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RESEARCH ARTICLE

Disentangling the effects of climate change in a mountain lake through community structure analysis

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Abstract

Pressures of climate change may trigger regime shifts in ecosystems. Identifying signs of these pressures before the critical transition remains challenging, and it could be useful to anticipate the regime shift. In this research, while exploiting the case of a lacustrine ecosystem, which passed from an unvegetated, phytoplankton-dominated state to a macrophyte-dominated regime, we analyzed the role of warming as a slow driver when far from the regime shift. To this end, we combined the analysis of the time series of the driver of pressure and of the response variables with the qualitative analysis of the lake community food web structure. Predictions obtained about the response of plankton populations to simulated press perturbations due to warming were consistent with observed variation in their levels of abundance, confirming that warming was a slow driver and unveiling the mechanistic basis of its effects. This case study suggests a novel approach to interpret early changes in ecosystems subjected to slow drivers of pressure, extending the toolkit beyond the analysis of statistical signature and manipulative experiments. Climatic variations gradually alter the external conditions that ecosystems face, and the approach presented here could help monitor their responses to climate change.

In ecosystems, climate change may trigger sudden and persistent community re-organizations that have been classified as regime shifts (Hare and Mantua 2000; Kortsch et al. 2012; Boucek and Rehage 2014; Zhao et al. 2017; Woolway et al. 2020; Calamita et al. 2024). They occur when the ecosystem is exposed to a progressively increasing pressure of a driving variable (Carpenter et al. 1999, Scheffer et al. 2001a; Scheffer et al. 2001b; Biggs et al. 2009; Lindegren et al. 2012; Dakos et al. 2024; but *see* Capon et al. 2015). Initially, the ecosystem adjusts smoothly to the rising pressure; however, once that pressure passes a critical threshold, an abrupt discontinuity emerges (Andersen et al. 2009; but *see* Hillebrand et al. 2020), driving a transition from one community configuration to another (Scheffer et al. 2001a; Scheffer et al. 2001b; Narwani et al. 2019).

The study of regime shifts in ecosystems has developed along two main directions: (1) analyzing the consequences that emerge once critical thresholds are exceeded (Wagner and Adrian 2009; Hillebrand et al. 2020); and (2) detecting early-warning signals, i.e., changes that precede the transition (Stelzer et al. 2021; Dakos et al. 2024 and references there in). These early signs are difficult to detect because, when far from the regime shift, a system may show little change (Jeppesen et al. 1990; Carpenter et al. 2011), and variations in the response of variables can hardly be causally connected to the continuous source of change.

Manipulative experiments in which the driver is defined were conducted to explore mechanisms of ecosystem response (Stelzer et al. 2021). Yet comparatively little attention has been paid to how sustained driver pressure propagates through an ecosystem when it is still far from a tipping point, and how its propagation shapes the response of the variables (Jarvis et al. 2016).

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This paper considers this issue while focusing on a small oligotrophic lake (Lake Scuro, northern Italy) as a case study. In summer 2003, this waterbody underwent a transition from an unvegetated, phytoplankton-dominated state to a macrophyte-dominated regime (Bertani et al. 2016). Since no macrophytic vegetation was observed in the lake before 2003, the rapid spread of the benthic charophyte *Nitella gracilis* (Smith) Agardh 1828 was considered an extraordinary and abrupt event, of which Bertani et al. (2016) demonstrated the tipping-point nature. The exceptionally high temperature recorded in July 2003 (Luterbacher et al. 2004; Schär et al. 2004) combined with low rainfall resulted in a marked reduction of the hydrometric level of the lake; light thus penetrated down to the lake bottom, promoting the massive growth of *N. gracilis*, with cascading effects on the food web (Bertani et al. 2016). Because a regime shift may follow fluctuations of a driving variable (Scheffer et al. 2001a; Scheffer et al. 2001b), the scenario described for Lake Scuro indicates temperature as a possible driver. According to regime shift theory, temperature must have been gradually increasing, imposing continuous pressure on the ecosystem (Carpenter et al. 1999; Scheffer et al. 2001a; Scheffer et al. 2001b). Since temperature alters parameters governing the growth rate of plankton species (Adrian et al. 2006; Rasconi et al. 2015), plankton populations might have shown the signs of this continuous pressure as variations in their patterns of abundance.

Within this framework, the specific aims of this research are to (1) confirm that Lake Scuro experienced a sustained warming trend before the regime shift, (2) identify the type of stress that rising temperatures imposed on plankton populations, (3) predict through a qualitative analysis of the community food web how the stress could have altered the population abundances, (4) assess whether the predicted changes match those actually observed, and (5) highlight complex responses due to the concomitant effects of gradual temperature change and of the regime shift.

These specific objectives converge on the main goals of investigating the role of temperature as a slow driver in Lake Scuro and offering a tool to detect its early effects by reconstructing the mechanism through which variables respond to it. This research seeks to enhance the understanding of cause-and-effect relationships in lakes while simultaneously offering a broader framework for how ecosystems respond to pressure.

Methods

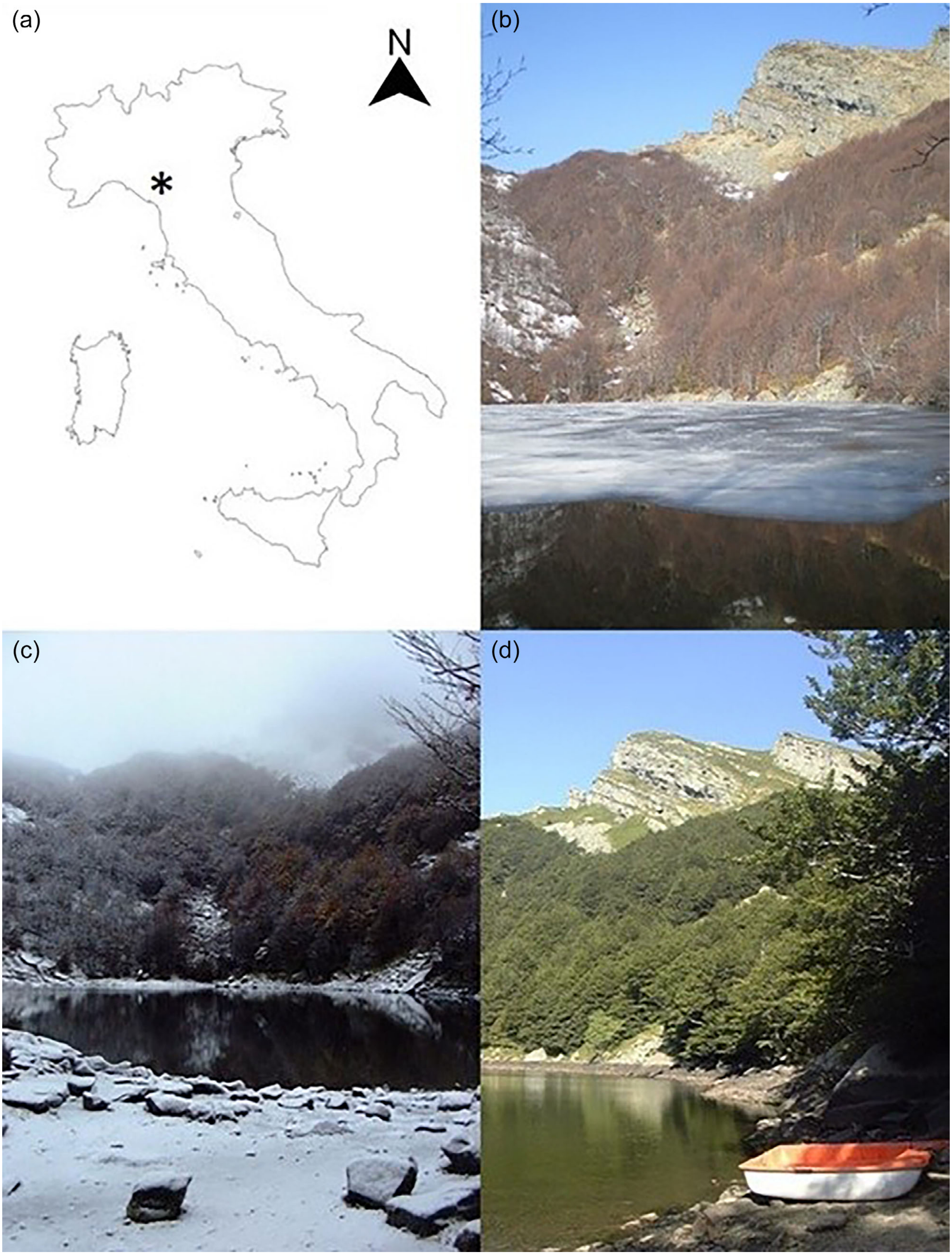
Study area

Lake Scuro (Fig. 1) is an oligotrophic dimictic lake belonging to the Tuscan Emilian Apennine National Park. Its surface extends over 1.2 ha, and its maximum depth is 9.4 m. Ice cover used to last from November to May (Moroni 1962) but this pattern has changed in recent years due to altered climate conditions. The lake is naturally fishless, with occasional releases for recreational purposes. However, these releases were limited and coincided with fishing events that removed most introduced fish, preventing any self-sustaining populations from establishing in the lake (Bertani et al. 2016). Lake Scuro is part of the Long-Term Ecosystem Research Network (LTER, site IT09 Mountain Lakes). Limnological surveys were conducted since the late 1950s (Rossetti et al. 2010) and more regularly during the open water season (May–November) in the years 1986, 1989–1994, 1998, 2003, 2007–2009, and 2012. The lake still shows pristine conditions, and its simple food web, with few trophic levels, facilitates the identification of changes in community structure and may help hypothesize the associated driving mechanisms (Primicerio et al. 2007; Rossetti et al. 2010). The metazoan heterotrophic plankton (copepods, cladocerans, and rotifers) is characterized by three main trophic components: microfilter feeders, macrofilter feeders, and polyphagous species with predatory habits (Ferrari et al. 1992). The remote location of this lake prevents the occurrence of most of the impacts typical of human-altered areas, and it makes the presence of tourists minimal so that local pressures, which might confound the influence of climatic patterns, are negligible. Long-term limnological time series are available for this site, including several years before and after 2003, thus providing a unique opportunity to follow long-term dynamics.

Research strategy

In this research, we exploited the time series of (i) zooplankton population abundances; (ii) chlorophyll *a* (Chl *a*) concentration as a proxy for phytoplankton abundance; and (iii) lake epilimnion temperature. We reconstructed the trends of these variables from empirical data collected during a long-term ecological sampling conducted between 1986 and 2012 by one of the authors (Rossetti et al. 2010; *see* also Lanucara et al. 2016; Rogora et al. 2018; Morabito et al. 2018). Time series comprised seasonal (open-water period) mean values of zooplankton species abundances, Chl *a* concentration, and epilimnion temperature. Supporting Information A shows the entire dataset.

We analyzed zooplankton and Chl *a* time series searching for significant variations using 2003 and 1994 as reference points to execute "before and after" statistical comparisons. The former year marks when the critical transition occurred, encouraging our investigation into significant variations in the abundance of plankton components before and after the regime shift. Bertani et al. (2016) indicated that the decline of phytoplankton due to the competitive advantage of *N. gracilis* led herbivore raptorial rotifers to decrease as a cascading effect, which they credited as due to the regime shift. However, this group began its steady decline in 1994. Because of this, we selected 1994 as a second reference point. We executed a multivariate critical transition test on the community data to evaluate whether a critical transition may have occurred also in 1994. Furthermore, we ran a step change analysis on the



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temperature data to assess the linearity of its trend before 2003 so that it may be identified as a possible source of continuous pressure that acted on the lake earlier than the 2003 heatwave.

Based on the ecology of the zooplankton, as deduced from the literature (Table 2), we identified potential press perturbations on temperature-sensitive species. Finally, we predicted the response of the populations inhabiting Lake Scuro to these press perturbations using the qualitative technique of loop analysis (Levins 1974; Puccia and Levins 1985), which considers the structure of interactions in the plankton community. During model construction, we conveniently aggregated the populations in trophic species and represented the community as alternative signed digraphs. We focused on the community structure as a possible locus of explanation because observed variations in the abundances may be the net effects of direct and indirect impacts of press perturbations that propagate from target variables to the rest of the community. The response of the zooplankton populations predicted by the models was then compared with the observed variations in population abundances.

Statistical analyses

We performed "before and after" tests by applying the Wilcoxon–Mann–Whitney routine included in the R Base Package (R-4.05.0). First, we compared zooplankton species abundances and Chl *a* concentrations before and after 2003, the year of the regime shift; next, we considered 1994 as a reference year to carry out the "before and after" comparisons. In fact, changes detected in some species (e.g., the raptorial rotifers), which were causally attributed to the 2003 regime shift, have been underway since 1994 (Bertani et al. 2016). We then used the outcomes of these tests as a benchmark for model predictions.

Testing for critical transitions

We applied the functions *segmented* (Muggeo 2008) and *cpt.mean* in the package changepoint (Killick and Eckley 2014) in the R statistical environment to assess whether points of abrupt change characterized the water temperature in the period of investigation (1986–2012). Because we applied a linear regression model to highlight a possible linear increase in the epilimnion temperature, the function *segmented* helps identify change points in regression models. We also applied the function *changepoint* to explore change points in the structure of the data independently of their representation through a regression model. To explore potential critical points before 2003, we performed a multivariate critical transition test on the community data using the *multiEWS* function from the *EWSmethods* in the R package (O'Brien et al. 2023). Additional details can be found in the Supporting Information H. In this case, the analysis clearly indicates the year in which the trend of the values changed compared to the past.

Model construction

Models were constructed as signed digraphs, which describe directed trophic interactions (Puccia and Levins 1985). They were created from data collected and observations made during a long-term ecological research project, along with information from the literature on the species' feeding habits. The grazing chain from inorganic nutrients to zooplankton species with predatory habits constitutes the backbone around which the food web models have been constructed. A node for inorganic nutrients (N) stands at the bottom of the web. Nutrients are exploited by phytoplankton, represented using two nodes: phytoplankton $< 20\ \mu$m (P1) and phytoplankton $> 20\ \mu$m (P2). P1 is the prevalent food source for microfilter feeders (crustaceans, C1 and rotifers, R1) and it can also feed macrofilter feeders, such as the cladoceran *Daphnia longispina* O. F. Müller, 1776 (DL) and the calanoid copepod *Eudiaptomus intermedius* (Steuer 1897) (EI). *Daphnia longispina* and EI can also feed on P2, as raptorial and predatory rotifers (RR and PR) do. Finally, raptorial rotifers and carnivorous micro-crustaceans, such as the last copepodite stages and adults of cyclopoid copepods, prey upon microfilter feeders (C1 and R1, respectively) and raptorial rotifers (RR). Several alternative models have been developed, each differing in which of the following interactions they include or omit, or in what combinations: no exploitation of phytoplankton $< 20\ \mu$m (P1) by *D. longispina* and *E. intermedius* (DL and EI), and predation by predatory micro-crustaceans (PC) upon microfilter-feeder rotifers (R1). Since it is unclear from the literature whether these interactions are stable components of the food web, we did not consider them as pillars. Instead, we used them to set up alternative schemes for the Lake Scuro food web. Supporting Information B summarizes the trophic criteria used to group the species in trophic components.

Loop analysis

Loop analysis is a qualitative technique that uses signed, directed graphs (digraphs) to represent networks of interacting variables (Levins 1974; Puccia and Levins 1985). A signed digraph depicts interactions between components (variables or nodes) using two connection types: arrows that represent positive effects (→) and circle-headed links (⊸) that represent negative effects on one another's rate of change. If a

(Figure legend continued from previous page.)

Fig. 1. Lake Scuro: (**a**) map of Italy with an asterisk indicating the location of the study site; (**b**) partial melting of the ice cover in spring (May 7, 2008); (**c**) autumn mixing period (October 23, 2003); and (**d**) marked decrease in the hydrometric level coinciding with the maximum thermal stability of the water column in summer (July 21, 2003).

variable limits its own rate of change, a negative self-link will appear on the node that represents it in the model.

Loop analysis predicts the direction of change in the level (e.g., biomass, number of individuals, spatial extension) of the variables in response to parameters' alterations targeted to one or more components (i.e., press perturbations; *see* Bender et al. 1984; Montoya et al. 2009). When a press perturbation increases the rate of change of the target variable, the latter is said to undergo a positive input. A negative input occurs when a press perturbation reduces the rate of change of that component (Puccia and Levins 1985). Inputs may percolate to other nodes connected to the target variables through the network, and their effects are summarized in a table of predictions. These effects are given only as a direction of change for the level of the variables: increase (+), decrease (−), or no change (0). For the sake of simplicity, the table of predictions denotes variations in response to positive parameter inputs (i.e., perturbations that increase the rate of change of target variables). Predictions about negative inputs can be obtained by simply reversing the signs in the table.

In models with a small number of components and/or a limited number of connections, expected changes in the variables can be tracked through the graph anatomy (Bodini 2000). This procedure is detailed in the Supporting Information C. When variables and connections increase, multiple pathways of interactions emerge, and the probability that they have opposite effects increases as well (i.e., some paths exert positive effects while others have negative effects) so that predictions may remain ambiguous (Puccia and Levins 1985). To address this issue, we used the *LevinsAnalysis* software (Scotti et al. 2020). The program runs numerical simulations by assigning random values to the coefficients of the community matrix, i.e., the numerical counterpart of the graph, where arrows are replaced by +1, circle-headed links by −1, and zeroes indicate no interaction. Values are extracted from a uniform distribution in the interval (0, 1], and at each simulation run, they enable calculating path strength. Hence, even in the presence of multiple positive and negative paths, their cumulative effect never remains ambiguous. After the simulations, the algorithm returns the direction of change (increase, +; decrease, −; no change, 0) for the level of any variable on a percentage basis (Supporting Information C). The *LevinsAnalysis* software also executes a stability analysis over the entire set of matrices created in every simulation and returns the number of stable matrices, building the tables of predictions on the subset of stable matrices. Finally, we applied this simulation framework to detect the system response to concurrent inputs. Several species were likely targeted by the effects of climate change, and we faced a problem of concomitant multiple press perturbations. Considering the autecological features of the species belonging to the lake's community (Supporting Information E), we identified those components that may have undergone press perturbations. Through simulations, we could predict the overall response of the system to these multiple stressors (details on the procedure are in the Supporting Information C, section "Concurrent inputs").

Null models

The signed digraphs that we developed to describe the interaction structure in the lake community were further validated by testing the occurrence of non-random matching between their predictions and the significant changes in biomass trends. To this end, for each graph we generated 99 random networks (i.e., null models) using the Erdős-Rényi algorithm (Erdős and Rényi 1959). In the randomized networks, we preserved the original numbers of nodes and interactions but rewired the topology at random. We conducted the comparison between original food webs and null models for the effect of concurrent inputs. We tested whether realistic models outperformed random models in predicting abundance changes of the main trophic groups when they returned complete correspondence with the observed variations. Significant differences were assumed if less than 2.5% of random models predicted an equal or larger number of abundance changes than the realistic ones for the studied period (i.e., two-tailed test, Bodini et al. 2018).

Results

Trends of abundance and between-period comparisons

Figure 2 illustrates the trends of the trophic groups that we incorporated as variables in the models. The dashed line marks the year 2003, when the transition from phytoplankton to benthic macrophyte dominance occurred (i.e., the regime shift, Bertani et al. 2016).

We performed the "before and after" comparisons around the year 2003 using the Wilcoxon–Mann–Whitney tests to assess changes in population abundances associated with the regime shift (Table 1, "Tests before and after 2003").

Only Chl *a* and raptorial rotifers showed significant differences: both the concentration of the former and the abundance of the latter decreased significantly after 2003. Raptorial rotifers started to decline in 1994 (Fig. 2e) and continued their downward trend until 2012 (*see* also Bertani et al. 2016 and fig. 7B therein); for this reason, we performed "before and after" comparisons using 1994 as a new reference year (Table 1; Supporting Information Fig. F1).

The test before and after 1994 indicates that the abundance of microfilter-feeder microcrustaceans (C1), predatory microcrustaceans (PC), and microfilter-feeder rotifers (R1) increased significantly. Concomitantly, the abundance of raptorial rotifers (RR) decreased. Macrofilter-feeder microcrustaceans (mainly represented by *D. longispina* and *E. intermedius*), predatory rotifers (PR), and Chl *a* concentration did not vary before and after 1994. We used these results as benchmarks to validate model predictions.

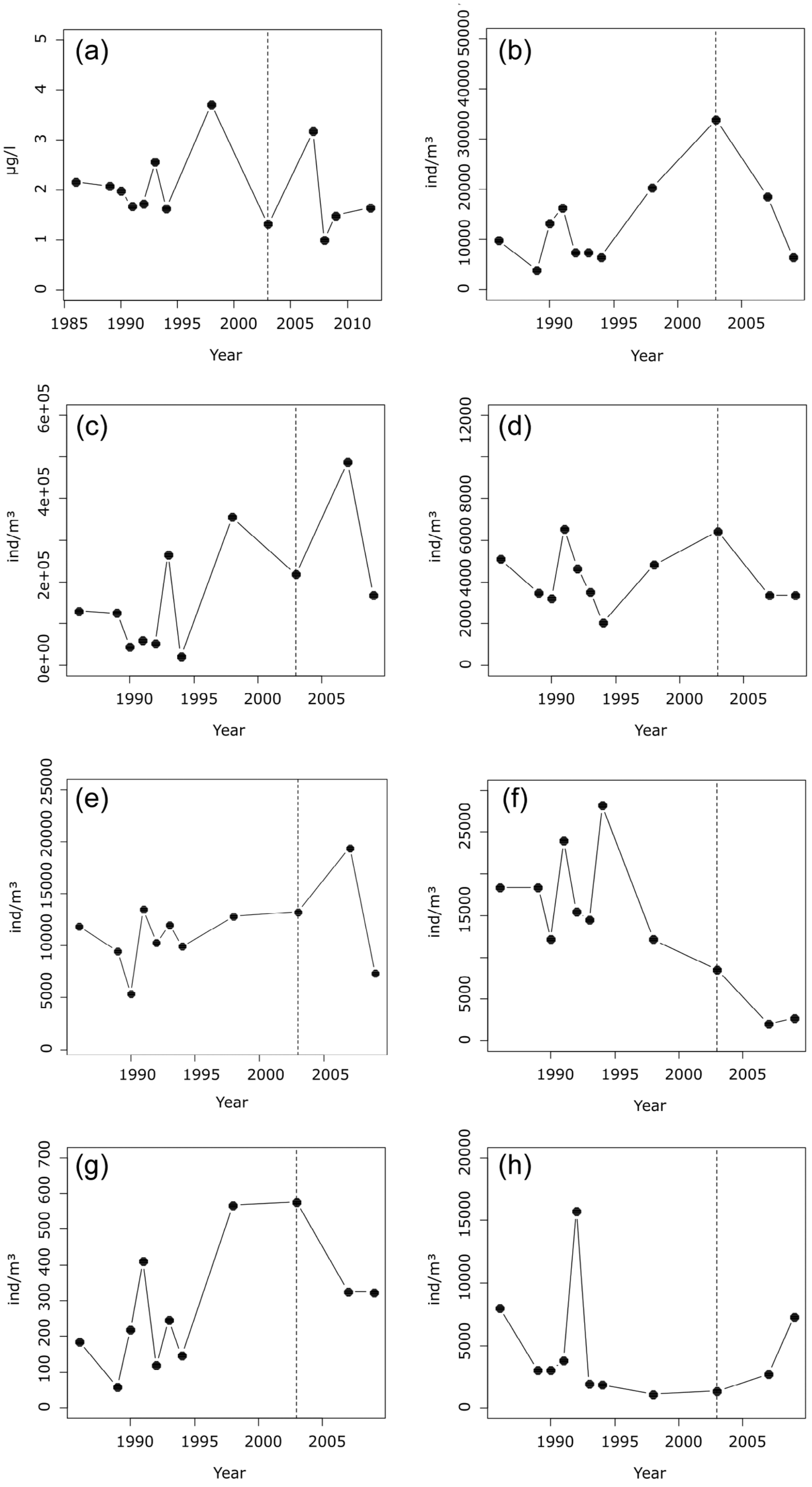

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Table 1. Results of the Wilcoxon–Mann–Whitney tests on the abundance of heterotrophic planktonic groups and chlorophyll *a* concentration in Lake Scuro. We compared the data across two reference years: before vs. after 2003, and before vs. after 1994. Significant differences are in bold.

Test before and after 2003

Group	Node name	Period Alternative hypothesis	*W*	*p*-value
Phytoplankton (chlorophyll *a*)	**P1 + P2**	**1986–1998 > 2003–2012**	**32**	**0.047**
Microfilter-feeder microcrustaceans	C1	1986–1998 ≠ 2003–2009	7	0.376
Daphnia longispina	DL	1986–1998 ≠ 2003–2009	13	0.921
Eudiaptomus intermedius	EI	1986–1998 ≠ 2003–2009	8	0.467
Predatory microcrustaceans	PC	1986–1998 ≠ 2003–2009	4	0.133
Microfilter-feeder rotifers	R1	1986–1998 ≠ 2003–2009	4	0.133
Raptorial rotifers	**RR**	**1986–1998 > 2003–2009**	**24**	**0.006**
Predatory rotifers	PR	1986–1998 ≠ 2003–2009	14	0.776

Test before and after 1994

Group	Node name	Period Alternative hypothesis	*W*	*p*-value
Phytoplankton (chlorophyll *a*)	P1 + P2	1986–1994 ≠ 1998–2012	27	0.445
Microfilter-feeder microcrustaceans	**C1**	**1986–1994 < 1998–2009**	**5**	**0.054**
Daphnia longispina	DL	1986–1994 ≠ 1998–2009	13	0.927
Eudiaptomus intermedius	EI	1986–1994 ≠ 1998–2009	8	0.315
Predatory microcrustaceans	**PC**	**1986–1994 < 1998–2009**	**2**	**0.012**
Microfilter-feeder rotifers	**R1**	**1986–1994 < 1998–2009**	**2**	**0.012**
Raptorial rotifers	**RR**	**1986–1994 > 1998–2009**	**28**	**0.003**
Predatory rotifers	PR	1986–1994 ≠ 1998–2009	21	0.230

Temperature trend and critical transition tests

To verify if temperature continuously increased up to the 2003 regime shift, we reconstructed the trend of the lake's epilimnion temperature from 1989 to 2003, which linearly increased (p-value = 0.05; *see* linear regression model in Supporting Information Fig. F2). Step change analysis did not identify any breakpoints before the 2003 regime shift (Supporting Information Figs. H1, H2). Furthermore, the function *segmented* (Supporting Information Fig. H2) confirms that the lake temperature followed a progressive, linear increase from 1986 to 2003. The results of the multivariate critical transition test on community data show fluctuations in the strength of the indicators (Supporting Information H) over time. Although some of these indicators singularly surpassed the threshold of critical transition in the years before 2003, the bulk of the indicators exceeded the threshold of critical transition starting from 2003 and persisted through 2007 (Supporting Information H).

Effects of the increasing temperature: Press perturbations and their target components

The gradual increase in water temperature may have generated press perturbations (inputs, in the loop analysis language) on species' growth rates. To identify possible entry points for these inputs, we reviewed the autecology literature about Lake Scuro species to determine how their demographic traits respond to warming. The review revealed temperature-driven changes in species' growth rates (Table 2). Increasing temperature and fluctuations may significantly reduce the growth rate of large-size phytoplankton (Zohary et al. 2021). Accordingly, we simulated a negative input on P2 (−P2). By contrast, the size of freshwater phytoplankton tends to shrink when temperature increases, which means that small phytoplankton cells are favored (Primicerio et al. 2007; Rasconi et al. 2017; Zohary et al. 2021), resulting in a positive input on P1 (+P1). *Diaphanosoma brachyurum* (Liévin, 1848) is the most representative warm stenotherm species among microfilter-feeding

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Fig. 2. Abundance time series of the main zooplankton groups and chlorophyll *a* concentration between 1986 and 2012: (**a**) chlorophyll a; (**b**) microfilter-feeder microcrustaceans; (**c**) microfilter-feeder rotifers; (**d**) *Daphnia longispina*; (**e**) *Eudiaptomus intermedius*; (**f**) raptorial rotifers; (**g**) predatory microcrustaceans; (**h**) predatory rotifers. The dotted line visualizes the year 2003. Values are averages that were calculated over the season of the open waters (May–November, *see* Supporting Information A).

Table 2. Possible effects of warming on Lake Scuro main trophic groups and associated press perturbations on their growth rate (inputs).

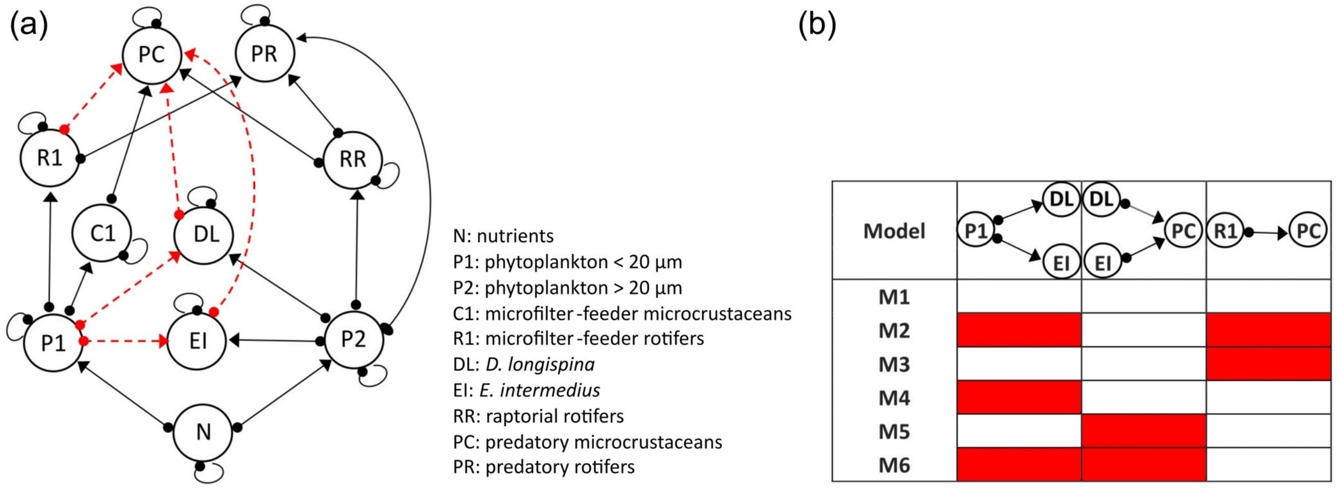
Food web compartment	Label	Warming effect	Press	References
Phytoplankton < 20 μm	P1	Longer ice-free periods and extended growing season; phytoplankton size shrinking	+	Primicerio et al. 2007, Mousing et al. 2014, Rasconi et al. 2017, Zohary et al. 2021
Phytoplankton > 20 μm	P2		−	
Microfilter-feeder microcrustaceans	C1	More favorable conditions for *Diaphanosoma brachyurum*, the most representative species among warm stenotherm microcrustaceans	+	Wærvågen and Nilssen 2011, Bertani et al. 2016
Microfilter-feeding rotifers	R1	Advanced timing of spring peaks; higher reproductive rates	+	Winder and Schindler 2004a, Winder and Schindler 2004b, Edmondson 1965, Strecker et al. 2004, Katkov and Fussmann 2023
Daphnia longispina	DL	Possible reduction in hatching of resting eggs and growth rate (preference for colder temperature)	−	Dziuba et al. 2020, Radzikowski et al. 2018
Eudiaptomus intermedius	EI	Shortening of the life cycle and anticipation of reproduction, doubling the number of generations per year	+	Primicerio et al. 2007
Raptorial rotifers	RR	Negative effect of increasing temperature on the cold stenotherms *S.* Gr. *Stylata-pectinata* and *P.* Gr. *Vulgaris-dolichoptera*, two of the most representative raptorial rotifers	−	Katkov and Fussmann 2023, Laugaste et al. 2010, Dupuis and Hann 2009
Predatory microcrustaceans	PC	Shortening of development time	+	Gophen 1976, Jamieson 1980, Maier 1989, Gerten and Adrian 2002

crustaceans (C1). An increasing water temperature is expected to positively affect its growth rate (Wærvågen and Nilssen 2011; Bertani et al. 2016), and we considered a positive input on variable C1 (+C1). On the contrary, *Synchaeta* gr. *stylata-pectinata* sensu Ruttner-Kolisko (1974) and *Polyarthra* gr. *vulgaris-dolichoptera* sensu Ruttner-Kolisko (1974), two raptorial rotifers, are cold stenotherm species. Consequently, they are negatively affected by increasing temperatures (Katkov and Fussmann 2023; Laugaste et al. 2010; Dupuis and Hann 2009). Coherently, we considered a negative input on RR (−RR). Among microfilter-feeding rotifers (R1), *Keratella cochlearis* (Gosse, 1851) and *Conochilus* gr. *unicornis-hippocrepis* sensu Ruttner-Kolisko, 1974 are the most abundant species. Winder and Schindler (2004a, 2004b) found that the abundance in spring peaks of *K. cochlearis* increases strongly due to long-term warming. Moreover, according to Edmondson (1965), *K. cochlearis* shows higher reproductive rates when the temperature increases. Other authors found the same positive effect of temperature on the growth of these species (Strecker et al. 2004; Katkov and Fussmann 2023), and we then simulated a positive input on this component (+R1). *Mesocyclops leuckarti* (Claus, 1857) is by far the most abundant predatory crustacean in Lake Scuro. Its development time shortens at higher temperatures (Gophen 1976; Jamieson 1980; Maier 1989; Gerten and Adrian 2002), and this suggests considering a positive input on it (+PC).

No indication of specific temperature influence was documented for the predatory rotifers (PR), mostly represented by *Asplanchna priodonta* Gosse, 1850 and *Ploesoma hudsoni* (Imhof, 1891). *Daphnia longispina* and *E. intermedius* are macrofilter-feeding crustaceans. The hatching of *Daphnia*'s resting eggs and its growth rate increase with colder temperatures (Dziuba et al. 2020; Radzikowski et al. 2018) while early ice-free seasons shorten the life cycle of *E. intermedius*, allowing it to anticipate reproduction and its transition from univoltine to bivoltine life cycle (Primicerio et al. 2007). We then simulated a negative input on DL (−DL) and a positive press perturbation on EI (+EI). Therefore, increasing temperatures may have resulted in five positive inputs (+P1, +C1, +EI, +R1, +PC) and three negative inputs (−P2, −DL, −RR) on the plankton community (Table 2).

The signed digraphs of the Lake Scuro plankton community

The feeding habits of the planktonic organisms allowed aggregating the species into trophic groups (Supporting Information Table B1) and identifying their most likely interactions. When knowledge about interactions is uncertain—because the literature reports different mechanisms of action—plausible alternatives occur. Accordingly, six signed digraphs emerged as possible configurations for the plankton community (Fig. 3; Supporting Information D). They reflect different hypotheses about the feeding behavior of the species, suggesting variations in the linkage arrangement according to



Model	P1 → DL P1 → EI	DL → PC EI → PC	R1 → PC
M1			
M2	🟥		🟥
M3			🟥
M4	🟥		
M5		🟥	
M6	🟥	🟥	

Fig. 3. Signed digraph describing the structure of the plankton community in Lake Scuro in its alternative configurations (Supporting Information D). Continuous black links in the digraph (**a**) identify the backbone of model M1. Dashed red links show possible interactions that served to build alternative graphs as table (**b**) synthesizes.

the direct knowledge of one of the authors (GR) and the literature (Supporting Information Table B1).

Model simulations and species responses to press perturbations

It remains uncertain which of the press perturbations (Table 2) actually occurred and how strongly each one acted. To identify which may have mostly influenced the community's dynamic, we ran a simulation routine that systematically combined the inputs (i.e., press perturbations) and calculated the model variables' overall response to each scenario of concurrent inputs (Supporting Information C, "Concurrent inputs"). All possible combinations of press perturbations were simulated. Then, we compared the expected

Table 3. Variables' responses to inputs simulated on the rows in the models M1 and M4. The sign next to each row label indicates the type of input simulated on that variable. The sign at the intersections of rows and columns gives the expected direction of change for the column variable when the row variable undergoes the indicated press perturbation. Numbers in the cells are the percentages of cases that yielded the indicated response over all simulation runs. The last row (signs) reports the overall expected response of each column variable to the concomitant inputs signaled in the rows. Predictions in agreement with the results of the "before and after 2003" comparison are marked in light gray.

Model M1	**N**	**P1**	**P2**	**C1**	**R1**	**DL**	**EI**	**RR**	**PC**	**PR**
+P1	−95.976	100	−44.467	97.988	98.793	−44.467	−44.467	−93.763	41.65	20.121
−P2	98.189	13.682	−100	38.229	98.793	−100	−100	−46.881	−19.92	−86.922
+C1	61.972	−99.598	96.781	99.396	−62.374	96.781	96.781	−52.917	98.793	−74.245
+R1	94.97	−95.171	12.072	−19.718	98.793	12.072	12.072	−64.185	−98.592	100
+PC	−85.312	39.034	61.167	−99.799	83.702	61.167	61.167	−98.994	100	−44.064
Mean	14.769	−8.411	5.111	23.219	63.541	5.111	5.111	−71.348	24.386	−17.022
Signs	0*	0*	0*	?+	+	0*	0*	−	?+	0*
Model M4	**N**	**P1**	**P2**	**C1**	**R1**	**DL**	**EI**	**RR**	**PC**	**PR**
+P1	−52.539	100	−89.637	95.648	99.586	46.114	42.798	−87.358	31.606	−24.767
−P2	56.684	81.554	−100	78.446	100	−60	−67.254	−67.254	6.528	−70.984
+C1	11.088	−100	99.793	96.062	−83.212	−4.663	−0.311	−40.518	96.477	−29.326
+R1	52.332	−83.212	46.529	−5.285	93.161	−48.394	−49.016	−64.145	−92.332	99.378
+PC	−63.523	8.601	37.409	−98.964	55.44	60	62.28	−94.611	99.793	−61.451
Mean	0.808	1.389	−1.181	33.181	52.995	−1.389	−2.301	−70.777	28.414	−17.43
Signs	0*	0*	0*	?+	+	0*	0*	−	?+	0*

Number of correct predictions = 9 (out of 10). Pseudo p-value of the whole results (alternative hypothesis: greater): 0.01.

variations in the level of the variables with the outcomes of the "before and after" tests (Table 1, "before and after 1994"), in search of the best matching between expected responses and observed variations (*see* Supporting Information E). The combination of positive inputs on P1, C1, R1, and PC, along with the negative input on P2, when simulated on models M1 and M4, yielded predictions that matched most of the changes observed in the time series (Table 3).

We used null models to assess if the graphs M1 to M6 generated unique predictions. We contrasted their performance in predicting the changes that were observed in the level of the variables with that of random networks with equivalent structural properties. The comparison was conducted considering only the predictions generated in response to multiple inputs that yielded the best matching with observed variations (i.e., inputs +P1, −P2, +C1, +R1, +PC; Table 3). Ninety-nine random graphs were simulated (Supporting Information Fig. E1). Models M1 and M4 significantly outperformed their random counterparts (p-value = 0.01) while the number of correct predictions was not significantly different from that of random networks in all the other cases (Supporting Information Fig. E1). The only prediction that appears to diverge from the observed variations concerns N (inorganic nutrients) To assess the robustness of predictions obtained from models M1 and M4, we removed nutrients from the two graphs and ran the simulations. The models returned a complete match between observation and predictions, indicating a substantial reliability of the graphs as representations of the community structure, despite the excess simplification at the bottom of the food web (Supporting Information E, Model M7–M8). To explore the effects of the regime shift, we modeled a new community structure including the benthic charophyte *N. gracilis* (NG), which rapidly spread in Lake Scuro since 2003 (Bertani et al. 2016). This macroalga competes with phytoplankton for the inorganic nutrients. Accordingly, we added a resource-consumer interaction between NG and N to the graphs M1 and M4. Macrophytes can also inhibit phytoplankton growth through allelopathy (Bertani et al. 2016). Because the literature is divided on the significance of this latter mechanism (Van Donk and Van de Bund 2002; Mulderij et al. 2007; Rojo et al. 2013), we represented the new community in two alternative configurations: (1) *N. gracilis* acts solely as nutrients consumer, and (2) *N. gracilis* both consumes inorganic nutrients and suppresses phytoplankton groups P1 and P2 through allelopathy. We added NG to models M1 and M4, which provide the most reliable community configurations. Model M7, whose predictions completely matched the results of the "before and after 1994" tests could not be used because without N, NG would be disconnected from the food web in the absence of allelopathy. Alternative graphs that include *N. gracilis* are presented in the Supporting Information G. Table 4 summarizes the results of the simulations for the model NG1. Only the outcomes of model NG1 are shown here because of its better matching with the pre- and post-2003 variations.

Discussion

Temperature, press perturbations, and ecosystem response

The 2003 heatwave was considered responsible for the reorganization of Lake Scuro's plankton community (Bertani et al. 2016). However, our statistical tests revealed that, comparing abundances before and after the regime shift, only the levels of phytoplankton (P1 and P2) and raptorial rotifers (RR) changed significantly (Table 1, "Tests before and after 2003"). Instead, most showed significant variations before and after 1994 (Table 1, "Tests before and after 1994"), which we considered as a demarcation point because it was then that raptorial rotifers began their steady decline, which continued beyond the year 2003 (Table 1). To assess whether variations in the population levels could be early effects of the increasing temperature, we simulated press perturbations according to the influence that temperature may have exerted on the growth rate of zooplankton populations (Table 2). Predictions

Table 4. Responses of the variables in the model NG1, which includes the benthic macrophyte *Nitella gracilis* (NG). The first column lists the simulated concurrent inputs and their signs. The last row (signs) indicates the overall expected response of each column variable to the concomitant inputs signaled in the rows. The predictions in agreement with the results of the "before and after 2003" comparison are marked in light gray.

	N	P1	P2	NG	C1	R1	DL	EI	RR	PC	PR
+NG	−99.817	−98.901	−92.125	100	−91.758	−50.733	−92.125	−92.125	64.652	−55.861	−88.828
+P1	−95.055	100	−27.106	−95.055	99.267	98.901	−27.106	−27.106	−97.436	43.956	39.377
−P2	99.634	−26.557	−100	99.634	19.048	95.604	−100	−100	−41.392	−40.659	−94.872
+C1	54.579	−98.901	97.253	54.579	100	−64.286	97.253	97.253	−54.029	99.634	−81.685
+R1	94.322	−97.253	−11.905	94.322	−29.853	98.901	−11.905	−11.905	−59.707	−98.718	99.451
+PC	−89.377	50.183	72.711	−89.377	−99.451	86.264	72.711	72.711	−99.634	99.817	−35.531
Mean	−5.952	−28.572	−10.195	27.35	−0.458	44.108	−10.195	−10.195	−47.924	8.028	−27.015
Signs	0*	?−	0*	?+	0*	?+	0*	0*	?−	0*	?−

Number of correct predictions = 7 out of 11. Pseudo p-value of the whole results (alternative hypothesis greater): 0.01.

from models M1 and M4 matched almost completely observed changes (Table 3), corroborating the hypothesis that the populations changed in response to the increasing temperature before the regime shift occurred. The only prediction that appears to diverge from the observed variations concerns N (inorganic nutrients). This component is expected to remain largely unchanged, which aligns with the conclusion of Morabito et al. (2018) that the level of inorganic nutrients in the lake has remained stable over the years. However, we conducted a before-and-after test from which significant changes emerged instead (Supporting Information Tables F1, F2). In the "before and after 1994" comparison, PO_4^{3-} and NO_3^{2-} did not vary significantly, whereas SiO_2 and NH_4^+ significantly decreased. The discrepancy between model predictions and observations may be due to the excessive simplification of the graphs in relation to the complexity of the nutrients-phytoplankton interactions. This limitation is due to the lack of information and data (only data about Chl *a* were available to us) needed to single out the main phytoplankton groups as variables in the models. With specific data at our disposal, we could reconstruct a few fundamental phytoplankton-nutrient interactions based on algae preferences and the role of single nutrients as limiting factors. The models would have predicted the effects of press perturbations on specific nutrient forms, making the comparison with the outcomes of the statistical test possible.

Water temperature may not be the only parameter at play in the lake. However, the remoteness of this waterbody has been preventing the occurrence of intense anthropogenic impacts, which must thus be considered negligible. The lack of surface tributaries makes the hydrometric level of the lake strongly dependent on precipitation and temperature (Rossetti et al. 2010). When we examined the possible role of precipitation, the absence of any significant trend suggests that this variable did not exert continuous pressure, and we discarded it as a possible driver. Variations that preceded the regime shift could be due to some critical transition that may have occurred before 2003, rather than being the response to the progressively increasing temperature. We applied a multivariate critical transition test on the community data to assess this hypothesis, and the results indicate possible critical transitions in 2003 and 2007 (Supporting Information H). Setting different parameters reflecting different time intervals in the time series highlighted a possible critical transition in 1998; these signs intensified in 2003 and continued through 2007. In no case, however, did critical transitions characterize the earlier 1990s, and, in particular, 1994 was not critical. We also analyzed the response of the plankton community to the increasing temperature through a step change analysis on epilimnion temperature. Results highlight nonlinear relationships between zooplankton abundances and temperature, marked by breakpoints or thresholds (Supporting Information H; Supporting Information Fig. H3). These nonlinear relationships suggest that, despite the linear change in temperature, the response may be nonlinear. However, we present this conclusion with caution, as the short and fluctuating time series available may have led to false positives in the analysis (Litzow and Hunsicker 2016). Possible nonlinear responses of plankton populations to temperature variations, as indicated by the step change analysis, do not discard the validity of the predictions we obtained with loop analysis. This technique, in fact, makes predictions under the moving equilibrium approach: in the presence of non-linearity, predictions hold for changes in average values (Puccia and Levins 1985). According to this, we performed our analyses using time series of variables' averages (Puccia and Levins 1985; Bodini 2000).

The response of the plankton community after the regime shift

The community structure incorporating the benthic charophyte *N. gracilis* (NG) yielded predictions that only partially aligned with the observed changes before and after 2003 (*see* Table 1 and Supporting Information G). Among the four alternative graphs, model NG1 returned the best matching, but several predictions still did not match the statistical analysis. Expected variations in the level of R1 (microfilter-feeder rotifers) and PR (predatory rotifers) differ from what the before and after tests indicate (*see* Table 1, "Tests before and after 2003", and Table 4). The scarcity of data points after 2003 remains a critical constraint and may have determined these discrepancies. Moreover, although the concept of a moving equilibrium can be applied in non-linear systems, the occurrence of multiple critical transitions (in 2003 and 2008; *see* Supporting Information H) may have influenced the community's response. Such critical transitions may have triggered a rapid sequence of parameter changes that the system's variables could not keep pace with, a speculative conclusion that warrants detailed investigation in future studies. Nevertheless, graph NG1 provides clues about some of the events that occurred in the Lake Scuro after 2003. The enhanced light availability caused by the heatwave stimulated the growth of *N. gracilis* and determined its rapid spread (Bertani et al. 2016). We simulated this increase in the benthic macroalga's growth rate by considering a positive input on NG. The +NG row of Table 4 lists the responses of the model variables (model NG1). The level of NG is predicted to increase (Table 4, row +NG, column NG) in agreement with the observation of massive growth of *N. gracilis* (Bertani et al. 2016). Only observational data were collected about the spreading of benthic algae. However, they are sufficient for a qualitative assessment, considering the lack of any sign of macrophyte occurrence before 2003. A decline in phytoplankton is predicted (Table 4, row +NG, columns P1 and P2), in agreement with the significant decrease of the Chl *a* concentration observed after 2003 (Table 1, "Tests before and after 2003"). However, the expectation about RR

(Table 4, row +NG, column RR) diverges from the observation that this component further declined after the regime shift (Table 1, "Tests before and after 2003"): the model predicts more raptorial rotifers in response to the positive press perturbation on NG. After 2003, Lake Scuro may have been subjected to the concomitant effects of the temperature press and the enhanced light availability at the bottom of the lake that favored *N. gracilis*. Accordingly, we performed simulations combining the inputs on temperature-sensitive traits and a positive input on NG. Small-sized algae (Table 4, row "signs," column P1) would decrease following all the inputs, whereas large-sized phytoplankton (Table 4, row "sign," column P2) were predicted not to change. Overall, the level of the algal populations may have decreased, in agreement with the significant reduction in Chl *a* (Table 1, "Tests before and after 2003"). The effects of inputs due to increasing temperatures offset that of the positive input on NG and, overall, RR was expected to decrease (Table 4, row "signs," column RR) in agreement with the observed data (Table 1, "Tests before and after 2003"). The predictions of this model suggest that the outburst of *N. gracilis* did not impair the raptorial rotifers. On the contrary, the augmented growth rate of the macroalga cascaded positively on their abundance. Moreover, the inputs due to the temperature rise reduced the abundance of RR. Therefore, the overall decrease of RR as detected by the statistical analysis (Table 1, "Tests before and after 2003") can be explained by a preponderant effect of the temperature over that of the improved growth conditions for *N. gracilis*. The concomitant action of these press perturbations, on the other hand, yielded an overall increase for NG (Table 4, row "signs," column NG), in agreement with the observed spreading of the macroalgae. The decline of raptorial rotifers that was deemed a consequence of the regime shift (Bertani et al. 2016), confounding causes and effects (Bodini 2000), can be better interpreted instead as the net effects of the two concomitant press perturbations. These results highlight that community structure analysis, when adequately supported by empirical data, can help unveil mechanisms of responses to perturbations (Bodini 2000; Rodriguez et al. 2021). The decline of raptorial rotifers began around 1994 and continued after the regime shift, following a trajectory imposed by the pressure due to the increasing temperature. The structure of interactions allows reconstructing the trophic cascade mechanisms: improved conditions for the benthic macroalga would increase raptorial rotifers, challenging the idea that the spreading of the macroalga impaired this latter group via the trophic cascade (Bertani et al. 2016). Nonetheless, combining better growth conditions for *N. gracilis* and the inputs due to the increasing temperature, the models predict an increase of *N. gracilis*, and a decrease of raptorial rotifers as well as of phytoplankton. We suggest that the mismatch between model predictions and statistical tests—particularly the predicted changes in R1 and PR, which contrast with the absence of significant variation before and after 2003—would require additional data and analysis.

Stability

Two community structures characterized Lake Scuro before and after the 2003 regime shift, primarily different because of the presence of the benthic alga *N. gracilis* after the regime shift. While zooplankton abundance fluctuated, species composition remained stable. Bertani et al. (2016) suggested that the transition to a macrophyte-dominated regime did not involve alternative stable states. However, our food web reconstruction indicated that alternative community models were qualitatively stable, with over 90% stability of the simulated matrices (Supporting Information D and G). Both community configurations showed a stable equilibrium, suggesting the possibility of alternative stable states. Intermediate, unstable conditions were not examined, nor did we examine the presence of hysteresis—commonly associated with state transitions—as the shift observed in Lake Scuro appears to resemble the smooth (linear) transitions described by Andersen et al. (2009).

Moving equilibrium to study slow drivers

In our analysis, we did not exploit statistical signatures of the time series of the plankton populations (our response variables), nor did we apply manipulative experiments, the two approaches that are commonly used to explore early effects of the drivers of change (Stelzer et al. 2021; Dakos et al. 2024). Statistical signatures alone rarely elucidate the causal mechanisms that link environmental drivers to biological responses (Stelzer et al. 2021). Conversely, manipulative experiments where drivers are under control often fail to reproduce the gradual, long-term changes seen in nature; for example, treatments may push species toward extinction or impose driver gradients far steeper than those experienced in situ (Jarvis et al. 2016; Stelzer et al. 2021). In this study, we took a new approach. First, we mapped the temperature-sensitive traits through which rising temperatures could exert pressure on target organisms. Then, we translated that pressure into predicted shifts in abundance. Finally, we compared those predictions with the abundance changes observed in the long-term time series. Manipulative experiments are called for to overcome the complexity that is often prohibitive for a complete understanding of the dynamics of early changes, which could hardly be elucidated from computational modeling (Stelzer et al. 2021). We have shown that part of this complexity can be resolved using a modeling approach. Ecosystem components exhibit complex responses because they are embedded in a network of interactions that modulate the effects of press perturbations (Bodini et al. 2024). Loop analysis exploits the structure of this network and allows predicting variables' responses considering the multiple pathways responsible for the spreading of pressures and feedback that modulate their expression. We have shown how the early effects of a slowly acting driver can indeed be predicted. To function as reliable early-warning signals of a regime shift, however, these predictions must be combined with the analysis of statistical signatures. Integrating the two approaches therefore allows us both to foresee the regime shift and to uncover the mechanisms through which the driver exerts its influence.

Model predictions have been obtained using the moving equilibrium approach (Puccia and Levins 1985), that is, the variables are at equilibrium with the parameters that change slowly enough so that the state variables can adjust and stay near equilibrium. A driver that varies progressively over time makes parameters change gradually, a scenario that falls within the validity conditions of the moving equilibrium. We identified temperature as a slow-acting driver by analyzing its long-term trajectory, which showed a linear increase over time, a behavior typical of these gradual drivers (Scheffer et al. 2001a; Scheffer et al. 2001b; Dakos et al. 2024). The matching we obtained between predicted and observed variations in the abundance of plankton populations confirms that the moving equilibrium approach was appropriate, and that temperature acted as a slow driver.

To our knowledge, the moving equilibrium has never been applied to investigate early effects of slow drivers. This case study offers evidence of its potential applications to investigate ecosystem responses to stress conditions besides equilibrium analysis and transient dynamics, especially under climate change where the external environmental conditions of ecosystems shift gradually. Loop analysis is most informative when the system is far from a critical transition, although the community that emerged after the regime shift in Lake Scuro displayed moving-equilibrium dynamics that offered a plausible explanation of its behavior. Moving equilibrium may also contribute to deepen the analysis of the “ghost equilibrium” (van Geest et al. 2007), which was introduced to study the dynamics of alternative stable states in eutrophic shallow lakes.

Lake Scuro was chosen as a case study because it experienced very little human disturbance. However, in most ecosystems, the effects of human pressures are not negligible. Although many case studies focus on a single driver that changes monotonically (e.g., the steady warming observed in Lake Scuro), real-world systems often face multiple, interacting drivers. In the presence of multiple drivers, contradictory effects may arise, thus confounding causes and effects (Dai et al. 2012). Combining loop analysis with simulations, as demonstrated in this study, can help in disentangling the effects of multiple drivers, especially when press perturbations can be attributed to specific drivers, and their target variables are identifiable.

Conclusions

Climate warming exposes ecosystems to pressures that gradually impair their resilience so that they become prone to regime shifts. Far from regime shifts, however, capturing the signs of these pressures is challenging. This was the focus of our research, which examined the effect of warming on a lacustrine ecosystem before it passed from an unvegetated, phytoplankton-dominated state to a macrophyte-dominated regime.

The increasing trend over time indicated that temperature exerted gradual pressure on the ecosystem. We forecasted the response to this pressure considering likely effects on temperature-sensitive traits of the species in the lake and simulating press perturbations on their growth rate accordingly. Loop analysis allowed us to predict consequences of these press perturbations on the level of abundances, which we compared with observed variations, obtaining a good match. The meteo-climatic forcings on Lake Scuro are shared by many high-altitude lakes, and our conclusions are likely to apply in these ecosystems.

By considering press perturbations due to warming and those associated with the regime shift, as they spread in the food web, loop analysis allowed disentangling different causes for effects that were previously considered simply cascading effects of the regime shift. Accordingly, loop analysis can be used as a diagnostic tool to study causes and effects.

Because loop analysis provided reliable predictions about the effects of warming on Lake Scuro, and it applies in the framework of moving equilibrium, we think this approach is useful to study the effects of slow drivers and thus enlarges the toolkit to investigate the effects of climate change, although more research is needed to confirm and explore in full its potential.

Author Contributions

Giulia Bertoletti conceptualization, methodology and simulation, editing. Marco Scotti programming and simulation, review and editing. Giampaolo Rossetti data curation, review, and editing. Antonio Bodini conceptualization, original draft preparation, editing.

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Conflicts of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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