

Zooplankton provide early warnings of a regime shift in a whole lake manipulation

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Abstract

Regime shifts are massive changes among alternate ecosystem states. Predicting regime shifts is difficult, but statistical indicators such as increasing variance or autocorrelation may provide early warnings of their impending onset. We conducted a 4 yr lake manipulation to test for early warnings prior to a food-web transition by adding largemouth bass (*Micropterus salmoides*) to a lake dominated by small fishes, and thereby drive a trophic cascade that altered zooplankton biomass, community composition, and body size. Declining catches of small fishes were associated with shifts to larger bodied species of *Daphnia*. We measured zooplankton biomass daily in a reference and manipulated lake and calculated variance and autocorrelation of the zooplankton time series using 28 d rolling windows. We asked whether the variance in the manipulated lake (relative to the reference lake) increased and whether autocorrelation approached unity (a value suggested by theory) prior to the food-web regime shift. The variance and autocorrelation of zooplankton biomass were similar between the two lakes in the first 2 yr of the manipulation. During the third year, variance was much higher and autocorrelation approached unity for sustained periods in the manipulated but not in the reference lake. Variance and autocorrelation were similar between the two lakes during the fourth year as the food-web transition moved to completion. The joint response of variance and autocorrelation in year 3 provided an early warning of the food-web transition consistent with theoretical expectation.

Some aquatic ecosystems undergo massive, abrupt changes shifting to a new and fundamentally different state (Holling 1973; Carpenter 2003). These regime shifts are well-described in some of the smallest as well as largest systems considered by limnologists and oceanographers. For example, small, shallow lakes shift between turbid, algal dominated and clear-water, vegetated states (Scheffer et al. 1993). These shifts are induced by interactions among several factors, including nutrient dynamics, food-web interactions, and sediment resuspension (Scheffer 1998). Large regions of the ocean oscillate between states because of the interactions of climate and hydrographic conditions. These dynamics induce different oceanic regimes that may extend over decadal time scales and lead to distinct differences in primary production, food-web structure, and fisheries (Chavez et al. 2003; deYoung et al. 2008).

The theoretical foundation for regime shifts lies in nonlinear models that undergo sharp transitions between stable regions (Scheffer 2009). These stable regions are known as alternate attractors, with intermediate conditions being unstable such that a system in an unstable domain will move toward one or the other attractor in the case of two alternate stable states. Transitions among states typically exhibit hysteresis. In addition to direct evidence for regime shifts noted above, there is a concern that regime shifts may occur more widely because of large-scale environmental forcings such as climate warming, sea-level rise, and loss of biodiversity (Scheffer et al. 2001).

Regime shifts are a problem for ecosystem management and affect ecosystem services (Millennium Ecosystem Assessment 2005a). The rapid changes during regime shifts are difficult to predict or anticipate, because they often

extend beyond the range of historical experience (Carpenter 2002, 2003) and involve thresholds that are rarely known before they are crossed (Groffman et al. 2006). Regional and global environmental change is likely to cause future regime shifts in inland water and marine ecosystems affecting services such as fish production, pollution mitigation, and storm protection, with consequences for human well-being (Millennium Ecosystem Assessment 2005b; Carpenter et al. 2011).

One prospect for anticipating regime shifts comes from recent theory that provides evidence for measurable changes in key ecosystem variables before regime shifts (Carpenter and Brock 2006; van Nes and Scheffer 2007; Scheffer et al. 2009). Specifically, there are early warning indicators (EWI) in time series that foreshadow regime shifts (Scheffer et al. 2009). Most of these indicators are based on familiar statistics as, for example, the variance or autocorrelation of a time-series variable across a sequence of time windows. For variables measured at high frequency relative to the time scale of regime shifts, these indicators provide a means of anticipating and possibly forestalling unwanted changes (Biggs et al. 2009; Contamin and Ellison 2009). Despite rapid theoretical advances (Brock and Carpenter 2010; Dakos et al. 2011; Seekell et al. 2011), there have only been a few empirical tests of EWIs and these studies have mainly used either laboratory systems (Drake and Griffin 2010; Dai et al. 2012; Veraart et al. 2012) or retrospective analyses (Dakos et al. 2009; Bestelmeyer et al. 2011; Lindegren et al. 2012).

We tested EWI theory using a whole lake manipulation of a top-predator. We previously reported early warnings from temporal phytoplankton dynamics over the first 3 yr of the study (Carpenter et al. 2011). In this paper, we consider high-frequency observations of zooplankton over

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4 yr in a manipulated and reference lake testing for early warnings of a regime shift driven by cascading trophic interactions. Shifts in size structure and species composition of zooplankton are known responses to changes in predation (Brooks and Dodson 1965; Hessen et al. 1995; Carpenter et al. 2001), and these changes might be useful as EWIs. However, zooplankton community metrics often have high observation error (Kamarainen et al. 2008) that might obscure early warnings because noise is high relative to EWI signals. Properties amenable to high-frequency measurement, such as community biomass, might also exhibit compensatory species dynamics that effectively reduce early warning signals. In addition, a prior model of the system considered in this study indicated that while modeled zooplankton dynamics provided early warnings, the magnitude of warning was much lower than that observed for phytoplankton and planktivorous fish (Carpenter et al. 2008). The matter is further complicated if more than one critical transition is feasible as could occur for mid-level consumers, such as zooplankton. In this case, early warnings associated with zooplankton might be muffled or magnified depending on the direction of responses for the interacting components (e.g., planktivorous fish and zooplankton), as well as the relative resilience of these components (Brock and Carpenter 2010). In aggregate, because of measurement difficulties and complex interactions, early warnings of regime shifts from zooplankton are uncertain. Here, we use high-frequency (daily) data to test the prediction that zooplankton dynamics provide early warnings prior to an experimentally induced food-web regime shift. We hypothesized that zooplankton in the manipulated lake would exhibit early warnings via the indicators of increased variance associated with autocorrelation approaching one.

Methods

Study sites—Peter and Paul lakes (89°32'W, 46°13'N) were used as the experimental and reference systems, respectively, in this study. These lakes are located adjacent to each other at the University of Notre Dame Environmental Research Center in northern Michigan and have been extensively described in prior studies (Carpenter and Kitchell 1993). Paul Lake is dominated by largemouth bass (*Micropterus salmoides*) and also supports a small population of pumpkinseed sunfish (*Lepomis gibbosus*). This study was conducted from May 2008 through early September 2011. At the beginning of the manipulation in 2008, Peter Lake was dominated by a variety of small omnivorous fishes collectively referred to as planktivores. These species were pumpkinseeds, golden shiners (*Notemigonus crysoleucas*), fathead minnows (*Pimephales promelas*), dace (*Phoxinus eos* and *P. neogaeus*), brook stickleback (*Culaea inconstans*), and mudminnows (*Umbra limi*). A small number of largemouth bass were also present in the lake.

Manipulation—To shift the Peter Lake fish community to piscivore dominance, we gradually added largemouth bass to the lake over several years. Because of the presence of a few largemouth bass in Peter at the beginning of 2008,

we reinforced small fish dominance by adding 1200 golden shiners on 28 May 2008. The rationale for this addition was to forestall a possible rapid shift toward piscivore dominance, which might have limited the opportunity to observe early warnings. Subsequently, adult largemouth bass were added to Peter Lake as follows: 12 fish on 07 July 2008, 15 on 18 June 2009, 15 on 21 July 2009, and 32 on 23 June 2011.

Fish populations estimates—The adult bass populations in the reference (Paul) and manipulated (Peter) lakes were estimated by repeated mark and recapture sampling using electrofishing and angling (Schnabel 1938). The populations of smaller fishes were monitored daily using minnow traps that were deployed along the edges of each lake (30 traps in Peter, 20 in Paul). Minnow traps had mesh sizes of 6 mm and 25 cm openings. The number of each fish species collected in each trap was recorded daily between 10:00 h and 13:00 h from late May to early September. After inspection, fish were returned to the lake and traps were reset at ~ 0.5 m depth.

Zooplankton biomass and community structure—To obtain high-frequency data on zooplankton, we measured zooplankton biomass daily in both the reference and manipulated lakes using a gravimetric method. Total daily observations in each lake were 105 in 2008, 110 in 2009, 110 in 2010, and 111 in 2011. There were no missing values. The sampling period was between late May and early September in each year. This is the time where biological activity and food-web interactions are most dynamic and likely to provide early warning signals. Sampling daily throughout the year was not feasible given the constraints of personnel and remote locations of the lakes.

Based on past studies (Carpenter et al. 2001), changes in *Daphnia* mean length are a reliable indicator of cascading changes in the food web. We measured *Daphnia* lengths daily, but because of the effort required, we only did this analysis in the manipulated lake. We also characterized the zooplankton community weekly in terms of abundance and biomass using previously developed approaches (Carpenter et al. 2001).

Gravimetric determinations of zooplankton biomass were based on four replicate vertical net-hauls taken daily in each lake with a 153 μ m mesh net. Samples were divided in the laboratory after collection using a plankton splitter. Subsamples were then passed through a 2 mm mesh to remove *Chaoborus* spp. and concentrated on a pre-weighed filter. The other subsamples were preserved in ethanol to use for sizing and identifying *Daphnia* (see below). The filters with sample were dried and reweighed to obtain a direct estimate of the biomass of zooplankton. We checked these concentrates under a stereomicroscope and confirmed that the bulk of the material was zooplankton. Further, we measured chlorophyll *a* (Chl *a*) on an additional replicate filter and found that the contribution of phytoplankton (e.g., from filamentous or colonial algae) to the mass on the filter was trivial (always < 2% in the manipulated lake).

For each daily tow, one of the split samples was preserved in ethanol and examined for *Daphnia* spp. Twenty

individuals were measured daily to obtain a mean length, and once per week, species of *Daphnia* were determined by identifying 20 individuals using traits developed for lakes in the region (Balcher et al. 1984; Pennak 1989).

Detailed assessments of the zooplankton community abundance and biomass were made weekly by taking duplicate net-hauls in each lake using an 80 μm mesh net. These samples were pooled and preserved in a 1% Lugol's solution. Samples were subsequently enumerated with a stereomicroscope. We identified and counted cladocerans to genera. Copepods were categorized and counted as calanoids and cyclopoids except for two distinct species, *Epischura lacustris* and *Mesocyclops edax*, which we counted separately. Nauplii were counted as a single group across all copepod species. The biomass of each taxon was estimated using length–weight regressions (McCauley 1984). For each weekly sample, ~ 15 individuals in each taxon were measured to estimate mean length. Weekly samples provided context for the analysis of the daily gravimetric biomass, as well as daily length data, but note that the biomass data from the weekly data are not used in the early warning analysis.

Phytoplankton biomass—We measured Chl *a* daily at 0.5 m in each lake to estimate phytoplankton biomass. We filtered 200 mL of water through GF/F filters. Filters were frozen and later extracted in methanol. Chlorophyll concentrations in the extracts were determined with a fluorometer using standard methods (Marker et al. 1980).

Statistical analysis—We calculated variance and autocorrelation across moving windows as EWIs. The mean daily zooplankton biomass data were log-transformed prior to analysis but not otherwise manipulated (e.g., not detrended). Because the data were seasonal, we analyzed each year separately. Within each year, we calculated variance and autocorrelations for 28 consecutive d and then shifted the time window 1 d forward and repeated the calculation. This moving-window process was repeated through the entire time series. Autocorrelation for a lag of one was similarly calculated using 28 d time windows. Statistics were calculated using the moments function in R (R Development Core Team 2011) with a program written by the authors. The program used the 4 yr biomass series organized by year as input, calculated the individual statistics for each specified time window, and produced output for each statistic as a series of moving-windows values across the sample dates. The time window was set at 28 d based on a compromise between higher precision of the estimate for long time periods vs. resolution of changes in the statistics related to events in the time series (Carpenter et al. 2011). Changing the window lengths to 21 or 35 d, for example, did not substantially alter the statistical patterns. Short window lengths (7 and 14 d) obscured early warning signals; longer window lengths (42, 49, and 56 d) produced similar early warnings but provided less temporal detail.

Results

Comparisons of fish and plankton in the manipulated lake relative to the reference lake—Additions of largemouth bass

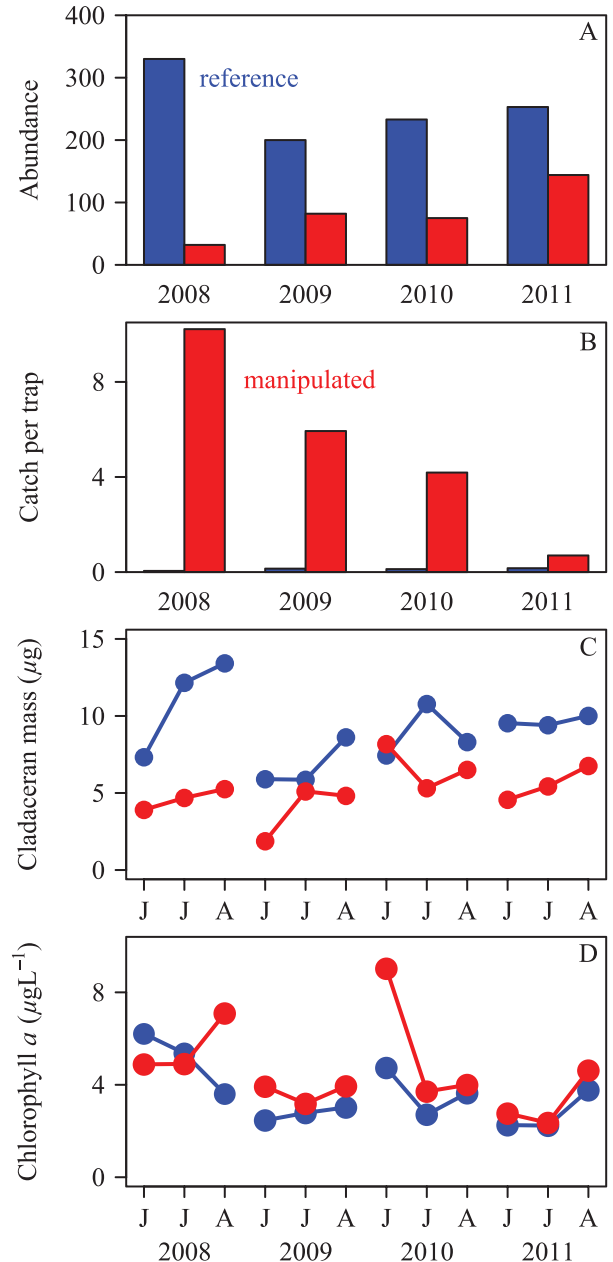


Fig. 1. Fish and plankton metrics related to the regime shift in the reference (blue) and manipulated (red) lakes. (A) largemouth bass population abundance in August of each year; (B) average catch per trap of small fishes from littoral minnow traps in each year; and monthly mean (C) cladoceran mass, and (D) chlorophyll *a* for each year (note years indicated at bottom).

to the manipulated lake resulted in a gradual increase in the bass population from 2008 through 2011 (Fig. 1A). In 2008 the adult bass population of the reference lake was 330 (95% CI = 276–411), while the population of the manipulated lake was 32 (we could not determine CIs). By the end of 2011, the adult bass population of the manipulated lake was converging toward the reference lake (Fig. 1A). Specifically, the population in the manipulated lake was 144 (95% CI = 100–219) relative to the reference lake with 253 adult bass (95% CI = 204–331). Note the

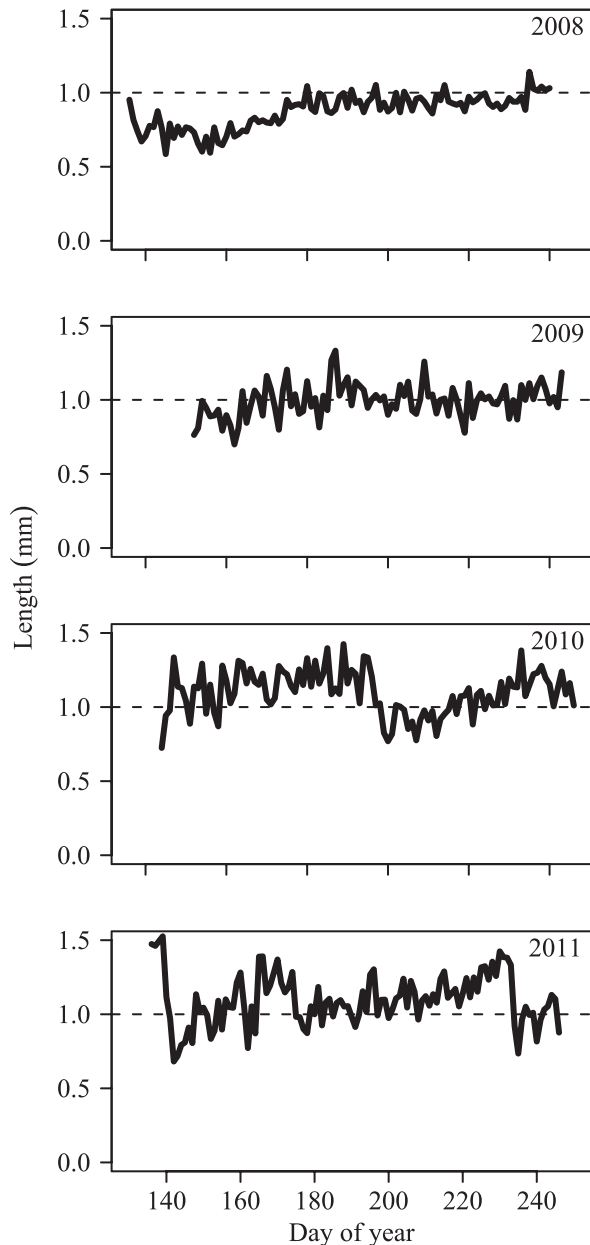


Fig. 2. Daily mean *Daphnia* spp. body size in the manipulated lake for years 2008–2011. The dashed line indicates 1.0 mm.

slight overlap in the confidence intervals of the population estimates.

Small fishes were abundant in the manipulated lake in 2008 and declined in subsequent years in concert with the increase in the bass population (Fig. 1B). In the reference lake, the abundance of small fish (largemouth bass young and pumpkinseeds) was low throughout the study. For example, in 2008 the abundance of small fishes was much greater in the manipulated than in the reference lake (t -test, $t = 20.4$, $df = 94$, $p < 0.0001$). In 2011 small fishes were still more abundant in the manipulated lake relative to the reference lake (t -test, $t = 5.1$, $df = 113$, $p < 0.0001$), but the difference was much smaller (Fig. 1B). Toward the end 2011 (last 25 d of sampling) the catches of small fishes in

the manipulated and reference lakes were similar (t -test, $t = 1.3$, $df = 45$, $p = 0.11$).

A key trait of the zooplankton in these communities is the average body size of cladocerans, which is related to both predation and grazing pressure (Carpenter et al. 2001). The reference lake had large-bodied cladocerans, including *Daphnia* spp. and *Holopedium gibberum*, while the manipulated lake started out with a mixture of small- and large-bodied cladocerans, including *Bosmina* sp., *Diaphanosoma birgei*, *Daphnia* spp., and *Holopedium gibberum*. Past research on these lakes indicates these communities can shift between dominance by small- and large-bodied zooplankton (Carpenter and Kitchell 1993), but the effect of initial composition on the rate of transition is not known. There was a strong difference in mean monthly body size between the reference and manipulated lakes in 2008 and early 2009, and this difference became smaller in subsequent years (Fig. 1C). For example, differences in mean body size ranged from 0.3 mm to 0.5 mm for 2008 through June of 2009, while the range was 0 mm to 0.3 mm for the period July 2009 through 2011. Although cladoceran body size became more similar, the reference lake cladocerans were still larger than those in the manipulated lake as indicated by t -tests of weekly size data comparing the reference and manipulated lakes each year (all $p < 0.05$).

Chl *a* was variable in the reference lake relative to the manipulated lake and there were substantial differences, particularly in August of 2008 and June of 2010 (Fig. 1D). Throughout 2011, monthly mean values were more similar (Fig. 1D). These monthly patterns are consistent with statistical tests comparing the reference and manipulated lakes each year. The manipulated lake was significantly higher than the reference lake in each year (t -tests: all $p < 0.05$) but differences between the lakes diminished. For example, the mean difference in chlorophyll was $1.8 \mu\text{g Chl } a \text{ L}^{-1}$ in 2008 compared with $0.8 \mu\text{g Chl } a \text{ L}^{-1}$ in 2011.

In summary, changes in the fish communities were notable in the manipulated lake and consistent with expectations that the addition of a piscivore would drive down populations of the smaller fish species. Changes in the plankton were less dramatic but consistent with the expectation that trophic cascades would propagate through the food web and create variability in the zooplankton and phytoplankton.

Daphnia body size and species in the manipulated lake—Daily observations of the mean length of *Daphnia* were similar to the observations of mean monthly cladoceran length but provided more detail on shifts in size for these key species. In 2008 *Daphnia* were typically < 1 mm in mean length until near the end of the sampling season (Fig. 2). In 2009 and 2010 *Daphnia* lengths tended to vary around 1 mm (Fig. 2). In 2011 body lengths were again variable around 1 mm, with short periods where mean body length was > 1.3 mm (Fig. 2).

These changes in body size are reflected in changes in species composition observed over the 4 yr (Fig. 3). The relative abundance of each species (excluding *D. retrocurva*) was different among years (one-way analysis of variance: all $p < 0.0001$). The smaller bodied species of *Daphnia*

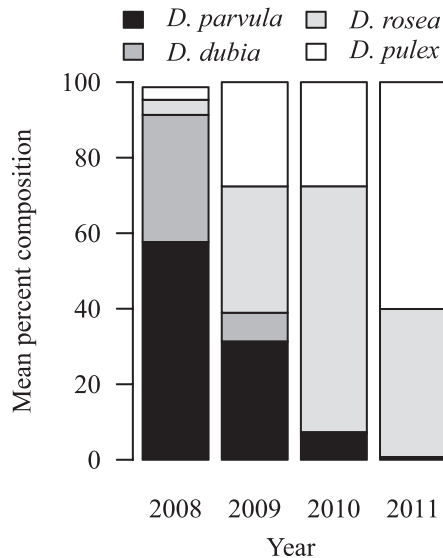


Fig. 3. Changes in the species composition of *Daphnia* in the manipulated lake based on the average of weekly samples for each year. Species include *Daphnia parvula*, *D. dubia*, *D. rosea*, and *D. pulex*. A fifth species, *D. retrocurva*, accounted for only a minor component of the community in 2008 and 2009 and is excluded from the graph. The bars for those 2 yr are slightly < 100% because of the exclusion.

parvula and *D. dubia* dominated in 2008. *D. parvula* remained important in 2009, but the relative abundance of the larger bodied species *D. rosea* and *D. pulex* increased in 2009 and especially 2010. These two species dominated the community in 2011. The *Daphnia* species in the reference lake are *D. rosea* and *D. pulex* based on many years of prior observation (Soranno et al. 1993). Hence, the *Daphnia* communities in the reference and manipulated lakes likely became more similar over the course of the study.

Zooplankton biomass and EWIs—Zooplankton biomass was relatively low in the reference lake (Fig. 4). In 2008 biomass increased until mid-summer and then declined (Fig. 4). In the other 3 yr there were no notable seasonal patterns (Fig. 4). In the manipulated lake, biomass was high early in the season and declined toward values similar to the reference lake in the second half of the season (Fig. 4). The timing of maximum biomass in the manipulated lake tended to be within the first 20 d of sampling in all years except 2008, when the biomass peak was later (~ day of year 160–170). The high early season biomass of zooplankton in Peter Lake was driven by cyclopoid copepods, which were numerous for the first few weekly samples of each year (data not shown).

The combined indicators of variance and autocorrelation were evaluated for early warning. The variance in the manipulated system was similar to the reference system in 2008 and 2009 (Fig. 4), and there was only a brief initial period in the manipulated lake where the autocorrelation was near 1 (Fig. 4). Variance in the manipulated system was substantially higher than in the reference lake in the first half of 2010, and during this same time period autocorrelation was > 0.9 (Fig. 4). In 2011 the variance

of the reference lake was higher than the manipulated lake, especially during the first part of the season (day of year 160–180), and autocorrelation was below 0.9 throughout the year (Fig. 4). For the years 2008 to 2010, there was a pattern of early to mid-summer rises in the autocorrelation and then a decline. This pattern was related, at least in part, to a decline in cyclopoid copepods (based on the weekly community data) from peak biomasses in late May or early June.

The increased variance and autocorrelation near 1 for zooplankton biomass during 2010 was consistent with expectation of an early warning of a regime shift. Consistent with this analysis we tested whether the daily running window variances in the manipulated lake minus the reference lake were > 0. The difference in variances was > 0 in 2010 (*t*-test, $t = 7.58$, $df = 83$, $p < 0.0001$) but not > 0 in the other 3 yr (2008 *t*-test, $t = 0.054$, $df = 83$, $p = 0.48$; 2009 *t*-test, $t = -1.80$, $df = 83$, $p = 0.96$; 2011 *t*-test, $t = -6.65$, $df = 83$, $p = 1.00$).

Discussion

The manipulated lake underwent a regime shift from dominance by small fishes to dominance by an opportunistic piscivore that dramatically reduced populations of small fishes (Carpenter et al. 2011). These changes cascaded through the food web. There was a shift in *Daphnia* species composition to larger bodied species and *Daphnia* length gradually increased over the course of the study. There were transitory blooms of phytoplankton (Carpenter et al. 2011; Seekell et al. 2012). Fish and plankton dynamics in the manipulated lake contrasted with the relatively stable conditions in the reference system.

An increase in variance along with autocorrelation approaching one for the zooplankton biomass time series in year 3 (2010) provided an early warning of the regime shift. These two indicators were consistent with theoretical expectations based on critical slowing down. The term ‘critical slowing down’ refers to the slower recover of systems from disturbances near thresholds, and this slower recovery is associated with increased variance and high autocorrelation (Scheffer et al. 2009). Near critical transitions, systems may also start to oscillate between alternate attractors (Brock and Carpenter 2010). This dynamic, known as flickering, may also be associated with increased variance and high autocorrelation.

Early warning was evident in the zooplankton dynamics during 2010 ≥ 1 yr prior to what we judge as the nearly full transition (in 2011) to the alternate state of a piscivore-dominated lake. However, we do not know the exact point of transition in the system (Carpenter et al. 2011), and so the amount of warning time is uncertain. In addition, the ecosystem may have been ‘committed’ to the transition by the middle of the third year when the warning signal was evident. In other words, bass dominance might have been inevitable at that point, and hence, while we have evidence of an early warning, we do not know whether that warning came too late for effective intervention.

Early warnings to the largemouth bass addition in Peter Lake were documented in prior papers for phytoplankton

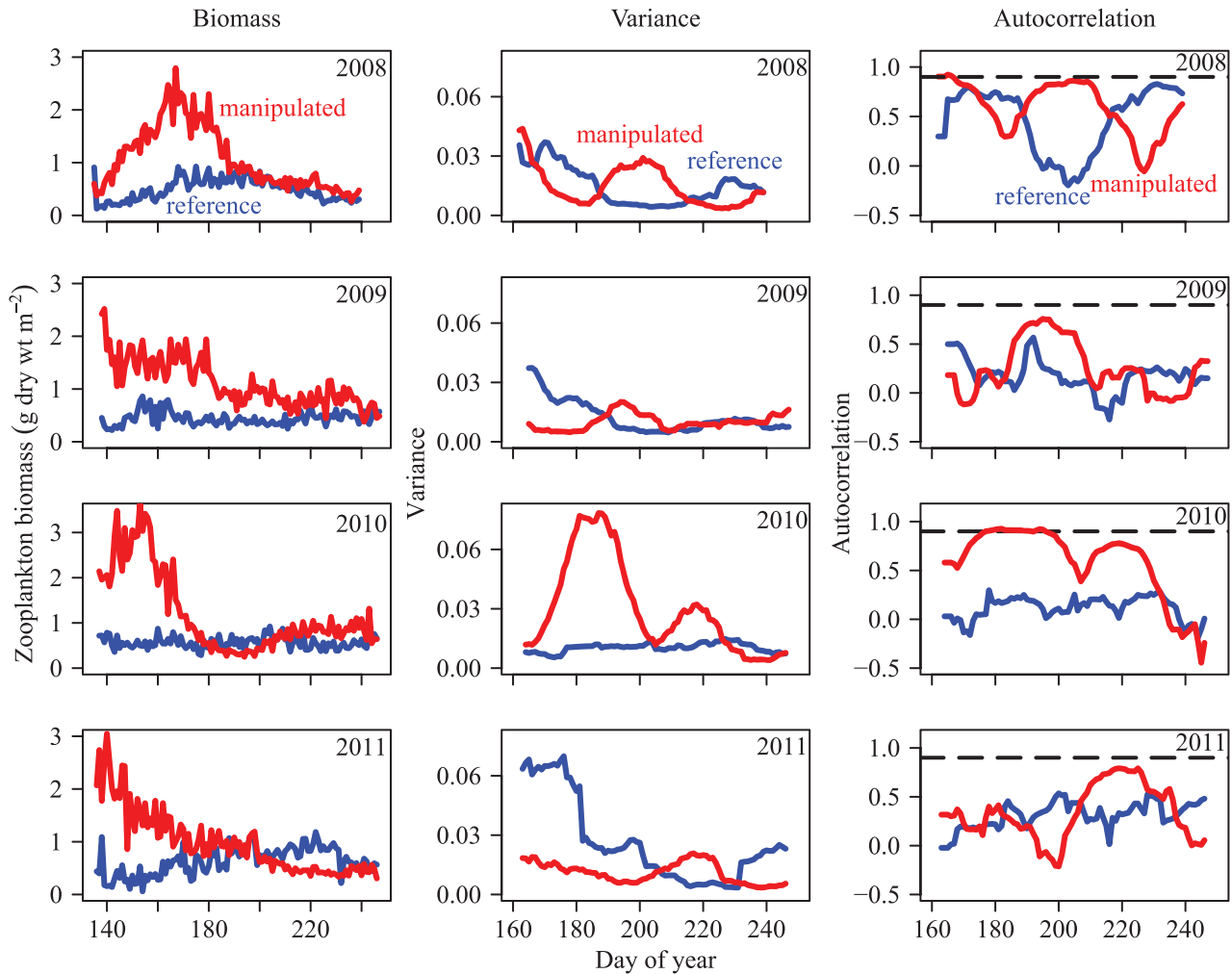


Fig. 4. Daily zooplankton biomass, as well as rolling windows of variance and autocorrelation, in the reference (blue) and manipulated (red) lakes for the years 2008–2011. Vertical dashed line at autocorrelation of 0.9 is a threshold for interpreting early warning.

and small fishes (Carpenter et al. 2011; Seekell et al. 2012). As with zooplankton, phytoplankton biomass (measured as Chl *a*) and catch rates in minnow traps were measured daily over several years. Carpenter et al. (2011), using statistical indicators like the ones in this study, found increased variance and lower return rate in 2009 and the first half of 2010. Seekell et al. (2012) tested for conditional heteroskedasticity (CH), which is nonrandom variance. Statistically significant CH was observed for Chl *a* though most of 2009 and the first half of 2010—similar to the Carpenter et al. (2011) result. For minnow catch data, Seekell et al. found even earlier warnings with significant tests in the latter half of 2008 and throughout much of 2009. These comparisons suggest the earliest warnings were evident in the small fishes, as might be expected given the immediate effects of bass predation on these populations. Phytoplankton provided earlier warnings than zooplankton, but the period of warning for phytoplankton overlapped with zooplankton (i.e., strong warnings in 2010). Zooplankton might be expected to respond sooner than phytoplankton because of cascading trophic effects, but, as discussed next, other factors may limit detection.

Of the three indicators (phytoplankton, small fish, and zooplankton), zooplankton were the least sensitive, with the caveat that somewhat different statistical methods were applied. This difference probably reflects the following: the greater sampling error associated with zooplankton, the use of gravimetric biomass rather than size or some other metric more sensitive to cascading interactions, and the potential for compensatory changes in this community. Although we did measure *Daphnia* lengths with high frequency in the manipulated lake, the lack of comparable data for the reference lake limits early warning analysis. Overall, the pattern of lower sensitivity in zooplankton relative to fish and phytoplankton is similar to the model analysis of Carpenter et al. (2008).

Specific zooplankton taxa might serve as better EWIs. The relative size and biomasses of *Daphnia* spp. are important in the lakes we studied (Carpenter et al. 2001). In other systems, such as the Baltic Sea, the copepods, *Pseudocalanus acuspes* and *Acartia* spp., exhibit early warning dynamics relative to a regime shift observed in that system (Lindgren et al. 2012). In the case of the Baltic, extensive monitoring data were available over several decades. The availability of long time

series for zooplankton species where measurements are frequent relative to the duration of regime shifts are the key need for evaluating the potential of indicator taxa.

Techniques for the analysis of EWIs are developing rapidly and several new methods emerged over the course of this study (Carpenter and Brock 2011; Seekell et al. 2011; Dakos et al. 2012). We used a relatively simple approach with two indicator statistics—variance and autocorrelation—which have been widely used in field and modeling studies (Carpenter et al. 2011; Dakos et al. 2012). Moving-window calculations of the coefficient of variation, which normalizes for the mean provided the same results as variance presented here. Return rate mirrored the autocorrelation statistic presented in this study. Moving-window analyses of kurtosis and skewness (not shown) were ambiguous, and we have found that these statistical indicators are less useful in providing early warnings. Dai et al. (2012) also found skewness did not provide early warnings in a laboratory yeast population undergoing regime shifts. CH tests for structure in variance and has proven useful in early warning analysis (Seekell et al. 2012). The CH method, however, requires large time windows (e.g., ≥ 50 observations) and was not successful at identifying early warnings in the zooplankton series of this study. Other approaches involve tests for nonlinearity and model-based approaches (Dakos et al. 2012). Essentially all the techniques for early warning analysis of time series require hundreds to thousands of observations. We had sufficient observations for the statistical indicators we used, but this limited the potential application of other methods, especially given the variability often associated with measurement of zooplankton biomass.

We assessed EWIs based on the combination of increased variance and autocorrelation approaching unity. Ditlevsen and Johnsen (2010) argue that joint responses of these signals are essential for detecting nonlinear regime shifts as opposed to noise-induced step changes in systems that would provide no early warning. Other researchers interpret early warning signals based on increased variance, positive trends in autocorrelation (as opposed to sustained autocorrelation near 1), and other statistics (Dakos et al. 2012). The robustness of EWIs has been supported by extensive theoretical analysis (Carpenter and Brock 2006; Van Ness and Scheffer 2007; Seekell et al. 2011) and several laboratory studies (Drake and Griffin 2010; Dai et al. 2012; Veraart et al. 2012). The basis for these indicators under field conditions remains to be determined. Ideally, repeated or replicated food-web manipulations would be analyzed to bolster evidence for early warning as well as provide a means for evaluating other statistical approaches. From a scientific perspective, the joint response of variance and autocorrelation provides strong evidence for a pending regime shift, but the result is limited to our single food-web manipulation. From a management perspective, large-scale manipulations that trigger robust EWIs are useful and suggest that action based on early warnings may be applicable to specific ecosystems. There remains a need to develop experience with EWIs to more firmly judge results scientifically and to use this approach in management.

The promise of EWIs rests partly on the potential for high-frequency monitoring of numerous attributes of

aquatic ecosystems (Hanson 2007; Porter et al. 2009; Ryan et al. 2011). There are increasing capabilities for making measurements of potential indicators by using developing sensor technology. Because there are many external drivers interacting with internal processes that may lead to regime shifts, the best indicator variables are often unknown. There is a need to assess possible leading indicators of regime shifts for many types of variables in different systems and circumstances. Further, the potential to use early warnings for intervention to prevent or reverse regime shifts (Biggs et al. 2009) must also be evaluated. Research in this area may, in time, provide tools to help managers promote where desirable, prevent where undesirable, or at least mitigate outcomes from regime shifts of aquatic ecosystems.

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