Multiple diversity–stability mechanisms enhance population and community stability in aquatic food webs

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Abstract. Biodiversity has been shown to increase the temporal stability of community and ecosystem attributes through multiple mechanisms, but these same mechanisms make less consistent predictions about the effects of richness on population stability. The overall effects of biodiversity on population and community stability will therefore depend on the dominant mechanisms that are likely to vary with the nature of biodiversity loss and the degree of environmental variability. We conducted a mesocosm experiment in which we generated a gradient in zooplankton species richness by directly manipulating dominant species and by allowing/preventing immigration from a metacommunity. The mesocosms were maintained under either constant or variable nutrient environments. Population, community, and ecosystem data were collected for five months. We found that zooplankton population and community stability is enhanced in species-rich communities in both constant and variable environments. Species richness increased primarily through the addition of species with low abundance. The communities that were connected to a metacommunity via immigration were the most diverse and the most stable, indicating the importance of both metacommunity dynamics and rare species for stability. We found little evidence for selection effects or overyielding as stabilizing forces. We did find support for asynchronous dynamics and statistical averaging, both of which predict destabilizing effects at the population level. We also found support for weak interactions, which predicts that both populations and communities will become more stable as richness increases. In order to understand the effects of biodiversity loss on stability, we will need to understand when different stabilizing mechanisms tend to operate but also how multiple mechanisms interact.

Key words: *asynchrony; mesocosm; metacommunity; overyielding; selection effects; species richness; stability; statistical averaging; weak interactions; zooplankton.*

INTRODUCTION

Biodiversity affects the stability of ecosystems, at least under some conditions. Here we define stability as a reduction in the temporal variability of an attribute within ecosystems. Specifically, we will focus on the stability of community attributes (e.g., aggregate variables such as the temporal variability of the biomass of a trophic level) and population attributes (e.g., the temporal variability of the biomass of a species). Many studies have found that diversity increases the stability of community-level attributes (Ives and Carpenter 2007, Griffin et al. 2009, Jiang and Pu 2009) although some have found negative or no effects of biodiversity on stability (Wardle et al. 2000, Engelhardt and Kadlec 2001, Gonzalez and Descamps-Julien 2004, Zhang and Zhang 2006, Jiang et al. 2009). In cases where biodiversity increased the temporal stability of communities, the mechanisms involved often vary (Table 1) and

are not necessarily independent or exclusive, making results from experiments difficult to interpret. Integrative studies that simultaneously test for multiple stabilizing mechanisms are few (Lehman and Tilman 2000, Tilman et al. 2006, van Ruijven and Berendse 2007, Li and Stevens 2010, Narwani and Mazumder 2012) and are generally limited to either terrestrial plant communities or to relatively simplified systems in the lab. Furthermore, previous experiments have all been done in "closed" communities where opportunities for community composition to vary due to immigration are largely absent, which can limit the degree to which different stabilizing mechanisms can operate.

In this study, we simultaneously explore all mechanisms that have been proposed to stabilize communities as richness increases that have also received some support in the literature. We categorize them into six main mechanisms (Table 1). *Increased asynchrony* is a mechanism that can operate to stabilize community attributes if species tend to oscillate more asynchronously with one another as species richness increases, thereby stabilizing community attributes such as trophic-level biomass. Increased asynchrony can result

TABLE 1. Mechanisms proposed for how diversity enhances community stability.

Mechanism for community stability	Test for mechanism	Effect of environmental variability	Effect on population stability
Increased asynchrony (Frost et al. 1995, Tilman et al. 1998, Yachi and Loreau 1999, Gonzalez and Loreau 2009)	Community synchrony $\varphi_x = (\sigma_{x\tau}^2)/(\sum_i \sigma_{xi})^2$; $0 =$ asynchronous; $1 =$ synchronous (Loreau and de Mazancourt 2008).	predicted to enhance compensation and insurance effects	destabilizing
Statistical averaging (Doak et al. 1998)	log(mean)–log(variance) slope $z > 1$; richness vs. summed variances negatively related.	neutral	destabilizing if $1 < z < 2$ (Tilman 1999)
Overyielding (Hector et al. 2010)	Species biomass increases with richness at a faster rate than standard deviation of biomass.	neutral	destabilizing for all values of $z < 2$, stabilizing only if $z > 2$ (Tilman 1999)
Selection effect (Steiner et al. 2005)	Stable species must dominate at high richness.	neutral	stabilizing only if stable species dominate
Facilitation (Doak et al. 1998, Mulder et al. 2001, Isbell et al. 2009, Romanuk et al. 2010)	No simple test, knowledge of species interactions is needed.	predicted to increase facilitation among species	stabilizing generally across many species
Many weak interactions; consumer–resource interactions in multi-trophic systems (McCann et al. 1998, Jiang and Pu 2009)	No simple test.	neutral	stabilizing generally across many species

from stronger compensatory dynamics between species driven by competition for resources, from altered predator–prey interactions in complex food webs that can increase asynchrony within trophic levels, or from insurance effects due to asynchronous responses of species to environmental variability and disturbance (Ives et al. 1999, Thébault and Loreau 2005, Downing et al. 2008, Gonzalez and Loreau 2009, Leary and Petchey 2009). *Statistical averaging*, also known as the portfolio effect, results from a probabilistic process that shows species are statistically more likely to oscillate asynchronously with other species as more species are added to a community. Statistical averaging operates when the slope (z) between the log variance and the log mean of a community attribute is greater than one (Petchey et al. 2002, Steiner et al. 2005, Vogt et al. 2006, Isbell et al. 2009, Jiang et al. 2009, Hector et al. 2010, Li and Stevens 2010). *Overyielding* is when a species grows better in species-rich communities relative to monocultures. Overyielding can stabilize communities if species' biomasses tend to increase faster than species' variability as richness increases (Valone and Hoffman 2003a, Isbell et al. 2009, Hector et al. 2010, Li and Stevens 2010). *Selection effects* can increase stability if a temporally stable species dominates at high species richness, but the reverse can also be true if temporally less stable species dominate at high species richness (Steiner et al. 2005, Polley et al. 2007). *Facilitation* stabilizes communities if positive interactions between species increase with richness, for example, if a species ameliorates the effects of a disturbance on other species and thereby reduces overall temporal variability of communities in response to disturbance (Vogt et al. 2006, Dovciak and Halpern 2010, Romanuk et al. 2010) Such facilitation may be strongly context dependent and has been shown to

operate primarily in response to enhanced environmental variability (Mulder et al. 2001) and can be sensitive to the degree of ecological realism of an experiment (Romanuk et al. 2009, Dovciak and Halpern 2010). Finally, *weak interactions* can stabilize communities because weak interactions tend to increase with richness and have been shown to dampen strong fluctuations of individual populations (McCann et al. 1998, Brose et al. 2006, Jiang et al. 2009, Jiang and Pu 2009, Allesina and Tang 2012).

One important distinction between these six mechanisms is that they make different predictions about whether the stability of community and ecosystem attributes is associated with similar effects on the stability of individual populations (Vogt et al. 2006, van Ruijven and Berendse 2007, Jiang et al. 2009, Jiang and Pu 2009). The degree to which biodiversity results in parallel stability responses between population and community attributes is likely to depend on the dominant mechanism(s) involved (Table 1; Jiang et al. 2009). Some mechanisms including increased asynchrony, statistical averaging, and overyielding predict that species richness stabilizes aggregate community properties but destabilizes populations under most conditions (Doak et al. 1998, Tilman et al. 1998, Tilman 1999, Yachi and Loreau 1999, DeWoody et al. 2003, Loreau and de Mazancourt 2008; Table 1). These patterns have been supported by many empirical studies (Tilman et al. 1998, Tilman 1999, Lehman and Tilman 2000, Caldeira et al. 2005, Steiner et al. 2011) although some have found that if populations are too destabilized they can actually destabilize rather than stabilize community attributes (Gonzalez and Descamps-Julien 2004, Jiang et al. 2009). On the other hand, selection effects, facilitation, and weak interactions predict that popula-

tions and communities will simultaneously be stabilized with increasing richness because with these mechanisms community attributes become more stable through the reduction of the average variability of populations through different means (Table 1).

The effects of biodiversity on the stability of population and community attributes will ultimately depend on the mechanisms involved, which will likely vary with environmental context and on the underlying factors influencing biodiversity. For example, environmental context matters because some mechanisms such as facilitation and increased asynchrony are predicted to be more important when environments are variable or are experiencing disturbance whereas other mechanisms do not differentiate with respect to environmental variability (Table 1). Other mechanisms will be sensitive to the factors underlying biodiversity gradients. For instance selection effects depend on the traits of dominant species whereas weak interactions depends more on the presence of rare species. Therefore different mechanisms will be more or less likely to operate if biodiversity is changing because of the gain or loss of dominant or rare species, or is influenced via a connection to a broader metacommunity that allows for compositional turnover and rare species (Howeth and Leibold 2010, Steiner et al. 2011).

In this paper, we illustrate how different mechanisms affect the stability of seminatural experimental pond ecosystems under different environmental and biodiversity contexts. We manipulated zooplankton richness by manipulating the richness and distribution of dominant species and by simulating immigration from a metacommunity. We maintained the different zooplankton richness treatments under either relatively constant and more variable environments by controlling the variability of nutrient supply.

To explore how species richness and environmental variability combined to affect stability, we quantified temporal stability at population, community, and ecosystem levels by calculating the coefficient of variation (CV) of zooplankton population biomass, community biomass (total phytoplankton and zooplankton biomass), and ecosystem gross productivity. We show that zooplankton richness stabilizes populations and communities in both constant and variable environments, and that multiple stabilizing mechanisms operate simultaneously and interactively. We also show that immigration from a metacommunity has a substantial stabilizing effect.

Material and Methods

Cattle tank pond ecosystems were established outdoors in April 2005 adjacent to the experimental pond facility at Kellogg Biological Station in Hickory Corners, Michigan, USA. The 300-L polyethylene tanks contained 16 L of silica sand as substrate and were fitted with a 1-mm screen mesh lid to control immigration by larger organisms. Each tank was filled with well water.

Nitrogen ($NaNO_3$) and phosphorus (NaH_2PO_4) were added to reach concentrations of 2250 µg N/L and 150 µg P/L to fall within the typical range of concentrations of natural fishless ponds near Kellogg Biological Station (Downing and Leibold 2010). Larval bullfrogs were added to each tank in June to minimize periphytic algae and to maintain nutrients in the water column.

Before zooplankton richness treatments were assembled, all tanks were first inoculated with a diverse mixture of microbes and phytoplankton collected and pooled from approximately 10 local ponds representing a range of abiotic and biotic conditions. Phytoplankton and microbial communities were filtered through a 30-µm mesh to remove zooplankton and macroinvertebrates and then allowed to establish for 10 days in the tanks before zooplankton were added.

We created a gradient of zooplankton richness using two different approaches. First, we established low zooplankton richness treatments of zero-, one-, and two-species communities by manipulating three "dominant" species previously identified as functionally important and among the most abundant species both regionally and within our experimental pond ecosystems: *Daphnia pulex* (Dap), *Scapholeberis mucronata* (Sca), and *Ceriodaphnia reticulata* (Cer) (Downing et al. 2008). Second, we established a high zooplankton richness treatment (N) by assembling a regional species pool from approximately 10 local ponds. We further modified the diverse regional pool (N) to create the $N - 1$ treatments (elimination of one dominant species at a time from the diverse regional pool) and the $N+$ treatment (adding species to the diverse regional pool via immigration from a metacommunity).

Using this combination of approaches, we generated 12 unique communities spanning a zooplankton richness gradient that varied with respect to the composition and abundance of dominant and rare species and connection to a metacommunity. The 12 treatments include zero species (no zooplankton), one species (monocultures of each of the three dominant species, Dap, Sca, and Cer), two species (all three possible pairwise combinations of the dominant species, Dap + Sca, Dap + Cer, Sca + Cer), $N - 1$ (diverse communities assembled from the regional species pool minus each of the dominant species, $N -$ Dap, $N -$ Sca, $N -$ Cer), N (diverse communities from the regional species pool that included the dominant species), and $N+$ (hyper-diverse communities including the dominant species and additionally receiving experimental immigration once every two weeks).

The dominant species were added to the experiment from cultures of single species created from existing lab populations, experimental tanks, and natural ponds. In order to create the diverse treatments ($N - 1$, N, and $N+$), we initiated the treatments with a diverse zooplankton inoculum pooled from natural zooplankton assemblages sampled from approximately 10 local ponds in which all three dominant species and any unintended predators were removed. The $N - 1$

treatments were created by adding two of the three dominant species back into the inoculum from stock populations resulting in the diverse inoculum minus one of each of the three dominant species ($N - 1$). The N treatment was established by adding all three dominant species back into the diverse inoculum. The $N+$ was established by adding all three dominant species back into the diverse inoculum and in addition received small inoculums of zooplankton pooled from the 10 local source ponds once every two weeks, mimicking natural dispersal within the regional pond metacommunity. The levels of immigration we imposed were approximately 0.5% per week and were sufficiently low that they should have negligible effects on any biomass inputs. Indeed, our calculations indicate that an immigrant individual was highly unlikely to be sampled at any subsequent time (approximately 0.02% per sampling event, assuming no mortality and depending on relative abundance in the inoculum). Thus, the immigration we imposed could allow for recolonization or a rescue effect if species go locally extinct (Mouquet et al. 2005), as well as seasonal compositional changes to occur in mesocosms, but would not be sufficient to maintain any measurable sink populations.

Each of the 12 zooplankton communities was replicated eight times for a total of 96 replicates. Half of the replicates were maintained under "constant" nutrient environments and half under "variable" nutrient environments. The environmental variability treatment was created to help distinguish between mechanisms and to determine if mechanisms such as increased asynchrony or facilitation become more important in variable environments as predicted (Table 1). Previous experiments indicate that 5% of nitrogen and phosphorus are lost per day from the water column in each mesocosm due to processes such as biological uptake, sedimentation, or denitrification. The mesocosms are isolated from natural supplies of nutrients (e.g., run-off, groundwater), therefore we experimentally added nutrients to all mesocosms assuming a 5% loss rate per day (e.g., Downing et al. 2008). In the constant environment, nutrients were added continuously via a slow drip to individual tanks of a dilute nutrient solution controlled by peristaltic pumps. In the variable environments, nutrient treatments received an entire two-week supply of nutrients via a pipette every two weeks, more typical of nutrient pulses associated with rainfall events. Therefore, while all mesocosms experienced the same degree of natural environmental variability due to natural weather patterns, the variable communities received enhanced variability in the form of biweekly pulses of nutrients, which represented the only form of environmental variability that was unique between constant vs. variable environments.

Tanks were sampled for population (biomass of individual zooplankton species), community (total phytoplankton and zooplankton biomass), and ecosystem (gross productivity) attributes 32 times at even intervals of 4–5 days beginning 11 May and continuing through 3 October. Zooplankton and phytoplankton communities were sampled by pooling 16 750-mL water column samples from each replicate with a tube sampler. A 300-mL sample was removed for phytoplankton analysis (chlorophyll a) and the remainder was collected on an 80-μm mesh screen and preserved in sucrose Lugols solution for microscopic enumeration. Juvenile and adult cladocerans were classified to genus or species. Larval and juvenile copepods were identified by stage (nauplii and copepedites) and were treated as a separate species for most analyses because of difficulties assigning them to a genus and because they are functionally unique life stages. All zooplankton count data were converted to biomass (μg/L) using standard length–weight regressions (McCauley 1984).

Ecosystem productivity and respiration was determined using diurnal oxygen cycles obtained with a YSI dissolved oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA) taken from each replicate at dawn, dusk, and the subsequent dawn (Downing and Leibold 2002). We report results only for gross productivity calculated by correcting estimates of net hourly productivity (oxygen production) during the day by addition of net respiration (oxygen consumption) at night.

Zooplankton and phytoplankton biomass (inedible >35 μm, edible <35 μm, and total) were log($X + 1$) transformed to meet the normality and homoscedasticity assumptions of ANOVA. The coefficient of variation (CV) was used to quantify temporal stability for individual zooplankton population biomass, total zooplankton biomass, total phytoplankton biomass, and gross ecosystem productivity. The remaining response variables met the assumptions of ANOVA without transformation. Seven replicates were dropped from further data analysis due to obvious contamination by unwanted species. The full ANOVA model included fixed effect terms for richness, environment, and richness × environment. They were conducted with and without the no-zooplankton (zero species) control. All statistical analyses were performed in SYSTAT (Version 12; Systat Software, Chicago. Illinois, USA).

The various stabilizing mechanisms outlined in Table 1 make testable predictions about the response of population and community properties to species richness and enhanced environmental variability.

Asynchronous dynamics

Detecting asynchrony (and similarly, negative covariance, compensatory dynamics, and insurance effects) between populations is challenging, which may partly explain the low detection rate of compensation or asynchrony in both nature and experiments (Houlahan et al. 2007, Downing et al. 2008, Loreau and de Mazancourt 2008, Gonzalez and Loreau 2009, Steiner et al. 2011). One central challenge is that correlation coefficients between species, which are often used to

show asynchrony, are not independent of species richness because the lower bound of correlations between species becomes less negative as species richness increases (Loreau and de Mazancourt 2008). Here we use a measure of community-wide synchrony introduced by Loreau and de Mazancourt (2008) that uses the statistic $\varphi_x = (\sigma_{x\tau}^2)/(\sum_i \sigma_{xi})^2$, where x is a temporal variable of interest for species i or community τ. Thus, $\sigma_{x\tau}^2$ is the temporal variance of a community-level variable (e.g., total zooplankton biomass) and $(\Sigma_i \sigma_{xi})^2$ is the maximum possible community biomass variance if all species were perfectly synchronized. If all species oscillate perfectly synchronously, $\varphi = 1$ and if all species oscillate perfectly asynchronously, $\varphi = 0$.

Statistical averaging

To evaluate the contributions of statistical averaging, we calculated whether the slope (z) between log(mean population biomass) vs. log(mean population variance) averaged across the experiment and for each species is greater than 1. Statistical averaging further predicts that populations will be destabilized as richness increases when $z < 2$ for individual populations (Cottingham et al. 2001, Petchey et al. 2002, Steiner et al. 2005, Li and Stevens 2010), and stabilized with richness if $z > 2$ for individual populations (Tilman 1999).

Overyielding

In order for overyielding to stabilize zooplankton communities, zooplankton biomass must increase with species richness, and at a faster rate than the standard deviation (SD) of biomass. If both these patterns are observed, the coefficient of variation (CV) of total zooplankton biomass should decline as richness increases because CV = (mean zooplankton biomass)/SD (Hector et al. 2010, Li and Stevens 2010).

Selection effect

The three dominant species (Dap, Sca, Cer) are the most likely candidates for a selection effect because they are also the most abundant (Fig. 1b). We calculated the average CV's of the three dominant species in monoculture to determine if these species were inherently more stable and could contribute to a selection effect. To further explore the selection effect we compared the average CV of all the species nested within each richness level to determine if species with the lowest CVs tended to be more abundant at high richness.

Facilitation, weak interactions

These mechanisms predict that community attributes are stabilized by reducing the population variability of many species simultaneously. Therefore we explored the reduction in population variability for each species across the richness gradient. To further differentiate between these mechanisms, we tested the prediction that facilitation causes reduced population variability in

variable as compared to constant environments as richness increases (Table 1).

RESULTS

The zooplankton treatments resulted in a strong richness gradient (Fig. 1a; $F_{5,76} = 156.96$, $P < 0.001$) with no substantial or sustained populations of unwanted species with the exception of seven replicates that were removed from further analysis, and the invasion of *Daphnia pulex* into several of the 0 richness treatment tanks (Fig. 1). Differences in richness were significant between all levels with the exception of 0 vs. 1 and $N - 1$ vs. N treatments (Tukey post hoc comparisons, $P < 0.05$). Richness was about twice as high in the $N+$ treatment, which was open to the metacommunity via immigration once every two weeks. In all richness treatments, the three species that were most abundant were also the dominant species we manipulated individually (Dap, Sca, Cer; Fig. 1b). Zooplankton biomass increased with zooplankton richness ($F_{5,76} = 14.18$, $P < 0.001$; Fig. 1b). Total phytoplankton biomass also responded to zooplankton richness ($F_{5,76} = 2.593$, $P = 0.032$), but not in a consistent direction. More specifically, edible phytoplankton declined consistently with increasing richness ($F_{5,76} = 8.350$, $P < 0.001$) while inedible phytoplankton increased with richness ($F_{5,76} = 2.84$, $P = 0.021$). Gross ecosystem productivity was not affected by zooplankton richness ($F_{5,76} = 2.081$, $P = 0.077$).

Stability tended to increase consistently as zooplankton richness increased for both average individual zooplankton population biomass ($F_{4,71} = 4.596$, $P = 0.002$) and zooplankton community biomass ($F_{4,71} = 2.900$, $P = 0.028$; Fig. 2a, b). The significant pairwise differences for population variability are between the $N+$ treatment and the one-species, two-species, and $N - 1$ treatments (Tukey post hoc test, $P < 0.05$) and for community stability the significant difference is between the $N+$ vs. one-species treatment (Tukey post hoc test, $P < 0.05$). The stability of total phytoplankton biomass declined with zooplankton richness (Fig. 2c; $F_{5,76} = 4.018$, $P = 0.003$) although this effect is not significant if the no-zooplankton treatment is removed from the analysis ($F_{4,71} = 1.757$, $P = 0.147$). This effect appears driven mainly by an increase in the variability of edible phytoplankton with increasing richness ($F_{5,76} = 6.260$, $P < 0.001$) because the CV of inedible phytoplankton does not vary with richness ($F_{5,76} = 0.755$, $P = 0.585$). The stability of gross ecosystem productivity did not vary with richness or environmental variability (Fig. 2d; $P > 0.05$). Stability at all levels is not influenced by environmental variability (ANOVA, $P > 0.05$ for environment and richness × environment effects).

Community synchrony between zooplankton populations decreased with zooplankton richness (Fig. 3a; ANOVA, richness effect $F_{3,52} = 8.68$, $P = 0.0001$). Post hoc tests reveal the significant difference is again between the $N+$ treatment and the two-species, $N - 1$,
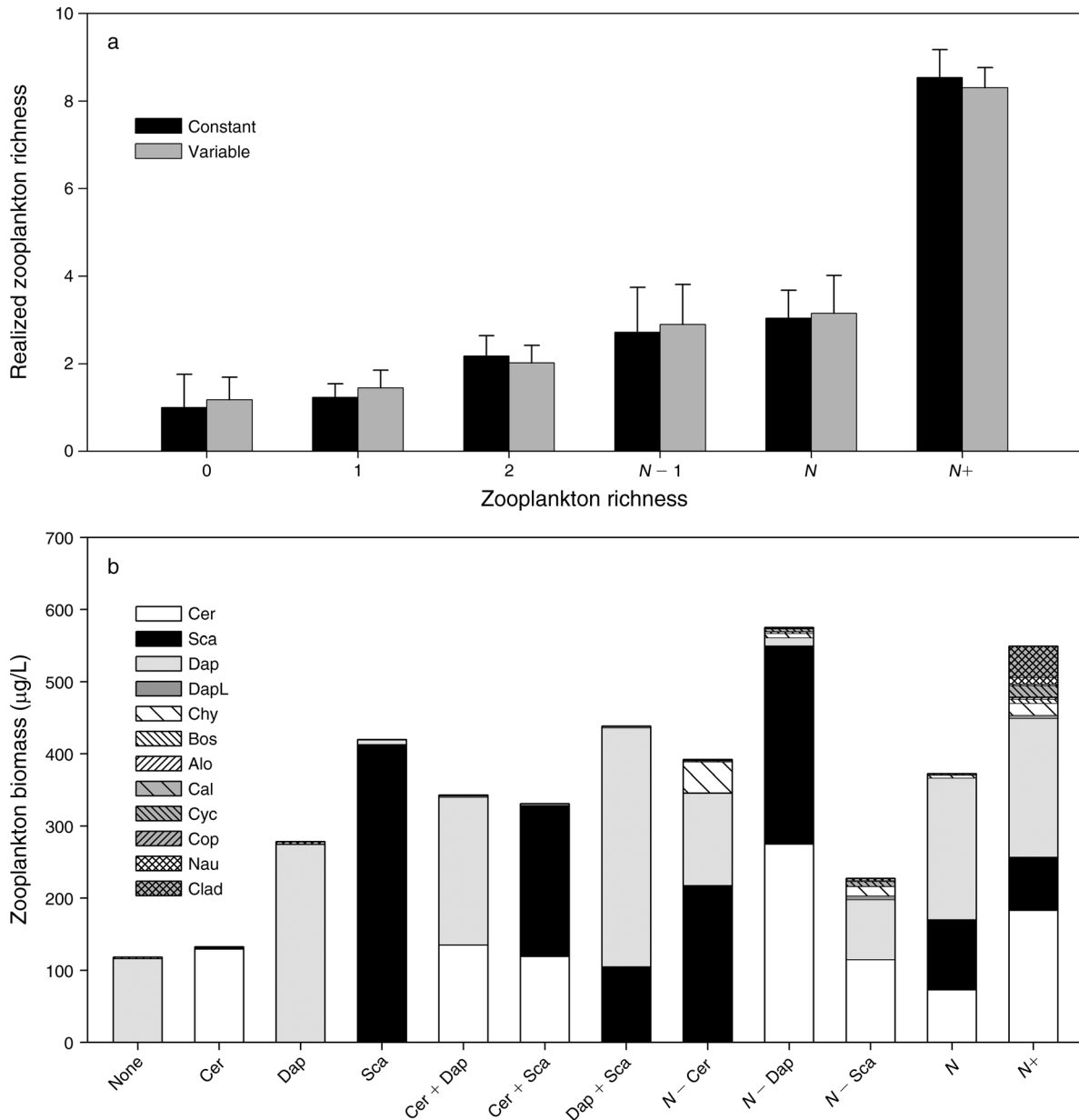
FIG. 1. (a) Treatment effects on zooplankton richness. Richness treatments are 1, monocultures; 2, two-species combinations; $N - 1$, diverse communities minus a focal species; N, diverse; $N+$, diverse + immigration. Error bars show SE. (b) Relative zooplankton abundance for zooplankton compositions. Zooplankton abbreviations are: Cyc, cyclopoids; Cop, copepodites; Nau, nauplii; Cer, *Ceriodaphnia*; Sca, *Scapholeberis*; Dap, *Daphnia pulex*; DapL, *Daphna laevis*; Chy, *Chydorus*; Bos, *Bosmina*, Alo, *Alona*; Cal, Calanoids; and Clad, minor cladocerans (*Simocephalus, Diaphanosoma, Macrocystic, Polyphemus,* and *Pleuroxus*).

and N treatments (Tukey post hoc tests, $P < 0.05$). We found no significant effect of environmental variability on asynchrony ($F_{1,52} = 0.12$, $P = 0.734$). We observed a marginally significant environment × richness interaction ($F_{3,52} = 2.55$, $P = 0.066$), suggesting richness may increase asynchrony to a greater degree in variable environments (Fig. 3a).

Our test for statistical averaging revealed that the average slope (z) across all zooplankton species was 1.949 ± 0.013 (mean $\pm$ SE; Fig. 3b; least-squares regression $F_{1,1294} = 23\,302$, $P < 0.001$, $R^2 = 0.947$). Z values for individual species ranged from 1.79 to 2.695. Exactly half of the 16 species had z values < 2 and the other half had $z > 2$ (Appendix). Of the eight species predicted to be destabilized with richness ($z < 2$), only two species actually showed this pattern (Appendix). The strength of statistical averaging does not change with environmental variability as indicated by similar z values (fluctuating environments, $z = 1.937 \pm 0.019$, least-squares regression $F_{1,638} = 10\,048$, $P < 0.001$, $R^2 = $
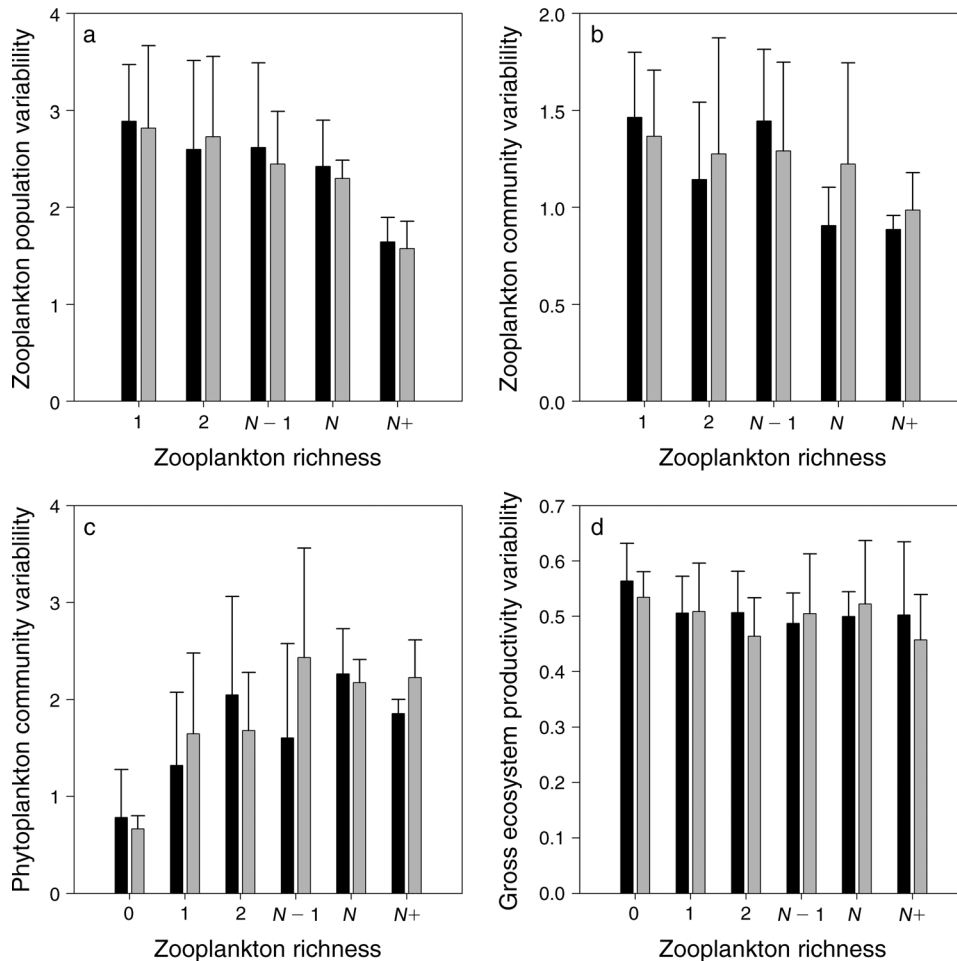
Fig. 2. Treatment effects on population, community, and ecosystem stability. Black bars show constant environments, gray bars show variable environments, and error bars show SE. (a) Zooplankton population variability calculated by average CV of biomass of individual populations, (b) zooplankton community variability calculated as the CV of biomass of total zooplankton biomass, (c) phytoplankton community variability calculated as the CV of biomass of total phytoplankton (chlorophyll a) biomass, and (d) ecosystem stability calculated as the CV of gross ecosystem productivity.

0.940; constant environments, $z = 1.962 \pm 0.017$, least-squares regression $F_{1,638} = 13\,725$, $P < 0.001$, $R^2 = 0.955$). Statistical averaging further predicts that variances across all species should decline with richness (Steiner 2005, Vogt et al. 2006) but our data do not show this trend (ANOVA on variances, $F_{4,71} = 0.3760$, $P = 0.825$).

To test for overyielding, we found that zooplankton biomass increased with richness (Fig. 1b; ANOVA $F_{4,71} = 2.759$, $P = 0.034$). However, overyielding is stabilizing only if zooplankton community biomass also increases faster than the standard deviation. We found the overall slope of the regression for mean biomass vs. mean standard deviation is not statistically different from one (slope = 0.95, 95% CI = 0.75–1.15; Fig. 3c; linear regression, $F_{1,79} = 89.89$, $P < 0.001$, $R^2 = 0.532$) indicating that biomass increases at the same rate as the standard deviation. The slope of regression in constant environments also indicates a regression slope

not significantly different from one (slope = 1.2, 95% CI = 0.91–1.44). However, the regression slope in variable environments is significantly less than 1 (slope = 0.67, 95% CI = 0.38–0.97), which indicates a potential role for overyielding in variable environments.

When testing for the role of selection effects on community stability we found the CVs, and thus the stability of the dominant species, in monoculture were indistinguishable from each other (Fig. 3d; ANOVA, $F_{2,42} = 0.626$, $P = 0.540$). To explore possible contributions of other species to a selection effect we calculated the CVs of all zooplankton species observed in the $N - 1$, N, and $N+$ treatments (Appendix). Most of the species other than the dominant species (Dap, Sca, Cer) are minor contributors to zooplankton community biomass and are thus not likely candidates for a selection effect (Fig. 1b, Appendix). However, the $N+$ treatment is unique from other richness treatments in that it has a much larger proportion of minor cladocerans (Fig. 1b)
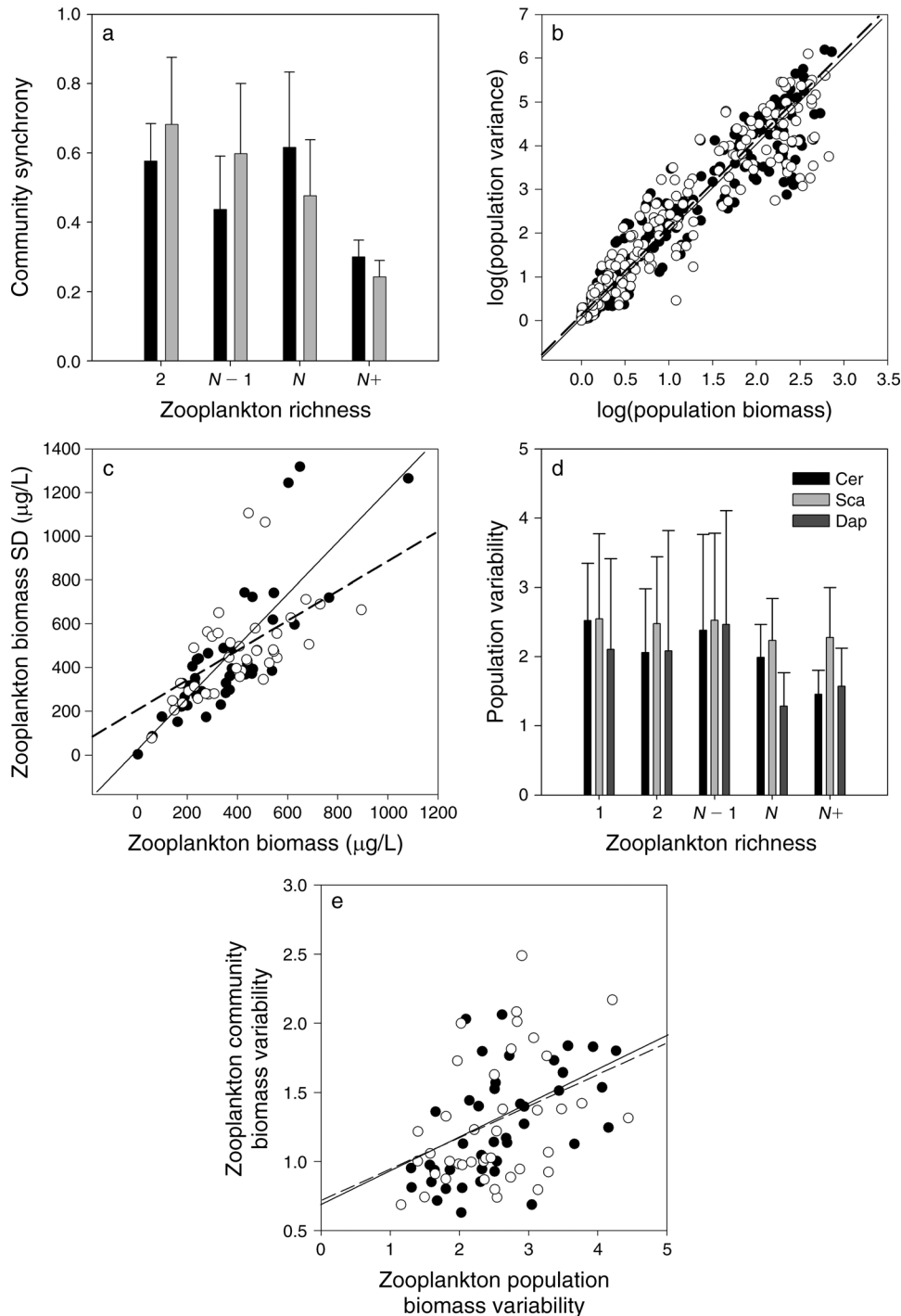
FIG. 3.   Evidence for stabilizing mechanisms. (a) Community synchrony with respect to zooplankton richness (1 = synchronous, 0 = asynchronous; black bars show constant environments, gray bars show variable environments, and error bars show SE). (b) Statistical averaging; mean–variance scaling relationship; solid circles and solid line show constant environments, while open circles and dashed line show variable environments. Biomass was measured in μg/L. (c) Overyielding; zooplankton biomass mean vs. standard deviation. (d) Statistical averaging; summed variances (CV) vs. richness; solid circles and solid line show constant environment, open circles and dashed line show variable environments, and error bars show SE. (e) Reduced population variability; zooplankton population variability (CV) vs. zooplankton community variability (CV); solid circles and solid line show constant environments, while open circles and dashed line show variable environments.

that might possibly contribute to a selection effect. We found that the minor cladoceran group has the second highest CV of all species, and it was statistically higher than *Bosmina*, *Ceriodaphnia*, *Chydorus*, *Daphnia*, and nauplii (ANOVA, species effect, $F_{9,53} = 2.683$, $P = 0.012$, pairwise differences determined via Tukey post hoc tests; Appendix), thus indicating the minor cladocerans are not contributing to a selection effect.

To test for a potential role of facilitation and weak interactions, we observed that population variability decreased on average across all species with richness, consistent with mechanism predictions (Fig. 2a). The population variability (CV) of each dominant species also showed a trend toward declining CVs with richness (Fig. 3d), however these trends were not significant for the individual focal species (ANOVA; species richness, $P > 0.05$ for all species combined; environment effect, $P > 0.05$ for all species; and richness × environment, $P > 0.05$ for all species). In addition, all 12 zooplankton species that were found in more than one richness level across the $N - 1$, N, and $N+$ treatments had the lowest CV in the $N+$ treatment (Appendix). Finally, we regressed the CV of zooplankton populations (averaged across all populations) vs. the CV of total zooplankton community biomass and found them to be positively related (Fig. 3e, linear regression, $F_{1,79} = 21.014$, $P < 0.001$, $R^2 = 0.210$).

DISCUSSION

We found that, as zooplankton richness gradually increased, both zooplankton population biomass and aggregate zooplankton community biomass became increasingly stable (Fig. 2). These effects were largely, though not entirely, due to the effects of low-level manipulated immigration ($N+$ treatment), which resulted in a treatment with double the richness over the next highest richness level (N). This makes it difficult to determine if the enhanced stability is due to a general effect of richness or due to the connection to a metacommunity. It is notable, however, that stability tends to gradually increase with each increasing richness level even though the significant differences are primarily between the $N+$ vs. the other treatments (Fig. 1). Thus, while we discuss our results in the context of richness increasing the stability of populations and communities, we are unable to truly differentiate between a richness effect per se and the effect of the $N+$ treatment created by connection to a metacommunity.

The $N+$ treatment was generated by allowing immigration from a metacommunity, which is a process known to influence species richness in natural ecosystems. Surprisingly, our manipulations focused on dominant species (Dap, Cer, Sca) had weaker effects than our low-level immigration manipulation. In contrast to the lack of strong effects of dominant species, rare species appeared more important for stabilization. This is best illustrated by contrasting high-diversity treatments with and without immigration. Immigration doubled the richness in the $N+$ treatments compared to the other N treatments through addition of rare species, and facilitated the enhanced stability we observed in the $N+$ treatments relative to all other richness treatments. Our results point to similar conclusions made by O'Gorman and Emmerson (2009) who found that species with relatively weak interactive effects on marine communities contributed similarly to ecosystem-level stability as those with strong effects. Understanding the particular ways that such effects work is a major challenge for future work, both theoretical and experimental.

Zooplankton richness tended to destabilize total phytoplankton biomass, however this effect was driven primarily by enhanced plant–herbivore oscillations in the presence of zooplankton as the richness effect disappeared when we dropped the no-zooplankton treatment. Zooplankton richness had no effect on the stability of gross ecosystem productivity. The prediction that stability of some variables (zooplankton populations and communities) might lead to stabilization at other levels (phytoplankton biomass and ecosystem productivity [Jiang and Pu 2009, Proulx et al. 2010]) was thus not supported in our study. Somewhat surprisingly, increased environmental variability in the form of nutrient pulses did not influence stability at any level although we had previously found a stabilizing effect of variability (Downing et al. 2008).

Our observation that zooplankton richness enhanced stability at the community level can be explained by at least three different mechanisms operating simultaneously: statistical averaging, increased asynchrony, and increased number of weak interactions. First, we find evidence for statistical averaging because the average mean–variance scaling relationship (z) across our experiment was 1.94, meeting the $z > 1$ requirement to invoke statistical averaging. This result lends further support to studies that have shown typical z values between 1 and 2 and suggests that statistical averaging is a consistent stabilizing mechanism across many different ecosystems (Tilman 1999, Cottingham et al. 2001, Tilman et al. 2006). We also found $z > 2$ for half of the species, which predicts populations stabilize with richness, while the other half had $z < 2$, predicting destabilization, and which indicates that statistical averaging does not have a consistent effect on zooplankton population stability (Appendix). Additionally, six of the eight species predicted to be destabilized as richness increases showed the opposite trend, indicating that theoretical predictions based on z values may be too simplistic to explain patterns of population stability in complex communities, perhaps due to the unrealistic assumption that species will have equal biomass in communities (Valone and Hoffman 2003b). Notably, in our experiment, different zooplankton species had highly unequal biomasses and richness increased pri-

marily by the addition of rare species (Fig. 1b), which likely weakens the statistical averaging effect.

Increased asynchrony between zooplankton populations also contributes to increased community stability with richness. The asynchrony effect is most apparent when contrasting open ($N+$) vs. closed (other richness treatments; Fig. 3a), suggesting that metacommunity dynamics can stabilize communities (Loreau et al. 2003, Steiner et al. 2011). Interestingly, we also observe a nonsignificant trend where richness enhances stability more in variable environments as compared to constant environments as predicted by the insurance effect (Table 1). Another prediction of asynchrony is that populations can become destabilized when strong negative covariance or compensatory dynamics occur (Tilman 1999), yet populations in our experiment were more stable as richness increased, a pattern also observed in other systems (Valone and Hoffman 2003b, Proulx et al. 2010). This suggests that other mechanisms that increase population stability could be operating, potentially overriding any decrease in population stability due to asynchrony and statistical averaging.

The pattern we observe of reduced population variability across most zooplankton species at high richness is consistent with facilitation (Doak et al. 1998, Mulder et al. 2001, Isbell et al. 2009, Romanuk et al. 2010) and weak interactions (McCann et al. 1998, Jiang and Pu 2009) as stabilizing forces. Facilitation cannot be ruled out but it does appear less likely because facilitation is predicted to be more important in variable environments and yet we observed no differences in population or community stability associated with environmental variability. Additionally, we know of no likely candidate mechanisms for direct facilitation among our zooplankton species. On the other hand, an increase in the number of weak interactions is consistent with our observation that zooplankton populations become more stable as species richness increases (McCann et al. 1998, Jiang et al. 2009). This mechanism is further supported by the pattern that increased richness in our experiment is due to the addition of rare species that are more likely to generate weak interactions in food webs. For example, increasing the number of weak consumer–resource interactions can help dampen strong consumer–resource interactions (McCann et al. 1998, Jiang et al. 2009, Jiang and Pu 2009). In addition, an increase in inedible algae can cause a reduction in edible algae through competition for nutrients similar to the pattern we observed in our experiment, which then in turn can weaken strong consumer–resource interactions (McCauley et al. 1999).

We found no evidence for a selection effect as a stabilizing mechanism in our experiment, and instead found that any selection effects in our open ($N+$) treatments were more likely destabilizing. Our data do suggest that overyielding may contribute to stabilizing zooplankton biomass in variable environments as richness increases, however this mechanism is not strong enough to generate an observable difference in stability between constant and variable environments as richness increases. Therefore while overyielding may provide a minor effect in variable environments, it cannot explain the overall pattern of enhanced stability in species-rich communities we observed in both constant and variable environments.

The various diversity–stability mechanisms all predict that community properties will be more stable in species-rich communities but make different predictions about the effects of richness on population-level stability (Table 1). In our experiment, we argue that asynchrony, statistical averaging, and weak trophic interactions contribute to stabilizing zooplankton communities under both constant and fluctuating environments. We do not find support for the selection effect and only weak support for overyielding in variable environments. Of the mechanisms potentially operating in our study, only weak interactions and facilitation predict populations will be stabilized indicating that it is important to understand the interaction and relative importance of different mechanisms in order to understand stability at different levels.

The lack of a strong environmental effect on stability at the population or community level was surprising given our previous results (Downing et al. 2008), which showed stability increased in variable environments due to an increase in asynchronous dynamics. The data we present here do show a marginally significant effect for asynchronous dynamics being enhanced most strongly by richness in variable environments, which supports our previous results. Previous empirical and theoretical work suggests that environmental variability should affect temporal stability but that this relationship is sensitive to the degree of environmental variability (Ives et al. 1999, Gonzalez and Descamps-Julien 2004, Thébault and Loreau 2005, Romanuk et al. 2009). In our experiment, it is possible that the enhanced environmental variability imposed experimentally through nutrient pulses could have been partially masked or counteracted by natural environmental variability or even perhaps by the richness manipulations that affected the entire experiment more strongly than the nutrient pulse treatment.

In summary, we found that species richness increased primarily through the addition of species with low biomass, that richness reached the highest levels in communities connected to a metacommunity, and that mesocosms connected to a metacommunity were the most stable. We found that zooplankton richness tends to increase population and community stability due to a combination of mechanisms that includes increased asynchrony, statistical averaging, weak interactions, and a possible role for facilitation. Our results are probably typical in that many ecosystems likely have multiple mechanisms operating simultaneously. However, the specific mechanisms that are most likely to dominate in different ecosystems will likely vary. For

example, some mechanisms like weak interactions may be more common in ecosystems with complex food webs or in ecosystems with strong connections to a meta-community that can increase rare species. Other mechanisms such as overyielding may be more important in predominantly single-trophic-level systems such as terrestrial grasslands. Knowing when different mechanisms are likely to operate and interact should help us better understand why the effects of richness on population and community stability vary across studies (Jiang and Pu 2009, Narwani and Mazumder 2012).

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SUPPLEMENTAL MATERIAL

Appendix

A table showing species-specific values for z, rank abundance, and coefficient of variation in $N - 1$, N, and $N+$ treatments (*Ecological Archives* E095-016-A1).