled conditions paired with the problem of high within treatment variability not unknown to mesocosm experiments and confounding effects by covariation of latitudinal specific external conditions when using space for time experiments.

In particular, I formally derived in the framework of MTE an expression (equation 3.5, Chapter III), which formalises two potential action paths on how nutrients and light might influence the temperature at which net autotrophic lakes switch to net heterotrophic lakes. The first more obvious path is through interactions influencing the physiological or apparent temperature sensitivity of GPP and ER. The second is through their influence on absolute metabolic rates, i.e. the ratio between GPP and ER at a reference temperature. Formulating our hypotheses in reference to the MTE had the advantage of direct comparability of our results to results from other studies on temperature effects on the metabolic balance (López-Urrutia et al., 2006; Iop; Yvon-Durocher et al., 2010b; Demars et al., 2011; Huryn et al., 2014; Welter et al., 2015). More importantly, our results could also be immediately translated to short-comings of the MTE; namely its lack of considering essential resources.

The found temperature dependence based on our pan-European space-for-time mesocosm experiment were intriguingly close to MTE predicted values, yet with slightly higher apparent

temperature dependency of GPP than the predicted physiological temperature dependency based on C3 land plants used by MTE. We ascribed this discrepancy to the positive correlation between temperate and day length, since temperature sensitivity of day length corrected values for GPP were lower and matched MTE predictions. Such an effect of day length serves as a good example as to how covariation of other drivers might serve to buffer the imbalance between GPP and ER predicted by the pure temperature effect. Contrary to our expectations, however, temperature dependence of metabolic rates did neither differ significantly between mesotrophic and eutrophic conditions nor between high and low water levels. Yet, overall variance in estimated activation energies among mesocosms, months and countries were rather large, despite the great care which was taken to standardize the experiment with a common protocol (Landkildehus et al., 2014). This variation could stem from idiosyncratic effects, since complex systems are sensible to small variations in the starting conditions. However, large variability could also hint to the power of covariation or interactions of factors differing over the latitudinal gradient to modify temperature dependence for single systems, as already described by De Castro and Gaedke (2008). Thus, we were cautious in interpreting the individual deviations since the large variance and the much smaller data base for single mesocosms, months or countries added additional uncertainty. However, the overall confirmation of a significant temperature dependence of metabolic rates despite the large variation within treatments hints to the fact that it is a strong signal in averaged large scale dynamics, an interpretation in line with findings by Dell et al. (2011). The most far reaching finding of our experiment was the dependence of the balance of absolute metabolic rates to the trophic status of a lake. Trophic state influences the switch point temperature from net auto- to net heterotrophy, which occurred approximately at 4 °C higher temperatures under mesotrophic compared to eutrophic conditions. While confirming that increasing temperature could lead to decreased carbon sequestration capacities in shallow lakes, we also showed that the nutrient status of lakes has a potentially important influence on the non-equilibrium dynamics of metabolic rates, by shifting the transition from a carbon sink to source (based on metabolic balance) to higher temperatures. Thus, including nutrient resources into MTE is likely to improve its predictive power for global change effects on ecosystems in general.

By explicitly adding non-equilibrium aspects of dynamic systems and the investigation of shifts in rate processes to the framework of threshold driven changes I widened the classical view of threshold-driven changes, which classically only considers sudden large changes in the equilibrium state of a state variable. However, adding the aspect of rate process imbalances is justified, considering the fundamental change in lake functioning when a transition from net auto- to net heterotrophy occurs. Hence, we refer to this type of threshold as functional threshold. Yet, there is also a straightforward link from functional thresholds to changes in equilibrium state, when depicting the system as simplified box model, as indicated in the introduction (Figure 1.2). Taking the box as the amount of stored carbon and GPP as input and ER as output rate of carbon, the found threshold temperature then marks the point where the non-equilibrium trajectory of

the system either heads to a theoretical new low or new high equilibrium state in carbon storage, acting as either source or sink of carbon along this trajectories. This box model is certainly a very simplified view, since other processes add and subtract organic carbon in lakes, like imports and export rates from and to the catchment, photo-oxidation or anaerobic respiration (Lovett et al., 2006). Sediment and the lake's water column would also be considered as separate boxes in more detailed models with their own exchange processes. It also stands to reason whether equilibrium states for lake carbon stores are at all relevant, since virtually all lakes receive some organic carbon from their catchment and many increasingly so under future climate scenarios (Jennings et al., 2009; Tranvik et al., 2009; Larsen et al., 2011). However, the simplified model serves to illustrate the potential importance of functional thresholds and its principle link to the more classical views on threshold-driven processes.

Follow up analysis should embed the found dependence of metabolic balance on temperature and trophic state into the context of the full flux network of carbon dynamics in shallow lakes to improve predictions on shallow lakes functioning as sink or source of carbon under future climate change scenarios. Another important and logical next step is to test the generality of the observed trophic state specific functional temperature thresholds with long-term observational data covering the entire yearly cycle. Our experiment ran from May to November. Thus, like so many studies and experiments we missed out winter metabolic dynamics, an almost uncharted territory in lake ecosystems research (Sommer et al., 2012). Since in winter light and carbon limitation might dominate metabolic dynamics (Huryn et al., 2014), the importance of temperature driven aspects need to be tested in order to be able to calculate annual metabolic budgets in lakes. Finally, very little is known about the influence of changes in stratification pattern and lake community composition on metabolism and metabolic balance (Tranvik et al., 2009). However, this is an important aspect to better understand as to how lake metabolism will change under climate change and eutrophication pressure, were shifts in thermal regime and community composition are already widely recognized (for review see Adrian et al. (2009), De Senerpont Domis et al. (2013)).

5.2 Time-for-time versus space-for-time versus experimental approaches

“How do you study an ecosystem no ecologist has ever seen?” This bold question asked by Williams and Jackson (2007) pointedly summarizes the challenges which ecologist encounter when they try to extrapolate current knowledge to infer future ecological systems. There are basically three empirical frameworks to investigate global change effects, which are also well know from climate change research: time-for-time and space-for-time approaches, as well as manipulative experiments (Kosten et al., 2009; Yvon-Durocher et al., 2010b; Huber et al., 2012; Stewart et al., 2013; Blois et al., 2013; Elmendorf et al., 2015). In time-for-time approaches

monitored variability in e.g. decadal long-term observational data towards certain drivers are mapped to potential future ecosystem trajectories. Similarly, space-for-time approaches use contemporary spatial gradients, while experiments rely on selective manipulation to understand future changes. The *C. kolensis* – *C. vicinus* case study based on decadal observational data is an example for a time-for-time approach where changes in nutrient availability, not temperature were identified as the major driver for the sudden change. In our experiment we combined a space-for-time approach with mesocosm experiments and were able to quantify trophic state specific switch point temperatures for metabolic balance. However, all three approaches have strengths and weaknesses with regard to their ability to extrapolate to future ecosystem trajectories. It is under debate, which of the methods give more faithful predictions of potential global change effects on ecosystems. While there seems to be qualitative agreement between the directions of predictions of the three approaches, there can be considerable quantitative differences (Elmendorf et al., 2015). Space-for-time approaches are thought to be prone to potentially overestimate effects (Blois et al., 2013; Elmendorf et al., 2015). By selecting a gradient for one driver, e.g. temperature, effects might get artificially enlarged, while under global change pressure they could be overridden or dampened by covariation in other factors, e.g. eutrophication (Blois et al., 2013; Davidson et al., 2015; Welter et al., 2015). Elmendorf et al. (2015) demonstrated good quantitative agreement between experimental and time-for-time approaches, while the effect size in the space-for-time approach was considerably larger. Interestingly Blois et al. (2013) demonstrated good agreement between space-for-time predictions with time-for-time predictions using palaeoecological data- covering across epochal time scales. This result points to the important aspect of temporal scale. How long does it take until changes show their full and lasting effect on both biotic and abiotic components of an ecosystem? Experimental and time-for-time predictions from long-term observational data might mirror short term climate change effects, while space-for-time predictions could mirror the long-term effects. Experiments often do not run long enough to capture time delayed effects, like lags in species response to temperature or slower changes in the biophysical environment, thus capturing non-equilibrium states (Elmendorf et al., 2015). The same problem applies to the comparison of inter-annual variability in long-term observational data or the analysis of extreme events, where important mechanisms like, migration, adaptation and thus the formation of new communities and their interactions might not have yet been fully established. Experiments are good at systematically disentangling effects of simultaneous changing drivers and are the only way to establish cause effect relations. Large mesocosm experiments, from all experimental set-ups cover the highest degree of realism and allow for hypothesis testing at quasi ecosystem level. However, mesocosms are well known for the 'wall effect', mainly caused by periphyton with its potential to considerably influence nutrient turnover and metabolic rates (Wetzel, 2001; Petersen, 2009). Space-for-time approaches on the other hand investigate systems well adapted to the respective current climate conditions in the respective geographical area, adding to the realism of observed temperature effects and thus overcoming uncertainties with which heated mesocosm experiments are burdened (Yvon-

Durocher et al., 2010b; Davidson et al., 2015). Again, space-for-time experiments are burdened with covariation of additional drivers such as systematic differences in day length, seasonal temperature development, humidity and global radiation. Those factors might well be irrelevant to expected global changes, while other important factors like difference in CO₂ level can not easily be varied along latitudinal gradients. Further, the historical development of the site and thus the species and communities might have taken place on considerable slower time scales than the pace of modern global change. Stewart et al. (2013) argues that blending different approaches can combine their strengths and mitigate their weaknesses, we followed this notion by combining mesocosm experiments with a space-for-time approach. The space-for-time aspect offered the possibility to set-up the mesocosm in lakes to ensure a natural and ambient water temperature while at the same time we could establish a large temperature gradient. The utilization of plankton and sediment inoculations from local lakes ensured that communities adapted to local climate conditions could develop. At the same time the experimental approach allowed us to test on those local communities the combined effect of a set of driving variables relevant for global change, namely temperature change together with trophic status and water level change. Yet some of the well know problems connected with space-for-time approaches and mesocosm experiments still persisted. The experiment was carefully set up to mimic all characteristic components of shallow lakes, including sediment, macrophytes and a 3 level food chain including planktivorous, but not piscivorous fish. Periphyton growth on the walls introduces uncertainty about how much of the metabolic activity of periphyton is captured in the dial oxygen curves, since periphyton tend to form a boundary layer to its environment. Further, our mesocosms were constantly mixed, this has the great advantage to know exactly the water volume that is considered for the calculation of metabolic rates, which is in natural system a great source of uncertainty (Staehr et al., 2010a). However, increased temperatures are expected to be accompanied by more frequency and longer lasting stratification events (Wagner and Adrian, 2011). This can have considerable effects on metabolic rates (Staehr et al., 2012; Obrador et al., 2014) and high nutrient conditions could lead to low oxygen conditions as was observed in mesocosm experiments in England (Moss, 2010). Set-up and sampling in our experiment were coordinated by a common framework, however due to the distance between the experimental locations, the sediment and the water to fill the mesocosms differed between countries. Further, between experiment locations were systematic differences in day length, seasonal temperature development and other climate parameters like humidity and global radiation. In summary, the combination of a space-for-time approach with mesocosms experiments proofed to have several new strong points no approach would have on its own, while some of the classical problems of each approach still remained. However, the close accordance of the found temperature effect on lake metabolism to other studies and predictions made by MTE gives great confidence in the generality of our findings. Ultimately, when coming back to the question by Williams and Jackson (2007) on “How to best study an ecosystem no ecologist has ever seen? While I have to conclude that a the *definite* answer can only lay in the future, elaborate computer models – supported by observational data and theory – give

evidence for our capacity to actually predict the expected changes and subsequent consequences even so with limited certainty. The 2 °C threshold in global air temperature rise, not to be exceeded to prevent major changes in the climate, has gained general acceptance in climatology (IPCC, 2013) and politics (FCCC/CP/2015/L.9/Rev.1, 2015) in the meantime. Globally lake surface summer water temperatures have already come close to that critical threshold in the most recent past decades (O'Reilly et al., 2015) – with noticeable consequences for lake ecosystems. However, more than only the changing climate exerts pressure on global ecosystems. This stresses the importance of new concepts, such as the Planetary Boundary Concept, which address ranges of critical thresholds, under consideration of all relevant stressors and their interactions, beyond which the functioning of the earth system is at risk. The results presented in this thesis regarding threshold-driven changes and their dependence on multiple drivers may inform such new concepts and further improve our ability to predict future ecosystems.

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Appendix A

Climate change effects on shallow lakes: design and preliminary results of a cross-European climate gradient mesocosm experiment

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Climate change effects on shallow lakes: design and preliminary results of a cross-European climate gradient mesocosm experiment

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Abstract. Climate change is expected to profoundly affect both temperature and net precipitation, with implications for lake water level. We describe the design of a harmonized, simultaneous, cross-European mesocosm experiment to elucidate the effects of climate change on community structure, functioning, and metabolism in shallow lakes at low and high nutrient levels with contrasting depths (1 and 2 m). We used cylindrical ($D = 1.2$ m) tanks that were either 1.2 or 2.2 m high, each having a 10-cm sediment layer. We inoculated the mesocosms with a mixed sample of sediment and plankton from lakes with contrasting nutrient concentrations and added macrophytes and planktivorous fish. Sediment was pre-equilibrated to the required experimental nutrient concentration. During the experiment the water level decreased with increasing temperature (up to 90 cm in the Mediterranean mesocosms) while conductivity increased. The average chlorophyll *a* concentration increased with temperature in the deep mesocosms but was more variable in the shallow mesocosms. Macrophyte

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abundance increased with temperature, while the oxygen data suggest that net primary production peaked at intermediate temperatures. We conclude that our experimental design has the potential for tracking the interacting effects of global warming and eutrophication in shallow lakes.

Key words: climate change, REFRESH project, macrophytes, nutrient enrichment, metabolism, water level effects, macroecology.

INTRODUCTION

Climate change is expected to significantly change temperature regimes and precipitation patterns across the world (IPCC, 2007; Bates et al., 2008), with implications for the nutrient status of lakes. In the northern temperate zone, loadings of phosphorus and nitrogen are expected to increase due to elevated precipitation and soil decomposition levels, leading to higher nutrient loss from land to lakes (Schindler and Vallentyne, 2008; Adrian et al., 2009; Jeppesen et al., 2009, 2011). Moreover, internal nutrient loading tends to increase in eutrophic lakes due to higher temperature and increased mineralization and as an indirect warming effect of prolonged or temporary thermal stratification (Jensen and Andersen, 1992; Søndergaard et al., 2003; Mooij et al., 2005; Wilhelm and Adrian, 2008). These changes favour the outbreak of algal blooms, often of toxic cyanobacteria (Huisman et al., 2004; Wagner and Adrian, 2009). However, there are also examples of shallow lakes showing lower nutrient concentrations in warm years due to higher water levels. For example, in Estonian lakes higher winter North Atlantic Oscillation (NAO) years, which are characterized by warmer and wetter winters, are associated with higher water temperatures but also with higher lake water levels (Nöges, 2004). In shallow Lake Võrtsjärv, high water level years are characterized by a lower P concentration due to weaker resuspension and leakage from bottom sediments, while the N concentration is higher as a result of lower denitrification rates in the deeper water column, leading to a higher N:P ratio and less favourable conditions for N-fixing cyanobacteria (Nöges et al., 2003).

In Europe global climate change is predicted to result in an approximately 25–30% decrease in precipitation and enhanced evaporation in the Mediterranean region. Pronounced interannual variation can be expected due to increasing frequencies and magnitudes of extreme drought events (Giorgi, 2006; Giorgi and Lionello, 2008). Droughts may reduce runoff, cause lower external nutrient loading, and potentially increase water clarity. However, such effects may be context dependent. For instance, in eutrophic lakes, higher evaporation, higher internal nutrient loading, and, possibly, reduced nitrification under low-oxygen conditions can lead to higher nutrient concentrations and lower water clarity (Jeppesen et al., 2009, 2011; Özen et al., 2010; Papastergiadou et al., 2010).

Climate warming may also affect trophic structure and dynamics. In warm lakes, top-down control by fish is strong due to the dominance of small and abundant planktivorous and benthivorous fish (Jeppesen et al., 2010a, 2012), enhancing zooplankton predator control and reducing grazing on phytoplankton (Gyllström et al., 2005; Meerhoff et al., 2007; Stefanidis and Papastergiadou, 2010).

Such changes may have an adverse influence on submerged macrophytes due to the deteriorating light conditions. Conversely, a lower water level can improve the light climate for macrophyte growth (Blindow, 1992; Nöges and Nöges, 1999; Coops et al., 2003; Beklioğlu et al., 2006), depending on lake morphology (Beklioğlu et al., 2006). A mesocosm study in a Turkish shallow lake showed that macrophytes resist increased nutrient loading, perhaps as a result of evaporation-triggered water level reduction, overriding the deleterious effect of periphyton- and phytoplankton-induced turbidity (Özkan et al., 2010). A similar outcome was observed in a recent experiment where water level and the presence or absence of fish were manipulated in a warm eutrophic shallow lake (Central Anatolia, Ankara) (Bucak et al., 2012). Results from both studies suggest that increased evaporation during summer in this region may help maintain the growth of submerged macrophytes in eutrophic shallow lakes despite the reduction in water clarity caused by fish predation. Bucak et al. (2012) concluded that the adverse effects of climate-driven eutrophication on water clarity may be counteracted by reduced water levels, provided that physical disturbance is not severe in the shallow margins of the lake. Climate change induced alterations in water levels may therefore be critical, both in shallow lakes in the Mediterranean and the continental part of the north temperate region where summer may become drier in the future. Consequently, water level effects need to be considered more explicitly in the analyses of climate change impacts on shallow lakes.

Space-for-time substitution is one of the most widely used approaches in ecological and climate change research. It accounts for key differences between ecosystems resulting from their location along broad latitudinal and altitudinal gradients and therefore allows assessment of how factors influencing ecosystem structure and function vary along these gradients (Meerhoff et al., 2012; Jeppesen et al., 2014). This approach has been used to analyse a series of lake surveys in Europe (Moss et al., 2003; Declerck et al., 2005; Gyllström et al., 2005; De Meester et al., 2006; Brucet et al., 2012), South America (Kosten et al., 2009, 2011), and in cross-continental studies (Jeppesen et al., 2007; Meerhoff et al., 2007; Kosten et al., 2012). The strength of this approach is that the biological assemblages *per se* have had time to evolve and adapt to the climate in which they live. However, it does have weaknesses in that biogeographical issues may be of importance and that correlative studies do not necessarily provide causal relationships (Jeppesen et al., 2014). Furthermore, the influence of co-variates of temperature and latitudinal variation on other climate-related variables such as seasonality and the length of the growing season may go undetected, and confounding factors that cannot be controlled may swamp the climate signal. Thus, alternative approaches are also needed to elucidate climate change effects more mechanistically, despite the challenging nature of the macroecological scales of interest.

A useful alternative is to combine correlative space-for-time substitution approaches with controlled experiments. A cross-European mesocosm experiment conducted in 1998 and 1999 (under the auspices of the SWALE project) in six lakes provides an example of such an approach. In this experiment, lakes were distributed from Finland to southern Spain, and the effects of fish addition and

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nutrient loading on shallow lakes were studied using a common protocol (Moss et al., 2004; Stephen et al., 2004). In these experiments, and contrary to the findings from latitude gradient studies, no differences in the degree of top-down control along the latitudinal gradient were observed, perhaps because of fixed fish densities. However, the variability of the experimental outcome from one year to the next increased with latitude, reflecting the greater variation in weather at the onset of the experiments (Moss et al., 2004). The SWALE study was restricted to a single depth and differed in the starting level of nutrient loading. Moreover, lake metabolism was not included.

This paper describes a refined design of a cross-European mesocosm experiment that accounts for the importance of water level fluctuation in driving ecological processes in lakes following global change. It examines the overarching effects of climate change on trophic structure and dynamics, as well as lake metabolism, at two nutrient levels and water depths (1 and 2 m) along a climatic gradient in six countries from Sweden to Turkey. The aim is to describe the highly standardized experimental design and sampling procedures, a key objective in terms of optimizing the comparison of results and reducing the risk of bias. We present the design of the experiment and some physico-chemical and biological data before discussing the strengths and weaknesses of the approach. This paper will be followed by a number of more detailed papers on the various physico-chemical variables studied.

METHODS

Experimental lakes and study period

For the experiment we selected a shallow, alkaline, clear freshwater lake in each of the six participating countries (Table 1). The lakes had: (i) a mean depth ≤ 4 m,

Table 1. Summary information on the study sites. Precipitation and air temperature cover the period 1 May to 1 November 2011

Experimental site	Coordinates	Climate	Altitude, m a.s.l.	Total precipitation, mm	Air temperature, °C
Sweden – Erken	59°49'59"N 18°33'55"E	Boreal	11	271	14.6
Estonia – Võrtsjärv	58°12'17"N 26°06'16"E	Boreal	35	298	14.9
Germany – Müggelsee	52°26'0"N 13°39'0"E	Transient maritime/continental	32	431	16.9
Czech Republic – Vodňany	49°09'14"N 14°10'11"E	Transient maritime/continental	395	401	15.3
Turkey – ODTÜ-DSİ Gölet	39°52'38"N 32°46'32"E	Transient/continental Mediterranean	998	223	18.7
Greece – Lysimachia	38°33'40"N 21°22'10"E	Mediterranean	16	252	23.4

allowing us to cover the natural patterns of temperature seasonality that characterize shallow lakes, (ii) low nutrient concentrations as we wanted to use natural lake water (where possible) and to run the experiment at both low and high nutrient concentrations, (iii) total alkalinity between 1 and 4 meq L⁻¹, (iv) low salinity (<1‰) and colour (<20 mg Pt L⁻¹). These conditions reflect natural abiotic characteristics of lakes across Europe, increasing the general relevance of our study. The experiments ran for six months in 2011 from spring (May) to the end of autumn (October/November), thus avoiding the ice-covered period at the northern sites. In all countries the experiments started on 9 May (day 1), enabling a synchronized sampling protocol to be employed.

Experimental set-up

The experimental set-up in each country encompassed two nutrient levels both with two different water levels, each represented by four replicates (16 mesocosms in total). A pontoon bridge with eight mesocosms (Figs 1 and 2) arranged in two rows divided by a boardwalk for ease of access was established in each lake. The treatment position was organized randomly but followed the same protocol in all countries (Fig. 1). The pontoon bridges were constructed from wooden or plastic boards and floating devices consisting of 32 plastic barrels, each 120 L. At one site the bridge was anchored at the bottom (the Czech Republic). At one end of the pontoon bridge a small working platform was established. For the free-floating systems the pontoon was anchored only at the platform end, which, irrespective of wind direction, functioned as a wave breaker and landing point for boats. To prevent birds from landing on or foraging in the mesocosms, fruit nets (thin net with, for example, 5 cm × 5 cm or 10 cm × 10 cm mesh sizes) were fixed above the mesocosms with a minimum height of 50 cm.

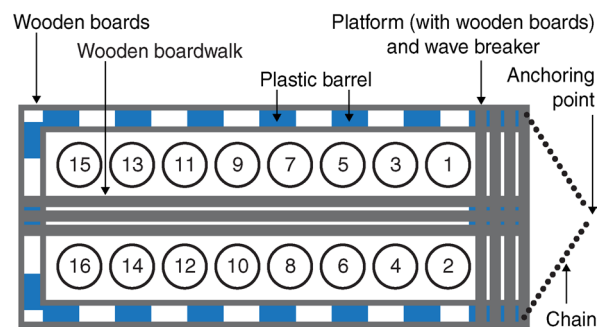


Fig. 1. Schematic representation of the floating pontoon bridge established at sites with prevailing wind exposure. A platform, functioning as a landing platform and wave breaker, is placed on the right side, which also serves as the anchoring end of the bridge. Randomly selected enclosure numbers are ascribed.

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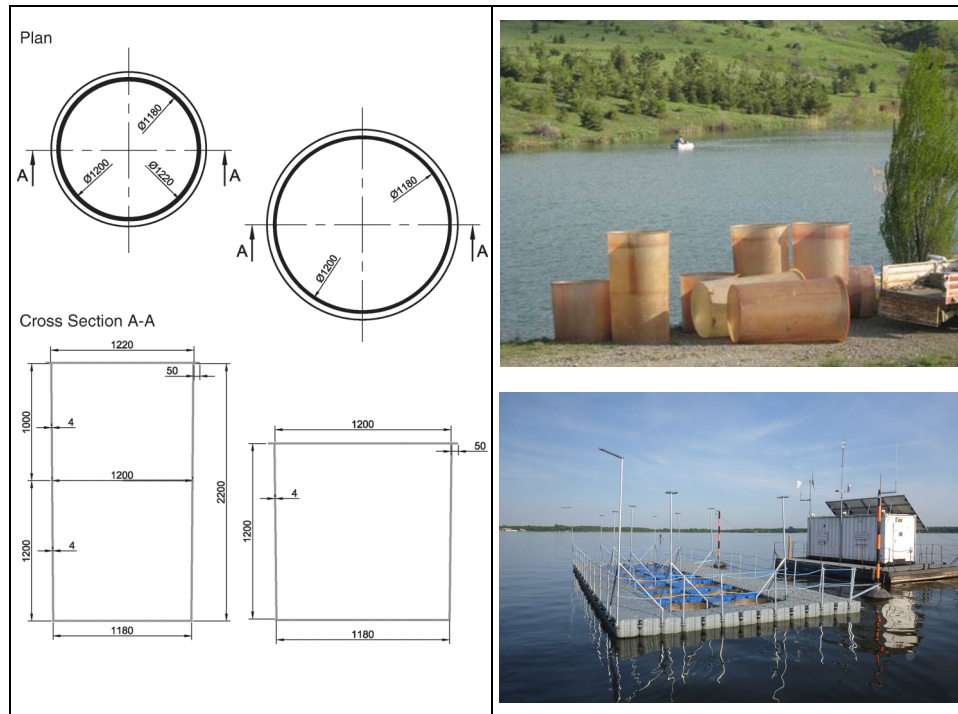


Fig. 2. Dimensions and photographs of the enclosures produced by Armaplast Composites & Plastics in Turkey and of the experimental set-up in Germany at Lake Müggelsee (photo: Thomas Hintze).

All mesocosms were constructed by the same manufacturer (Fig. 2) and consisted of cylindrical ($D = 1.2$ m) fibreglass (4 mm) tanks of two heights, 1.2 and 2.2 m. The robustness of the experimental set-up minimized the risk of loosing sites or treatments and offered a potential for reuse and prevented the diffusion of O_2 and CO_2 , thereby avoiding artefacts in the calculation of mass balances on oxygen and carbon. The upper edges of all mesocosms were attached to the pontoon bridges 20 cm above the water surface to avoid incursion of water during windy periods. The water level in the 1.2 m deep mesocosms was 1 m and in the 2.2 m deep mesocosms, 2 m.

Once established, a 10 cm deep sediment layer was added to each mesocosm. The sediment contained 90% (by volume) washed sand (grain size <1 mm) and 10% sediment from a mesotrophic lake situated close to the experimental site in each country. Large particles (e.g. plant fragments, mussels, stones, debris, etc.) were removed by sieving through a 10 mm mesh. Before the sediment was added, it was equilibrated to the two experimental total phosphorus (TP) treatment levels (25 and $200 \mu g TP L^{-1}$). This avoids transient states in sediment equilibration (resulting from different nutrient loading patterns in the original lakes) during the experiment observed in earlier experiments (E. Jeppesen and M. Søndergaard, personal observations). The pre-equilibration (done country-wise) commenced

during late autumn and winter of the previous year and ran until the start of the experiment. Two tanks were filled with a 10 cm sediment layer (ca 0.2 m³ sediment in each tank), and 20–50 cm water with low (25 µg L⁻¹) or high (200 µg L⁻¹) TP concentrations, respectively. This sediment:water ratio ensured proper exchange of nutrients between the water and the sediment. Following water addition, the sediment and water were mixed with a rake. The TP concentration was measured periodically (at least biweekly) and the water was replaced with fresh nutrient-rich or nutrient-poor water whenever the TP differed from the initial concentrations of 25 and 200 µg TP L⁻¹, respectively. This procedure was repeated until the TP concentrations in the water were at the desired levels (±25%).

Following the addition of sediment, the mesocosms were filled with 500 µm filtered nutrient-poor water from neighbouring lakes with P concentrations <25 µg TP L⁻¹. If this was not possible, water from another oligotrophic lake was transported to the enclosures or, less desirable, tap water was used (e.g. in Germany and the Czech Republic). The mesocosms were left undisturbed for four days to allow the suspended matters to settle.

To simulate a shallow, fully mixed lake, the water in the mesocosms was continuously circulated during the experiment using water pumps. Standard 2 to 5 W aquarium pumps were adequate to pump ca 300 L h⁻¹. To establish comparable mixing between deep and shallow mesocosms, pumps running at half power were used for the shallow (1 m) mesocosms. The pumps were placed with their inlets (via a PVC tube) in the middle of the mesocosm, ca 10 cm above the sediment, and their outlets 10 cm below the surface. Power for the pumps was obtained either by (i) a power cord from the shore (Czech Republic, Estonia, Sweden), (ii) a solar panel (placed horizontally to ensure similar irradiance irrespective of the direction of the floating pontoon bridge) in Greece (240 W, in combination with 12 V 250 Ah batteries) or solar panels from a nearby lake station (Germany), or (iii) a power supply with six parallel connected 120 Ah batteries changed three times a week (Turkey).

Inoculations

To standardize initial conditions and enable the potential for developing a diverse flora and fauna, the mesocosms were inoculated with a mixed sample of sediment and plankton from five local lakes in each country covering a nutrient gradient of 25–200 µg TP L⁻¹. The inoculum was added on day 4 after setting up the experiment and when the initial disturbance effect of adding water and sediment had subsided. For the inocula of plankton, five vertical net hauls (using a plankton net with a diameter of 20 cm and 50 µm mesh), covering the entire water column (bottom to the surface) without disturbing the sediment, were taken in each of the five selected lakes and pooled (per lake). The five samples were kept separately in 5 L barrels, which were filled with lake water from the sample lake. Plankton samples were kept cool prior to addition and were not stored longer than 24 h. The contents of the five 5 L barrels from the five lakes were carefully mixed and a 1 L subsample was added to each mesocosm.

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Five litres of sediment was also collected from each of the five lakes to add biota and/or resting stages of biota. The sediment was collected at a depth approximating the mean depth of the lake. To avoid introducing fish and large mussels (e.g. *Anadonta* spp. and *Dreissena* spp.), the sediment was sieved through a 10 mm mesh. Care was also taken to remove any fish eggs. The inoculum sediment from the five lakes was mixed thoroughly, and 1 L was added on day 4 to each enclosure, dispersed evenly on top of the TP equilibrated sediment–sand layer.

Submerged macrophytes and fish were added to the mesocosms three days after the sediment and plankton additions (day 7). Eight 5–10 cm long apical shoots of water-milfoil (*Myriophyllum spicatum*) collected from the experimental lake (or another lake in the region) were planted (evenly distributed) into each mesocosm. Water-milfoil was selected as it was present in lakes in all countries. In Germany the plants were collected in autumn of the previous year to ensure availability at the start of the experiment. These were kept in aquaria in the laboratory until use. Before introducing plants to the mesocosms, the plants were placed in soda mineral water (carbonated) for 15 min to remove snails and invertebrates. To ensure that shoots would sink into and remain in the sediment until root development (approx. 2 weeks), ca 5 g pebble stones were attached to the shoots by duct tape.

The original intention was to stock a fish species present in all countries and three-spined stickleback (*Gasterosteus aculeatus* L.), size 2–4 cm, was selected. However, it was not possible to catch sticklebacks in Greece, Turkey, and Sweden prior to the experiment, either due to low population numbers or ethical issues (legislation prohibiting the transfer of populations between sites). Thus, sticklebacks were imported from Germany and acclimatized for the Turkish experiment. Underyearling roach (*Rutilus rutilus* L.) and western mosquito fish (*Gambusia affinis* Baird and Girard) obtained from the study lakes were used in Sweden and Greece, respectively. Between 4 and 20 g of fish biomass (equating to six sticklebacks or mosquito fish or two roach) was added to each enclosure irrespective of nutrient level. We sought to attain a male:female ratio of 1:1, allowing breeding during the experiment. Before addition, the fish were measured (mm). Fish were caught at least 1–2 weeks before the experiment to ensure that they would survive handling. Length–weight relationships were established from the remaining pool (20 fish used). Any dead fish were replaced from a stock of reserve fish when necessary. Fish density, length, and weight were determined at the end of the experiment.

Nutrient loading

The experiment included two levels of nutrient concentrations representing mesotrophic and eutrophic conditions with four replicates at each depth. To adjust and maintain the concentrations, P and N were added to all mesocosms (Table 2) using Na_2HPO_4 and $\text{Ca}(\text{NO}_3)_2$ as the P and N source, respectively. The ratio of P and N added was 1:20 (by molecular weight). The initial nutrient addition was conducted on day 4 after the addition of the sediment and plankton inoculum. At this stage

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Table 2. Nutrient dosage to the mesocosms four days after establishment (initial) and thereafter monthly. The ratio between total phosphorus (TP) and total nitrogen (TN) addition is 1 : 20 (by molecular weight)

Mesocosm type	Initial P, mg mesocosm ⁻¹	Monthly P, mg mesocosm ⁻¹	Initial N, mg N mesocosm ⁻¹	Monthly N, mg N mesocosm ⁻¹
Shallow (1 m), low NP	0	5.1	0	102
Shallow (1 m), high NP	179*	81.6	1575***	1632
Deep (2 m), low NP	0	10.8	0	216
Deep (2 m), high NP	376**	172	3225****	3440

* 1020 L × 175 µg P L⁻¹; ** 2150 L × 175 µg P L⁻¹; *** 1020 L × 1.5 mg N L⁻¹;
**** 2150 L × 1.5 mg N L⁻¹.

only high nutrient mesocosms were dosed with the objective of reaching the high nutrient concentrations of 200 µg P L⁻¹ and 2 mg N L⁻¹. Thereafter, all mesocosms received monthly dosing of N and P (Table 2). The dosing levels and ratios followed those used in previous experiments (Gonzales Sagrario et al., 2005; Jeppesen et al., 2007). In addition, the tanks received input via precipitation; in the Czech Republic this was, on average, 3.4 mg TP and 76 mg total nitrogen (TN) per mesocosm per month, which are low compared to the added TP and TN in the high nutrient mesocosms (Table 2), but as much as 68% and 75% of the added TP and TN, respectively, in the low-dosed shallow mesocosms (Table 2). The addition of nutrients took place after sampling and ecosystem metabolism measurements had been concluded.

Sampling procedures

Sampling was initiated on day 7 following the addition of fish and macrophytes. Thereafter, samples were taken at monthly intervals in all countries. The entire water column, from the surface to approximately 5 cm above the sediment, was sampled randomly with a tube sampler (diameter 7 cm) at 10, 30, and 60 cm intervals from the mesocosm wall and pooled. For phytoplankton, chlorophyll *a*, and water chemistry analyses, the sample was taken outside the macrophyte stands. For zooplankton, samples were collected irrespective of whether plants were present or not at the sampling point as zooplankton tend to hide in the daytime among plants (Timms and Moss, 1984; Burks et al., 2002). If water depth in the enclosure was low, three to six extra samples were taken (10, 30, and 60 cm from the enclosure wall, at different points than the first sample) to obtain sufficient water. For chemical analyses a 500 mL sample was taken and kept dark and cool (5 °C) until reaching the laboratory where a 100 mL subsample was filtered. The filtered and unfiltered samples were stored frozen until analysis.

For phytoplankton, 50 mL glass bottles were filled with unfiltered water, 0.5 mL Lugol's solution was added, and the bottles were kept in dark until analysis. For zooplankton, 5 L of well-mixed pooled water was filtered through a 20 µm mesh,

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poured into a 50 mL bottle, and preserved with 2.5 mL Lugol's solution. After sampling, the remaining water was returned to the mesocosm.

Salinity, conductivity, temperature, dissolved oxygen, and pH were measured in situ in the centre of the mesocosms. A multi-parameter probe was used and water depth was averaged from measurements at four points.

On each sampling date and for each mesocosm, a species list of dominant and subdominant submerged macrophytes (not including filamentous algae) was produced. Percent Plant Volume Inhabited (PVI%) was calculated each month by visually estimating the percentage coverage and measuring average plant height of macrophytes using the formula:

$$\text{PVI\%} = \frac{\% \text{coverage} \times \text{average height}}{\text{water depth}}.$$

To determine macrophyte coverage, the enclosures were divided into quarters to allow estimation of area. If present, filamentous algae were included in the estimate of total macrophyte coverage. Following the final PVI% estimate and at the end of the experiment, the macrophytes were harvested by cutting the stands close to the sediment surface. Surplus water was removed from the harvested material and wet and dry weights (drying at 60 °C for 24 h) were measured. Benthic invertebrates were also sampled at this point by taking three separate Kajak cores (Plexiglass cores, Ø = 52 mm and core length = approx. 10 cm) per mesocosm or an Ekman grab. Samples were pooled, rinsed, and filtered through a 500 µm mesh in the field before being preserved in 96% ethanol in a 500 mL plastic beaker with a wide opening (one beaker per mesocosm).

From 15 July to 15 August a periphyton growth experiment was undertaken in all deep mesocosms. Artificial transparent polypropylene strips (21 mm × 297 mm each) with a slightly textured surface (IBICO®, Germany) were placed 30 cm from the mesocosm wall, 50 cm below the water surface (Köhler et al., 2010). A small weight was attached to the middle of each strip to ensure that they remained fixed at the required depth, even during a drop in the water level. After the strips were removed in August, they were kept cool and dark in a plastic container and frozen in the laboratory until analysis.

Processing of water chemistry and chlorophyll *a* samples

Total phosphorus, soluble reactive phosphorus, total nitrogen, ammonia, nitrate + nitrite, alkalinity, and chlorophyll *a* were determined using comparable standard procedures in the different laboratories.

Processing of plankton samples

At least two phytoplankton and two zooplankton samples from each mesocosm were counted: one sample representing the starting conditions and one sample

representing an integrated sample of the whole experimental period. The latter consisted of a mixed sample of subsamples (25% of the original sample volumes) from each of the monthly samples. The remaining 75% of the original sample volume was stored separately for subsequent analysis of seasonal dynamics.

Ecosystem metabolism

Metabolism in each mesocosm was estimated on a monthly basis. Oxygen, pH, and temperature were measured (upper 20 cm of the fully mixed water column) at least every second hour for a 24-hour period using a multi-parameter probe following the same mesocosm order each time. Light attenuation was measured once a month at 1 p.m. (every 10 cm down to <1% of the surface light) using a light meter. At the same time samples for alkalinity were taken. The gas exchange coefficient was estimated in late October or early November when temperatures, and thus respiration, were low. The oxygen concentration was lowered to ca 30% of saturation in the late evening by adding N₂. Oxygen recovery was then tracked during the night and the exchange rate calculated, taking respiration into account (for details about the method see Liboriussen et al., 2011).

Statistical analysis

In this paper we use averages over the entire duration of the experiment, which covered the period from May to October for all variables except for submerged macrophyte PVI% covering the period from July to October. We analysed these data with analysis of covariance with nutrient dosing and depth as fixed factors and water temperature as the covariate variable using the GLM procedure SAS (SAS Institute Inc., 2008). All variables except temperature were log-transformed.

RESULTS

Average water temperature during the experiment varied between 15.5 and 25.1°C (Fig. 3) and air temperature between 14.6 and 23.4°C, but these did not faithfully follow the latitude gradient (Table 1). While limited water level changes occurred between Sweden to Czech Republic (ranging from –3 cm to +12 cm), profound reductions were observed in Turkey and Greece (accumulating to 46 and 84 cm, respectively by the end of the experiment) (Fig. 3 shows a significant temperature effect, but no significant depth effect (Table 3)). This coincided with the high summer temperatures and low precipitation (223 and 252 mm, respectively, compared to 271–431 mm further north), reflecting the typically low summer water levels found in the Mediterranean region. Conductivity tended to be higher in the mesocosms at these two southern sites, but also in the mesocosms in Germany, probably due to the use of tap water. The analysis of covariance showed a significant temperature effect (Table 3).

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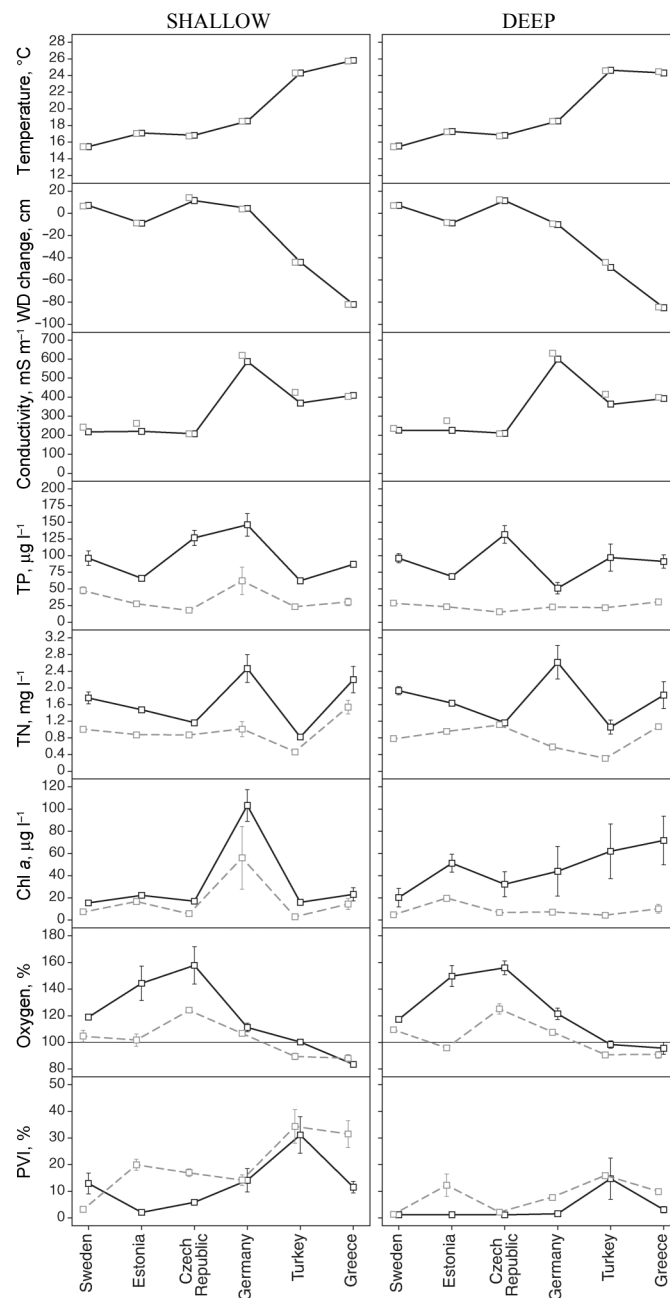


Fig. 3. Selected physical and chemical variables ordered country-wise according to increasing mean water temperature during the experiment, i.e. May–October 2011. Means (SE, standard error) of water temperature, maximum change in water depth (WD), conductivity, total phosphorus (TP), total nitrogen (TN), chlorophyll *a* (Chl *a*), mean diurnal oxygen saturation during the experiment in the high nutrient (full line) and the low nutrient mesocosms (dashed line). Also shown is plant volume inhabited (PVI%) for the period July–October. For the upper three panels SEs are not included as they are so low that they would be largely hidden in the symbols.

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Table 3. Analysis of covariance tests of significance using mean values for the study period for each mesocosm. Significant variables are in bold. Marginally significant values are in parentheses

	Water level change	Total nitrogen	Total phosphorus	Chlorophyll <i>a</i>	PVI% macrophytes	Conductivity	O ₂
Depth	0.84	0.25	(0.055)	0.18	(0.056)	0.81	0.74
Nutrients	0.99	0.27	0.0012	0.49	0.59	0.65	0.0001
Depth × Nutrients	0.99	0.52	0.37	0.27	0.43	0.94	0.83
Temp	<0.0001	0.03	0.35	0.97	<0.0001	<0.0001	0.0001
Temp × Depth	0.90	0.16	0.10	0.17	0.60	0.87	0.80
Temp × Nutrients	0.97	0.73	0.64	0.040	0.64	0.86	0.003
Temp × Depth × Nutrients	0.99	0.38	0.57	0.15	0.67	0.96	0.95

Low and high nutrient levels were maintained throughout the experiment (data not shown). Average TP in the shallow and deep low nutrient mesocosms was 35 and 24 $\mu\text{g P L}^{-1}$, respectively, and 103 and 90 $\mu\text{g P L}^{-1}$ in the shallow and deep high nutrient mesocosms, respectively. Nutrient dosing and depth, but not temperature, had a significant effect for TP in the analysis of covariance (Table 3). Total nitrogen averaged 0.96 and 0.81 mg N L^{-1} in the shallow and deep mesocosms with low nutrient addition, respectively. The corresponding figures were 1.65 (shallow) and 1.70 (deep) mg L^{-1} in the high nutrient mesocosms. The analysis of covariance showed a temperature effect, but no effect of dosing for TN (Table 3).

Chlorophyll *a* averaged 17.3 (shallow) and 8.8 (deep) $\mu\text{g L}^{-1}$ in the low nutrient mesocosms and 33 (shallow) and 47 (deep) $\mu\text{g L}^{-1}$ in the high nutrient mesocosms (Fig. 3). While the concentration in the deep mesocosms demonstrated an increasing trend from cold to warm lakes as expected, chlorophyll *a* values were more variable in the shallow mesocosms. The analysis of covariance showed that the interaction between temperature and nutrients was significant, but no depth effect was detected (Table 3).

Submerged macrophyte PVI% (average July to October) showed an increasing trend with temperature, particularly in the shallow mesocosms. The analysis of covariance revealed temperature to be significant and depth to be marginally significant, whereas no nutrient effect was observed (Fig. 3, Table 3).

The northern mesocosms were generally supersaturated with oxygen during the study period and saturation tended to peak at intermediate temperatures during the experimental period, while saturation in the mesocosms in Greece and Turkey was below 100% (Fig. 3). The mixed effect model showed saturation to be significantly affected by depth, temperature, and interactions between nutrients and temperature (Table 3).

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DISCUSSION

Our experiment illustrates how careful design, preparation, and sampling procedures can be used to optimize the results from cross-national mesocosm experiments. The more detailed results from the experiment are yet to be published, but some overall conclusions can already be drawn. To avoid compromising these publications, in this overview we have restricted our analysis to averages for the study period, which potentially can hide important interactions. As expected, we observed a strong gradient in temperature (air temperature 14.6–24.3 °C, water temperature 15.5–25.1 °C) as an average for the experimental period and an accompanying large change in water level due to variation in net precipitation. While no significant difference was found in the water level in the mesocosms from Sweden to the Czech Republic, it fell by 46 and 84 cm in Turkey and Greece, respectively, with major implications for the shallow mesocosms. The temperature gradient did not follow latitude. The Czech and Turkish sites were located at higher altitude and were therefore exposed to cooler climatic conditions relative to their latitudinal counterparts (Germany, Greece).

As expected, the phosphorus levels differed significantly among high and low nutrient treatments. We attribute this to: (i) the thorough pre-equilibration of the sediment to the experimental P concentrations over a period of several months prior to the start of the experiment using a standardized protocol, and (ii) standardization of the sediment composition (1 : 10 ratio of sediment to sand). Although some minor variation occurred, our procedure ensured that relatively similar P concentrations were maintained across sites. Experience from a Danish long-term mesocosm study has shown that the same sand–sediment combination as used in the present study (but without pre-equilibration) results in prolonged high internal loading (particularly of phosphorus) in the systems under low nutrient concentrations but in P retention under high nutrient concentrations (M. Søndergaard et al., unpublished results). Ideally, the same type of sediment should have been used in all countries, but this was not possible for practical reasons.

Nitrogen also tended to be higher in the mesocosms receiving high nutrient doses. Nitrogen concentration increased with temperature, possibly resulting from reduced nitrogen retention under warmer conditions as carbon can be limiting under high assimilation rates (Kosten et al., 2012).

Since our primary goal was to produce similar nutrient concentrations at contrasting depths, nutrients were dosed per volume (Table 2). While this provides good opportunities for comparing shallow lakes with similar N and P concentrations but contrasting depths, the drawback is that the biota has more total nutrients available in the deep tanks, which makes the comparison, including the nutrient balances, of deep and shallow tanks difficult.

For chlorophyll *a* we found the interaction between nutrients and temperature to be significant, with nutrients enhancing the effect of temperature. This concurs with several recent studies based on space-for-time analysis of large data sets (Jeppesen et al., 2007, 2010b; Moss et al., 2011), time series (Wilhelm and Adrian,

2008; Wagner and Adrian, 2009), experiments (see review in Stewart et al., 2013) and modelling (Mooij et al., 2005; Trolle et al., 2011); for a review, see Jeppesen et al. (2014).

The overall PVI% of macrophytes increased with increasing temperature, particularly in the shallow mesocosms. Concurrently, Bucak et al. (2012) found in a shallow mesocosm experiment in Turkey that water level reductions lead to faster growth of macrophytes due to improved light conditions for the plants. The water level reduction in the deep mesocosm experiments of Bucak et al. (2012) was, however, not accompanied by increasing macrophyte coverage. Macrophyte growth and colonization depth are, in general, positively affected by temperature (Rooney and Kalff, 2000), but whether this growth potential is realized depends on a number of factors, including the trophic structure, periphyton and plant grazer abundance, and winter climate (Jeppesen et al., 2014), which were largely standardized in our study.

We also sought to describe system production and respiration from diurnal variation in oxygen and CO₂ exchange based on alkalinity measurements and diurnal variation in pH. Generally, there is a good relationship between oxygen saturation and net production in fully mixed lakes and mesocosms (Nielsen et al., 2013). Our results indicate higher net production for the study period in the mesocosms in the northernmost four countries, peaking in Central Europe, than in Greece and Turkey. This concurs with other findings, suggesting that net production decreases from cold to warm lakes (Kosten et al., 2010).

In conclusion, the experimental design described is suitable for studying how changes in nutrients affect the biota and metabolism in shallow lakes at contrasting water levels and in different climate zones. A considerable effort was required to standardize the experiments and to ensure low risk of failure. During the course of a year we discussed and prepared a detailed sampling protocol and considered the types of mesocosms to be used. Detailed protocols are particularly necessary when groups with very different backgrounds and experience are running a joint experiment. Our design also has some drawbacks. (1) Due to budget constraints our mesocosms were relatively small, but scale is of considerable importance (Schindler, 1998). We had a simple fish community structure (1 species), with no piscivores. At such a small scale, adequate conditions for piscivory cannot be created. It is evident from a number of recent studies that the proportion of piscivores decreases with decreasing latitude (Jeppesen et al., 2010a, 2010b; Meerhoff et al., 2012), and the experiment cannot account for this. In larger (and more costly) systems, such problems could be minimized and stocking of piscivorous fish would be possible, enabling natural development of planktivorous fish. By ensuring full mixing of the water column as in natural shallow lakes that are typically polymictic, scale was, in part, compensated for. (2) We lost several mesocosms, either because they sank during heavy storm events or leaked water due to damage. In total, 6 of the 96 mesocosms were lost. The storm effect could have been avoided by running the experiment on land (perhaps in buried tanks), but such tanks tend to get warmer than those placed in water.

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Despite these drawbacks we conclude that the system presented here offers great potential to study the effects of global warming and eutrophication on in-lake processes and dynamics in shallow lakes. The thorough preparatory phase encompassing the design of the experiment and writing of protocols has been essential for the success of this multi-national, multi-cultural experiment.

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Zusammenfassung

Im Fokus der Ökosystemforschung standen lange Zeit kontinuierliche und lineare Prozesse, ganz dem Paradigma *Natura non facit saltus* – die Natur macht keine Sprünge, entsprechend. Beobachtungen basierend auf Langzeitdaten haben jedoch gezeigt, dass kleine Veränderungen in treibenden Einflussgrößen abrupte Neuorganisationen von Ökosystemen auslösen können, wenn Grenzwerte überschritten werden. Abrupte Veränderungen an einem Grenzwert, die zu einer Neuorganisation eines Ökosystems führen, werden auch als „Regime-shift“, bezeichnet und nach ihrer Dynamik in abrupte und diskontinuierliche Regime-shifts unterteilt. Die Existenz Grenzwert getriebener Regime-shifts erhält im Zeitalter des Anthropozäns besondere Relevanz, da die Eingriffe des Menschen weltweit zu systematischen Veränderungen in treibenden Einflussgrößen von Ökosystemen führen und damit die Wahrscheinlichkeit erhöhen, dass solche Grenzwerte überschritten werden könnten. Grenzwert getriebene Veränderungen können dabei prinzipiell auf allen Organisationsebenen eines Ökosystems stattfinden und sowohl den Gleichgewichtszustand eines Systems als auch den funktionellen Status betreffen. Dabei ist zu erwarten, dass eine solche Neuorganisation des Ökosystems wichtige Ökosystemdienstleistungen wie z.B. die Bereitstellung von klarem Wasser oder die Kohlenstoffbindung entscheidend verändern. Die Existenz nicht linearer und diskontinuierlicher Prozesse ist aus der dynamischen Systemtheorie bekannt und ihre Dynamiken um den Grenzwert in der Bifurkationstheorie wohl beschrieben. Auch aus der empirischen Ökosystemforschung gibt es einige gut dokumentierte Fälle von sprunghaften Veränderungen und den damit verbundenen, grundlegenden Neuorganisationen. Die Frage nach der tatsächlichen Relevanz solcher nicht linearen und diskontinuierlichen Veränderungen in komplexen, natürlichen Ökosystemen, in denen viele Prozesse sich wechselwirkend beeinflussen, ist jedoch noch ein aktives Feld der Forschung. Seeökosysteme sind von vielen schnell veränderlichen physikalischen, chemischen und biologischen Prozessen geprägt, gleichzeitig integrieren Seen Einflüsse aus ihrem gesamten Einzugsgebiet, dadurch sind sie nicht nur besonders sensibel gegenüber Umweltveränderungen sondern können auch umweltbedingte Veränderungen frühzeitig anzeigen. Im Allgemeinen wissenschaftlichen Konsens sind besonders klimabedingte Veränderungen, sowie Eutrophierung als weltweit relevante, akute Belastungsfaktoren für Seeökosysteme anerkannt.

Im Fokus der vorliegenden Arbeit steht, in drei Studien, die Analyse von Grenzwert getriebenen Veränderungen in Flachseen. Dabei realisieren die gewählten Analyseansätze eine enge Verknüpfung zwischen aktuellen ökologischen Theorien und empirischer Datenanalyse. Eine enge Verknüpfung zwischen Theorie und Daten hat dabei nicht nur den Vorteil die kausale Interpretation von empirischen Daten zu erleichtern, sondern erlaubt auch, durch die Ableitung theoriebasierter Hypothesen, diese zu überprüfen und zu verbessern. Die enge Anbindung empirischer Analysen an theoretische Ansätze und Konzepte trägt damit unmittelbar dazu bei das mechanistische Verständnis über die funktionellen Zusammenhänge in Ökosystemen zu

verbessern.

Die erste Studie verbindet empirische Datenanalyse, basierend auf Langzeitdaten vom Müggelsee (Deutschland), mit theoretischen Konzepten aus der Populationsökologie und der Regime-shift Theorie. Die Studie erforscht die Dynamiken und Ursachen, die zu einem abrupten Dominanzwechsel zwischen den beiden Wintercopepoden Arten *C. vicinus* und *C. kolensis* geführt haben. *C. kolensis* und *C. vicinus* sind im Nahrungsnetz, in einer sogenannten Intraguild-Predation (IGP) Beziehung, eng mit einander verbunden, d.h. beide konkurrieren um Algen als Nahrungsquellen, dabei ist *C. kolensis* der überlegene Konkurrent; auf der anderen Seite stehen die Naupliien von *C. kolensis* unter dem Prädationsdruck von *C. vicinus*. Von besonderem Interesse waren in der Studie die potenziellen Einflüsse der ansteigenden Durchschnittstemperatur und die Veränderungen im Trophiegrad des Sees zu differenzieren. Im Ergebnis zeigt die Analyse, dass der Dominanzwechsel das Resultat eines komplizierten Wechselspiels zwischen der Reduktion im Nährstoffgrad und den internen Populationsdynamiken war, während die Veränderungen in der mittleren Temperatur nur untergeordnete Bedeutung hatte. Durch seine größere Konkurrenzstärke konnte *C. kolensis* seinen Status von einer seltenen zur dominanten Copepodenart verändern, in einer Zeit, in der sich die Biomasse des Phytoplanktons stark reduzierte. Dies war allerdings erst möglich, nachdem der Prädationsdruck durch *C. vicinus* unter einen kritischen Grenzwert gefallen war. Damit lässt sich der abrupte Anstieg in der Abundanz von *C. kolensis* einem abrupten Regime-shift zuordnen, wohingegen die Abundanzen von *C. vicinus* quasi linear der Verminderung in der Algenbiomasse im See folgen. Damit ist diese Studie nicht nur ein wichtiges Beispiel für die Relevanz abrupten, nicht linearer Veränderungen in Seeökosystemen, sondern zeigt auch, dass IGP-Beziehungen ein wichtiges, jedoch häufig vernachlässigtes, Konzept darstellen um Populationsdynamiken in Seeökosystemen zu verstehen.

In der zweiten Studie stand die Frage im Fokus wie in Flachseen Veränderungen in Wassertemperatur, Nährstoffstatus und Wasserstand gemeinsam die Bruttopräprimärproduktion (Gross primary production, GPP), Gesamtrespiration (Ecosystem respiration, ER) sowie ihr gegenseitiges Gleichgewicht beeinflussen. Die Studie basiert auf Daten, die im Rahmen eines europaweit durchgeführten Mesokosmen-Experiments, von sechs verschiedenen Arbeitsgruppen erhoben wurden. Die Hypothesen über den zu erwartenden Effekt der drei Einflussgrößen wurden im Rahmen der Metabolic Theory of Ecology (MTE) hergeleitet. Es ist zu erwarten, dass sich im Zuge der globalen Veränderungen sowohl Temperatur, Trophiegrad als auch Wasserstand und damit die Lichtverhältnisse in Flachseen verändern. Während der separate Einfluss aller drei Größen auf die Metabolismusrate in Flachseen schon untersucht wurde, ist über ihren gemeinsamen Effekt, ihre gegenseitige Beeinflussung sowie ihren Einfluss auf die Fähigkeit eines Seeökosystems Kohlenstoff zu binden, wenig bekannt. Der gewählte „space-for-time“ Ansatz im Aufbau des Experiments erlaubte dabei den Effekt zweier Trophiegrade (mesotrophisch und eutrophisch) sowie zweier Wassertiefen (1 und 2 m) über einen großen Temperaturgradienten hinweg zu vergleichen. Über den gesamten untersuchten Temperaturgradienten betrachtet war

der Einfluss der Temperatur auf die ER größer als auf die GPP. Dadurch haben im Mittel alle Systeme ihren Status von netto autotrophen Systemen zu netto heterotrophen System geändert. Der Versuch hat damit gezeigt, dass ein Anstieg in der Wassertemperatur dazu führen kann, dass zunehmend weniger CO₂ aus der Atmosphäre gebunden und schließlich sogar freigesetzt wird. Die Grenzwerttemperatur bei der der Wechsel von netto autotroph zu netto heterotroph stattfindet ist allerdings stark vom Trophiegrad abhängig und liegt für mesotrophe Systeme wesentlich niedriger als für eutrophe Systeme. Während also bezüglich des Temperatureffekts die qualitativen und quantitativen Ergebnisse des Versuchs die Vorhersagen im Rahmen von MTE bestätigen, zeigen sie gleichzeitig deutlich auf, dass die explizite Berücksichtigung von limitierenden Ressourcen in der MTE, die bislang fehlt, das Potenzial der Theorie, und damit ihre Vorhersagen über die Auswirkungen von gleichzeitigen Veränderungen in Klima und Trophiegrad auf Seeökosysteme, sowie deren Bedeutung in regionalen und globalen Kohlenstoffkreisläufen, wesentlich verbessern würde.

In einer dritten Studie wurden generische Frühwarnsignale (early warning indicators, EWIs) auf ihre Verlässlichkeit getestet diskontinuierliche Regime-shifts, basierend auf Langzeitdaten von 6 verschiedenen Ökosystemen, vorherzusagen. Unerwünschte sprunghafte Veränderungen in einem Seeökosystem rechtzeitig vorherzusagen und zu verhindern ist aus der Perspektive des Ökosystemmanagement wünschenswert. Eine solche Vorhersage kann aber schwierig sein, da die entsprechenden Systemvariablen im Vorfeld oft nur geringe quantitative Veränderung aufweisen und Grenzwerte, die von anderen Seen eventuell bekannt sind, sich nur bedingt übertragen lassen, da sie oft hoch systemspezifisch sind. Die Bifurkationstheorie sagt aber voraus, dass mit der Annäherung des Systems an einen kritischen Grenzwert, sich bestimmte statistische Eigenschaften in den Zeitreihen der Systemvariablen systematisch verändern. Diese Veränderungen sind unabhängig vom darunter liegenden Prozess und könnten somit als generische Frühwarnsignale dienen. Diese Studie zeigt im Ergebnis, dass zwar in vielen der Systemvariablen die EWIs entdeckt werden konnten und dies sogar einige Zeit bevor die diskontinuierliche Veränderung tatsächlich stattfand, allerdings war die Konsistenz zwischen den vier getesteten EWIs gering. Damit hat diese Studie deutlich gezeigt, dass die Hoffnung, bald generische, potentiell einfach zu handhabende Frühwarnsysteme im Ökosystemmanagement zur Verfügung zu haben, verfrüht ist und im Gegenteil sehr viel Systemverständnis im Vorfeld notwendig ist um EWIs richtig anzuwenden und zu interpretieren. Gleichzeitig gibt die Studie aber auch wichtige Impulse und Hinweise für die Weiterentwicklung von EWIs.

Die Ergebnisse der vorliegenden Arbeit bestätigen die Relevanz von Grenzwert getriebenen Veränderungen in Seeökosystemen, wenn anthropogene Einflüsse zu systematischen Veränderungen in wichtigen Einflussgrößen führen. Dabei können grenzwertgetriebene Veränderung auf allen organisatorischen Ebenen, von den Populationen bis hin zum gesamten Ökosystem, auftreten. Außerdem können grenzwertgetriebene Prozesse Systemvariablen, aber auch Veränderungen im funktionalen Status eines Sees betreffen. Die vorgestellten Ergebnisse können dazu beitragen

wichtige Theorien, wie die MTE, fortzuentwickeln und helfen damit, unsere Möglichkeiten die Folgen des Globalendwandels abzuschätzen und vorherzusagen, zu verbessern. Durch die Identifikation und Quantifizierung von grenzwertgetriebenen Veränderungen unter Berücksichtigung multipler, relevanter Einflussgrößen können die Ergebnisse aber auch im Rahmen von neuen Konzepten, wie etwa dem Planetary Boundary Concept, interessant sein. Die Ergebnisse zeigen auch, dass eine Vorhersage solcher Veränderungen bei weitem nicht trivial ist und erinnern somit daran, dass dem menschlichen Handeln im Umgang mit solch komplexen Systemen wie der Natur idealerweise das Vorsorge-Prinzip zugrunde liegen sollte.

List of publications

2013

Ulrike Scharfenberger, Aldoushy Mahdy, and Rita Adrian (2013). *Threshold-driven shifts in two copepod species: Testing ecological theory with observational data*. Limnology and Oceanography 58(2): 741 – 752. doi: 10.4319/lo.2013.58.2.0741

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