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

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Running title: Population and community stability in aquatic food webs

Abstract: 317 words

Main text: 4947 words

References: 50

ABSTRACT:

Species rich communities tend to have higher temporal stability of community (aggregate) properties. Many different stabilizing mechanisms have been identified which include asynchronous dynamics, statistical averaging, overyielding, selection effects, facilitation, and weak interactions. These mechanisms are predicted to stabilize community properties as species richness increases but they make less consistent and in some case opposite predictions about the effects of species richness on the temporal stability of populations. Additionally, different stabilizing mechanisms are expected to be more or less important under constant or variable environments and in single or multiple-trophic level communities. We conducted a mesocosm experiment using aquatic food webs to test how different mechanisms contribute to population and community stability under different conditions. We manipulated zooplankton richness under either constant or variable nutrient environments and collected time series data for population, community, and ecosystem properties spanning many generations. Our results suggest that zooplankton population and community stability are enhanced in species-rich communities but that stability was not affected by environmental variability. We observed that species richness increased primarily through the addition of species with low abundance, indicating the importance of rare species for stability. We found that increased asynchrony, statistical averaging, and weak interactions operate simultaneously to enhance community stability as richness increases in both constant and variable environments. Our results also suggest that some stabilizing mechanisms such as the selection effect and overyielding which can be important in single trophic level studies may not be as important in multi-trophic food webs. In contrast, mechanisms such as weak interactions may be more important in food webs and can counteract the destabilizing effect on populations resulting from other mechanisms. In summary, our results show that species richness can stabilize both populations and communities in aquatic food webs. Predicting the effects of richness and environmental variability on population and community stability will ultimately require knowledge of the relative contributions of various mechanisms under different environmental scenarios.

INTRODUCTION:

It is increasingly apparent that biodiversity can affect the stability of ecosystems, at least under some conditions. Many studies have found positive relations between diversity and resilience of community and ecosystem attributes such as biomass of entire trophic levels or productivity and respiration (Steiner et al. 2006, Mulder et al 2001, McDougall 2005, McNaughton 1977, Tilman 1996, Downing and Leibold 2010, Downing et al. in prep.). However others have found negative or no effects (Caldeira *et al.*, 2005; Engelhardt & Kadlec, 2001; Zhang & Zhang, 2006a; Allison, 2004; Wardle, Bonner & Barker, 2000; Griffiths *et al.*, 2001, Pfisterer 2002). Even in cases where positive effects have been observed the mechanisms involved also seem to vary (Table 1) and such mechanisms are not necessarily independent or exclusive (e.g. Lehman & Tilman 2000; Tilman *et al.* 2006; van Ruijven & Berendse 2007; Li & Stevens 2010). There thus seem to be multiple, possibly conflicting mechanisms acting to alter how biodiversity affects the stability of ecosystems.

An important component of the question is whether stabilization of such large scale attributes are associated with similar or different effects on the stability of individual populations that contribute to these large scale properties. (Vogt *et al.* 2006; van Ruijven & Berendse 2007; Jiang *et al.* 2009; Jiang & Pu 2009). The degree to which component populations show parallel responses to biodiversity with large scale aggregate community and ecosystem effects is likely to depend on the dominant mechanism(s) involved (Table 1, Jiang et al. 2009). Some stabilizing mechanisms, including increased asynchrony (which encompasses compensatory dynamics and insurance effects) and statistical averaging (also known as the portfolio effect), predict that species richness will stabilize community properties even though they may also tend to destabilize populations. These mechanisms are similar in that they require asynchrony between populations to increase with species richness however they differ with respect to whether enhanced asynchrony is due to biotic interactions or simple chance (Doak *et al.* 1998; Tilman *et al.* 1998; Yachi & Loreau 1999; DeWoody *et al.* 2003; Loreau & de Mazancourt 2008). Increased asynchrony resulting from compensatory dynamics is driven by species interactions such as competition for resources or altered predator-prey interactions in complex food webs (Leary & Petchey 2009) whereas increased asynchrony caused by the insurance effect is due to differential responses of species to environmental variability (Ives *et al.* 1999; Ives *et al.* 2000; Thébault & Loreau 2005; Downing *et al.* 2008; Gonzalez & Loreau 2009; Leary & Petchey 2009)(Table 1). A majority of studies find that increased asynchrony due to compensation or insurance effects tends to stabilize community level properties (Tilman *et al.* 1998; Tilman 1999; Lehman & Tilman 2000; Caldeira *et al.* 2005; Hector *et al.*

2010) except for a couple of studies where enhanced asynchrony destabilized populations to the point where asynchrony no longer stabilized communities (Gonzalez & Descamps-Julien 2004; Jiang *et al.* 2009).

Statistical averaging is another stabilizing mechanism that increases asynchrony between populations but in this case it is due to probabilistic processes rather than biotic interactions (Doak *et al.* 1998). Statistical averaging contributes to community stability if the slope (z) between log variance – log mean of the community property is >1. Theory further predicts that statistical averaging will tend to decrease population stability when z < 2 for individual populations, but increase population stability if z > 2 (Tilman 1999). Studies usually report a value of z between 1-2 and many studies have concluded that statistical averaging is at least one of several mechanisms contributing to community stability (Tilman 1999; 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 & Stevens 2010).

In contrast to mechanisms involving asynchrony, most other diversity-stability mechanisms predict that richness will stabilize communities while having either no effect or a stabilizing effect on populations (Table 1). Overyielding can lead to more stable communities if species' biomasses increase with richness faster than species' variability, but is neutral with respect to predictions on population level variability (Tilman 1999; Valone & Hoffman 2003a; Isbell *et al.* 2009; Hector *et al.* 2010; Li & Stevens 2010). Selection effects can stabilize populations and communities if an inherently stable species dominates species-rich communities (Steiner et al. 2005) but they can also destabilize populations and community attributes if the reverse is true (Polley *et al.* 2007). Facilitation among species may also stabilize populations and communities as richness increases through facilitating efficient use of resources (Vogt *et al.* 2006; Dovciak & Halpern 2010; Romanuk *et al.* 2010) or ameliorating the effects of disturbance (Mulder *et al.* 2001) which reduces large population fluctuations and in turn increase community stability. However, such facilitation may be largely context dependent as it has been shown to operate primarily in response to disturbance or enhanced environmental variability (Mulder *et al.* 2001), or at low enrichment levels (Romanuk *et al.* 2006), and can be sensitive to the degree of ecological realism of an experiment (Romanuk *et al.* 2009; Dovciak & Halpern 2010). Finally, an increase in the number of weak interactions/trophic complexity as richness increases in food webs is also predicted to stabilize populations and communities by reducing strong fluctuations of individual populations (McCann *et al.* 1998; Brose *et al.* 2006; Jiang *et al.* 2009; Jiang & Pu 2009).

The overall effects of biodiversity on the stability of communities and populations are also likely to depend on the factors that affect biodiversity itself. For example, selection effects depend on the

population attributes of dominant species and compensatory dynamics may depend on which species are maintained in a community. Additionally, enhanced local biodiversity due to connection to a broader metacommunity may have different consequences for stability (Howeth and Leibold 2010) than do other mechanisms for regulation biodiversity (e.g. extinctions of rare species, extinctions of dominant species, random extinctions etc.). It seems likely that explaining or predicting the overall effects of biodiversity on the stability of ecosystems and component populations is thus best thought of as a question of evaluating the relative strengths of these different mechanisms as they may contribute (either positively or negatively) to stability (Petchey et al. 2002). In this paper we illustrate how such a variety of forces act in such a way to affect the overall stability of semi-natural experimental pond ecosystems. We manipulated zooplankton biodiversity by manipulating the distribution of dominant vs subdominant species and by simulating immigration from a metacommunity and we examine their effects under either constant or pulsed nutrient inputs. We then quantified temporal stability as the coefficient of variation (CV) of zooplankton population biomass, community biomass (total phytoplankton and zooplankton), and ecosystem gross productivity to determine how zooplankton richness affects the temporal stability of populations, communities and ecosystems. We show that zooplankton richness stabilizes both populations and communities through multiple mechanisms operating simultaneously and interactively.

Material and Methods

Experimental design and set up:

Cattle tank pond ecosystems were established outdoors in April 2005 adjacent to the experimental pond facility at Kellogg Biological Station in Hickory Corners, MI. The polyethylene tanks held 300 L and contained about 16 L of silica sand as a bottom substrate and were fitted with a 1mm screen mesh lid to control immigration by larger organisms. Each tank was filled with well water to which nitrogen ($NaNO_3$) and phosphorus (NaH_2PO_4) was added to reach concentrations of 2250 µg N/ l and 150 µg of P/l which fall within the typical range of concentrations of natural fishless ponds surrounding Kellogg Biological Station (Downing & Leibold 2010). Larval bullfrogs were added to each tank in June to minimize the development of periphytic algae and to maintain nutrients in the water column.

To test the effects of species richness on population, community and ecosystem stability in constant versus variable ecosystems, we assembled communities with different zooplankton richness and crossed each community with either a constant or variable supply of nutrients. Previous

experiments have indicated that nutrients must be replaced at a rate of approximately 5% per day in order to maintain constant levels of nutrients in the water column. In the constant environment treatment nutrients were added via a continuous drip controlled by peristaltic pumps delivering low nutrient at a 5% per day loss rate. In the variable environment treatments we added a two week supply of nutrients via a pipette every two weeks which is more typical of nutrient pulses in natural ponds associated with rainfall events (Soranno *et al.* 1997). All mesocosms were maintained outdoors and were subject to identical natural environmental fluctuations such as those due to weather. Therefore, the pulsed nutrient additions represented the only form of environmental forcing that was unique among treatments.

In early April all tanks were inoculated with a diverse mixture of microbes and phytoplankton collected and pooled from approximately 10 local ponds. Phytoplankton and microbial communities were filtered through a 30 µm mesh to remove zooplankton and macroinvertebrates before being added to the tanks. The microbial and phytoplankton communities established for 10 days before we added the zooplankton richness treatments. Based on a previous experiment we identified three species, *Daphnia pulex* (Dap), *Scapholebris mucronata* (Sca), and *Ceriodaphnia* (Cer) as functionally important and dominant zooplankton species in local ponds and in tank experiments with potentially varying impacts on community stability (Downing *et al.* 2008). We used these 3 species as focal species in our experiment in addition to the larger regional zooplankton species pool to explore the role of species richness on population and community stability.

We created the following zooplankton richness gradient : 0 species (no zooplankton), 1 species (monocultures of the 3 focal species; Dap, Sca, Cer), 2 species (all 3 possible pairwise combinations of the focal species; Dap+Sca, Dap+Cer, Sca+Cer), N-1 (diverse communities minus each of the focal species; N-Dap, N-Sca, N-Cer), N (diverse communities), and N+ (hyper-diverse communities maintained with periodi c experimental immigration). Each unique zooplankton community was replicated 8 times with 4 replicates of each community maintained under either constant or variable environments for a total of 96 mesocosms. The monoculture treatments were established by generating cultures of single species from existing lab populations, experimental tanks and natural ponds. The diverse treatments (N-1, N, and N+) were all initiated with a diverse zooplankton inoculum pooled from natural assemblages of zooplankton sampled from approximately 10 local ponds. The diverse zooplankton assemblage was scanned under a dissecting scope to remove any unintended invertebrate predators. In the N-1 treatments each of the intended focal zooplankton species were also removed before the pooled inoculums were added to each tank. The N+ treatment was established with the diverse zooplankton

inoculum but also received small pooled inoculums of zooplankton every two weeks collected from approximately 10 local ponds. This process mimics natural dispersal that occurs within these pond metacommunities and greatly enhances diversity by allowing species to recolonize after local extinction in a mesocosm, and by allowing seasonal variation observed in natural ponds to occur in the tank ecosystems. Each of the 11 unique zooplankton compositions plus the no zooplankton control was crossed with the environmental variability treatment (fluctuating nutrient pulses vs constant nutrient drip), with 4 replicates per treatment for a total of 96 tanks.

Response variables:

Tanks were sampled for population (biomass of individual zooplankton species), community (total phytoplankton and zooplankton biomass) and ecosystem (productivity and respiration) properties 32 times at even intervals of 4-5 days beginning May 11 and continuing through October 3rd. Zooplankton and phytoplankton communities were sampled by pooling 16 750ml water column samples from each replicate with a tube sampler. A 300 ml sample was removed for phytoplankton analysis and the remainder was collected on an 80 μm mesh screen and preserved in sucrose Lugols solution for later microscopic enumeration in the lab. 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 OH, USA). Oxygen measurements were obtained from a 500 ml integrated water sample taken from each replicate in the morning, evening and subsequent morning at dawn and dusk. Net productivity is estimated as the change in O_2 concentration per hour between sunrise and sunset, and respiration is calculated as the net change in O_2 concentration between sunset and the following sunrise (Downing & Leibold 2002). We report results only for gross productivity which was calculated by correcting estimates of net hourly productivity during the day by addition of net respiration at night.

Data analysis:

A total of seven replicates were dropped from further data analysis due to excessive contamination. The remainder of the zooplankton manipulations were largely successful (no substantial

or sustained populations of unwanted species) and resulted in a strong zooplankton richness gradient as intended across treatments (Figure 1a, $F_{5,76}$ = 156.959, p < 0.001).

Zooplankton and phytoplankton biomass (inedible > 35 μm, edible < 30 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 most closely met the assumptions of ANOVA without transformation. The full ANOVA model included fixed effect terms for richness, environment, and richness x environment. They were conducted with and without the no zooplankton (0 species) control.

The various stabilizing mechanisms outlined in Table 1 make specific and testable predictions about the response of population and community properties to species richness and enhanced environmental variability. Our experimental design manipulating species richness and environmental variability combined with a high-resolution temporal data set allows us to test for the potential role of each of the mechanisms on zooplankton population and community stability as described below.

Asynchronous dynamics. Detecting asynchrony (and similarly negative covariance, compensatory dynamics and insurance effects) between populations can be 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 & de Mazancourt 2008; Gonzalez & Loreau 2009). 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 & de Mazancourt 2008). Here we use a measure of community-wide asynchrony introduced by Loreau & de Mazancourt (2008) that uses the statistic $\varphi = \frac{\sigma_{x\tau}^2}{(\sum_i \sigma_{xi})^2}$. This measure is independent of species richness and scales the observed variance of community biomass (numerator) over the maximum possible community biomass variance if all species were perfectly synchronized (denominator). 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 the slope (z) of the relationship between log (mean population biomass) vs log (mean population variance) the average z across the experiment as well as for each species. If z > 1 statistical averaging is predicted to stabilize communities. The effect of statistical averaging on population stability depends on the value of z such that when z < 2 for individual populations should become destabilized as richness increases (Cottingham *et al.* 2001; Petchey *et al.* 2002; Steiner *et al.* 2005; Li & Stevens 2010), and if z > 2 for

individual populations statistical averaging is expected to stabilize populations as richness increases (Tilman 1999).

Overyielding. In order for overyielding to stabilize zooplankton communities, we would expect both that zooplankton biomass increases with species richness and that biomass increases 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 & Stevens 2010).

Selection effect. If the selection effect is operating, stable species should dominate at high richness. To test for the selection effect we calculated the average CV's of the focal zooplankton species in monoculture (Cer, Dap, Sca) to determine if any of these species could therefore contribute to a selection effect. The focal species are also the most dominant across the experiment and therefore likely candidates for the selection effect (Figure 1b). To further explore the selection effect we compared the average CV of the all species nested within each richness level to determine if species with the lowest CV's were more abundant at high species richness.

Facilitation, weak interactions, and increased complexity of trophic interactions. These mechanisms all predict that community properties 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 is more important in variable environments by exploring if population variability is lower in variable as compared to constant environments as richness increases (Table 1).

Results

Richness and biomass

Our manipulations of species richness were largely successful with the exception of regular invasions by 1-2 species into the 0 richness treatment. (Figure 1a). In addition the N+ treatment which in which fortnightly additions of small amounts of zooplankton to simulate immigration from a metacommunity had substantially higher species richness than the N treatments without such immigration. Nevertheless these additional species never developed substantial biomass (Figure 1b). Our richness treatments also had substantial effects on biomass with a general increase with richness (Figure 1b). In all richness treatments, the dominant species were those we manipulated individually (D. pulex, Scapholeberis mucronata, and Ceriodaphnia ??,). The individual absence of these three dominant

species (in the N-1 treatments) allowed a number of additional species to invade even though they too remained at low biomass.

Richness and environmental fluctuations effects on stability

Stability as measured by the CV of variables also increased with zooplankton richness for both average individual zooplankton population biomass ($F_{4,71}$ = 4.596 p=0.002) and aggregate zooplankton community biomass ($F_{4,71}$ = 2.900, p = 0.028, Figure 2 a,b). The stability of phytoplankton community biomass declined with zooplankton richness (Figure 2c, $F_{5,76}$ = 4.018, p= 0.003) although this effect is not statistically significant if the no zooplankton treatment is removed from the analysis ($F_{4,71}$ =1.757, p= 0.147). The stability of gross ecosystem productivity did not vary with richness or environmental variability (Figure 2d, p>0.05). Stability at all levels is not influenced by environmental variability (ANOVA p > 0.05 for environment, and richness x environment effects).

Mechanisms

Community asynchrony quantified using the method of Loreau and DeMazancourt (2008) between zooplankton populations increased with zooplankton richness (Figure 3a, ANOVA, richness effect $F_{3,52}$=8.68, p=0.0001). We found no significant effect of environmental fluctuations ($F_{1,52}$= 0.12, p=0.734) on asynchrony. We also did not observe a significant environment x richness interaction ($F_{3,52}$= 2.55, p=0.066), although the trend is that richness increases asynchrony to a greater degree in variable environments (Figure 3a).

Our test for statistical averaging revealed that the slope z = 1.949 ± 0.013 (SE) averaged across all zooplankton species biomass (Figure 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 and exactly half of the 16 species had z-values < 2 and the other half had z > 2 (Appendix 1). Of the 8 species predicted to be destabilized with richness (z < 2), only 2 species actually showed this pattern (Appendix 1). The strength of statistical averaging does not change with environmental variability as the z-values are similar between environments (Fluctuating environments: z = 1.937 ± 0.019 (SE), Least squares regression $F_{1,638}$= 10,048, p < 0.001, R^2 = 0.940; Constant environments: z = 1.962 ± 0.017 (SE), Least squares regression $F_{1,638}$= 13,725, p < 0.001, R^2 = 0.955). Another prediction of statistical averaging is that variances across all species should decline with richness (Vogt et al. 2010, Steiner 2005) but our data do not show this trend (ANOVA on variances $F_{4,71}$ = 0.3760, p= 0.825).

To test for the overyielding mechanism we found that zooplankton biomass increased with richness (Figure 1b, ANOVA F$_{4,71}$ = 2.759, p = 0.034). However, in order for overyielding to stabilize zooplankton community biomass, zooplankton community biomass must also increase faster than the standard deviation. We found the overall slope of the regression for mean biomass versus mean standard deviation to be not significantly different from 1 (slope = 0.95, 95% CI = 0.75-1.15) (Figure 3c, linear regression F$_{1,79}$ = 89.89, p < 0.001, R^2 =0.532) indicating that biomass increases at approximately the same rate as the standard deviation. The slope of regression for the 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 the 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 that the CV's of the focal species in monoculture (Cer, Dap, Sca) are indistinguishable from each other (Figure 3d, ANOVA, F$_{2,42}$ = 0.626, p = 0.540) indicating no differences in the stability between the dominant species (Figure 1b). To further explore possible contributions of other species to a selection effect we calculated the CV's of all zooplankton species observed in the N-1, N and N+ treatments (Appendix 1). Most of these species are relatively minor contributors to zooplankton community biomass and are not likely candidates for a selection effect (Figure 1b, Appendix 1). The N+ treatment is unique from other richness treatments in that it has a much larger proportion of minor cladocerans (Figure 1b). We explored the possibility that the minor cladocerans as a group contributed to a selection effect in the N+ treatment by determining if CV of this group was lower compared to the other species in the N+ treatment. The minor cladoceran group has the second highest CV of all species, and 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 1).

To test for a potential role of facilitation and weak interactions we observed that population variability decreased on average across all species with richness (Figure 2a). The population variability (CV) of each of the 3 focal species also showed a trend towards declining CV's with richness (Figure 3d), however these trends were not significant for the individual focal species (ANOVA's for each species richness F$_{4,54}$, p> 0.05 for all species; environment effect F$_{1,54}$, p > 0.05 for all species; and richness * environment F$_{4,54}$, 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 1). Finally, we regressed average zooplankton population CV vs zooplankton total

community biomass CV and found that as average population CV increases, total biomass CV's also increase (Figure 3e, linear regression $F_{1,79} = 21.014$, $p < 0.001$, $R^2 = 0.210$).

Discussion:

Overall, we found that zoo;lankton richness stabilized both average zooplankton population and aggregate zooplankton community biomass. Community phytoplankton biomass was less stable as zooplankton richness increased and the stability of gross ecosystem productivity did not vary with zooplankton richness. Increased environmental variability in the form of nutrient pulses did not influence stability at the population, community, or ecosystem level. Therefore, a prediction that stability of some variables (zooplankton population and zooplankton community) might lead to stabilization at other levels (phytoplankton community biomass and ecosystem productivity) was not supported (Jiang & Pu 2009; Proulx et al. 2010). We found that the presence of zoo;lankton (regardless of rishness) rather than the richness of zooplankton was the major driver of plant-herbivore oscillations because the zooplankton richness effect on phytoplankton stability disappeared when we dropped the no zooplankton treatment. Consequently, we also infer that the stability of ecosystem productivity, which is driven primarily by phytoplankton in these systems (Downing & Leibold 2002), showed no change with zooplankton richness.

The increased stability of total zooplankton biomass can be explained by at least 3 different mechanisms that operate simultaneously: statistical averaging, increased asynchrony, and increased number of weak interactions/ increased complexity of trophic 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 required to invoke statistical averaging. This result lends further support to studies that have shown z values typically range between 1 and 2 and suggests that statistical averaging is a consistent mechanism that increases stability across many different ecosystems (Tilman et al. 1998; Tilman 1999; Cottingham et al. 2001; Tilman et al. 2006). The predicted effects of statistical averaging on population stability depend on the value of z, such that if z < 2 populations should be destabilized and if z > 2 populations will be stabilized. Our finding of an average z value of 1.94, and the observation that half of the species had z>2 and half had z<2 indicates that statistical averaging does not have a consistent effect on zooplankton population level stability (Appendix 1). Additionally, 6 of the 8 species predicted to be destabilized as richness increases based on their z-value < 2 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 & Hoffman 2003b). Notably, in our experiment zooplankton species were highly unequal with respect to their biomass and zooplankton richness increased primarily by the addition of rare species (Figure 1b) which makes it difficult to assess the strength of the statistical averaging effect.

Increased asynchrony between zooplankton populations also contributes to increased community stability with richness but this effect is only really apparent in contrasting open (N+) versus closed (other richness treatments). Interestingly, we also observe a non-significant trend towards richness enhancing stability more in variable environments as compared to constant environments as is predicted by the insurance effect (Table 1). Another prediction of the asynchrony mechanism 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 that has also been observed in other systems (Valone & Hoffman 2003b; Proulx et al. 2010). This suggests that mechanisms other than asynchrony are likely operating that also increase stability at the population level and potentially overriding any decrease in population stability due to asynchrony.

The pattern of reduced population variability across most zooplankton species at high richness is consistent with stabilizing mechanisms that include 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 & Pu 2009). Facilitation cannot be ruled out, however it 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 candidates for direct facilitation among our zooplankton species. An increase in the number of weak interactions is a more likely mechanism that is consistent with our observation that zooplankton populations in general 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 species which contribute little to overall zooplankton community biomass and are therefore more likely to generate weaker interactions with other members of the community (Figure 1b). For instance, increasing zooplankton richness may increase the number of weak consumer-resource interactions which can help dampen strong consumer-resource interactions (McCann et al. 1998; Jiang et al. 2009; Jiang & Pu 2009). An increase in the abundance of inedible or resistant algae may also help stabilize large population oscillations as zooplankton richness increases (McCauley et al. 1999). We found that inedible algae increased with zooplankton richness which also supports a role for weak interactions (ANOVA, richness effect, $F_{4,57} = 2.72$, $p = 0.036$).

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We found no evidence for a stabilizing selection effect 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 observed in both constant and variable environments for enhanced stability in species-rich communities.

The various diversity-stability mechanisms 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 did not find support for selection effects and only weak support for overyielding in variable environments. The data do support a role for asynchrony, statistical averaging, and weak trophic interactions that operate under both constant and fluctuating environments. Of these mechanisms, weak interactions is the only one that also predicts increased population stability with richness whereas the other mechanisms predict neutral or negative effects on population stability. Therefore the increase in weak trophic interactions appears to be an important mechanism that may even counteract the potentially negative effects of the other mechanisms on population stability.

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) where we found increased stability in variable environments due to an increase in asynchronous dynamics. The data we present here from shows a similar but non-significant trend as our previous work in that asynchronous dynamics appear to be influenced most strongly by richness in variable environments. 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 & Descamps-Julien 2004; Thébault & Loreau 2005; Romanuk *et al.* 2009). In our experiment it is possible that the environmental forcing imposed experimentally through nutrient pulses could have been partially masked or counteracted by environmental variability that affected the entire experiment through weather fronts, precipitation, temperature changes, etc. We believe environmental variability is likely important for understanding diversity-stability relationships and should remain a focus of additional experimental work.

In conclusion, we found that both population and community stability increased with species richness in constant and variable environments due to statistical averaging, an increase in asynchronous dynamics between populations and an increase in the number of weak interactions. We argue that other ecosystems are also likely to have multiple mechanisms operating simultaneously and that the overall patterns of stability at both the population and community level will require an understanding of the relative importance of different mechanisms and their possible interactions. In addition, our results show that rare species are important for stabilizing population and communities because species richness increased in our system due primarily to the addition of species with relatively low abundances. Finally, we suggest that different stabilizing mechanisms might dominate in different ecosystems (e.g. single versus multi-trophic, aquatic versus terrestrial) which might help explain observations that the effects of richness on population and community stability differ across experimental/ecosystem groupings (Jiang & Pu 2009).

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Comment [5]: I think we also need to break out the three different composition treatments for the 2 and N-1 richness in our figures. We can put them in contact with each other to emphasize that they refer to the same treatment though.

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Comment [6]: Do we want to emphasize the effects of openness as a source of diversity?

TABLE 1: Mechanisms proposed for diversity- community stability

MECHANISM FOR STABILIZING COMMUNITY PROPERTY	TEST FOR PRESENCE OF MECHANISM	PREDICTED EFFECT OF ADDED ENVIRON- MENTAL VARIABILITY	PREDICTED EFFECTS ON POPULATION STABILITY
Increased asynchrony (compensation, insurance effect) (Frost *et al.* 1995; Tilman *et al.* 1998; Yachi & Loreau 1999; Gonzalez & Loreau 2009)	Community asynchrony $$\varphi = \frac{\sigma_{x\tau}^2}{(\sum_i \sigma_{xi})^2};$$ 0 = complete asynchrony; 1 = complete synchrony (Loreau & 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 $z < 2$, stabilizing if $z > 2$ (Tilman 1999)
Overyielding (Hector *et al.* 2010)	Species biomass increases with richness at a faster rate than standard deviation (SD) of species biomass	Neutral	Neutral (Tilman 1999)
Selection effect (Steiner *et al.* 2005)	Stable species must dominate at high richness	Neutral	Stabilizing on average due to dominance by one or a few stable species
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 & Pu 2009)	No simple test	Neutral	Stabilizing generally across many species

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Figure 1. Treatment effects on a) zooplankton richness. Richness treatments; 1 = monocultures , 2 = 2 species combinations, N-1 = diverse communities minus a focal species, N = diverse, N+ = diverse + immigration, b) relative zooplankton abundance (µg/l) averaged across all compositions within each richness treatment. Zooplankton abbreviations; Cyc = cyclopoids, cop = copepodites, nau = nauplii, cer = *Ceriodaphnia*, sca = *Scapholebris*, dapp = *Daphnia pulex*, dapl = *Daphna laevis*, chy = *Chydorus*, bos = *Bosmina*, alo = *Alona*, cal = Calanoids, Minor cladocerans = *Simocephalus, Diaphanosoma, Macrocystic, Polyphemus,* and *Pleuroxus.*

Figure 2. Treatment effects on population, community, and ecosystem stability. Black bars = constant environments, grey bars = variable environments. 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 (chl a) biomass, and d) ecosystem stability calculated as the CV of gross ecosystem productivity.

Figure 3: Evidence for stabilizing mechanisms. a) Community asynchrony with respect to zooplankton richness; black bars = constant environments, grey bars = variable environments b) Mean-variance scaling relationship as a test for statistical averaging; closed circles and solid line = constant environments, open circles and dashed line = variable environments c) summed variances versus richness as a test for statistical averaging, ; closed circles and solid line = constant environment, open circles and dashed line = variable environments d) zooplankton biomass versus zooplankton standard deviation as a measure of overyielding, e) zooplankton population variability(CV) versus zooplankton community variability (CV) as a test for reduced population variability mechanism; closed circles and solid line = constant environments, open circles and dashed line = variable environments
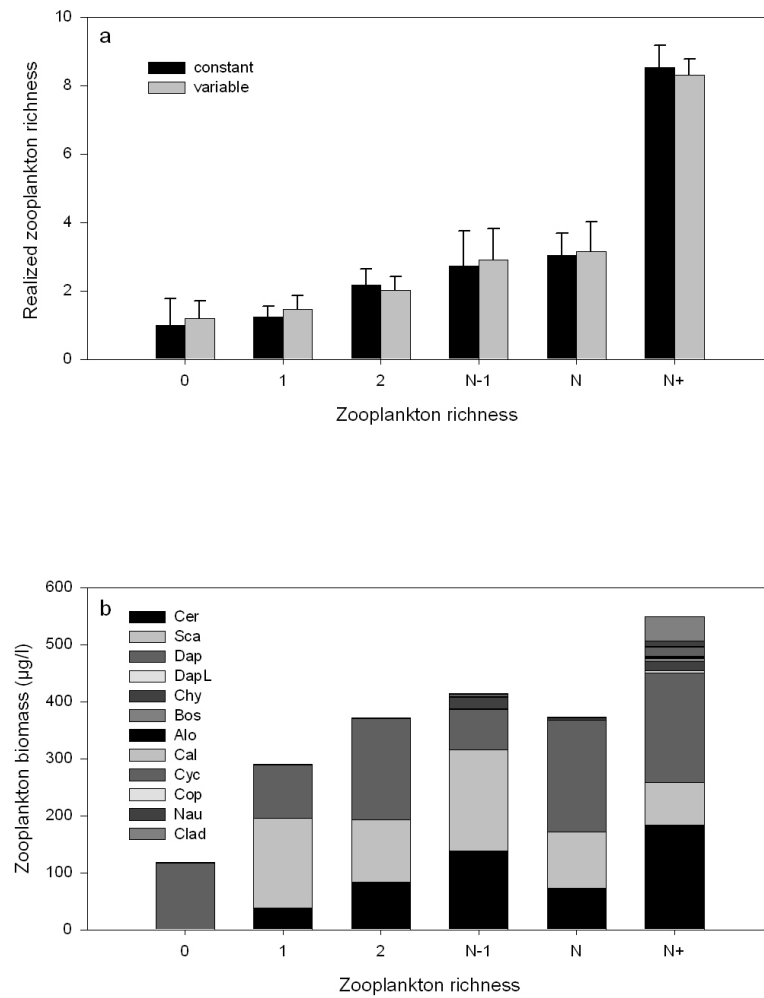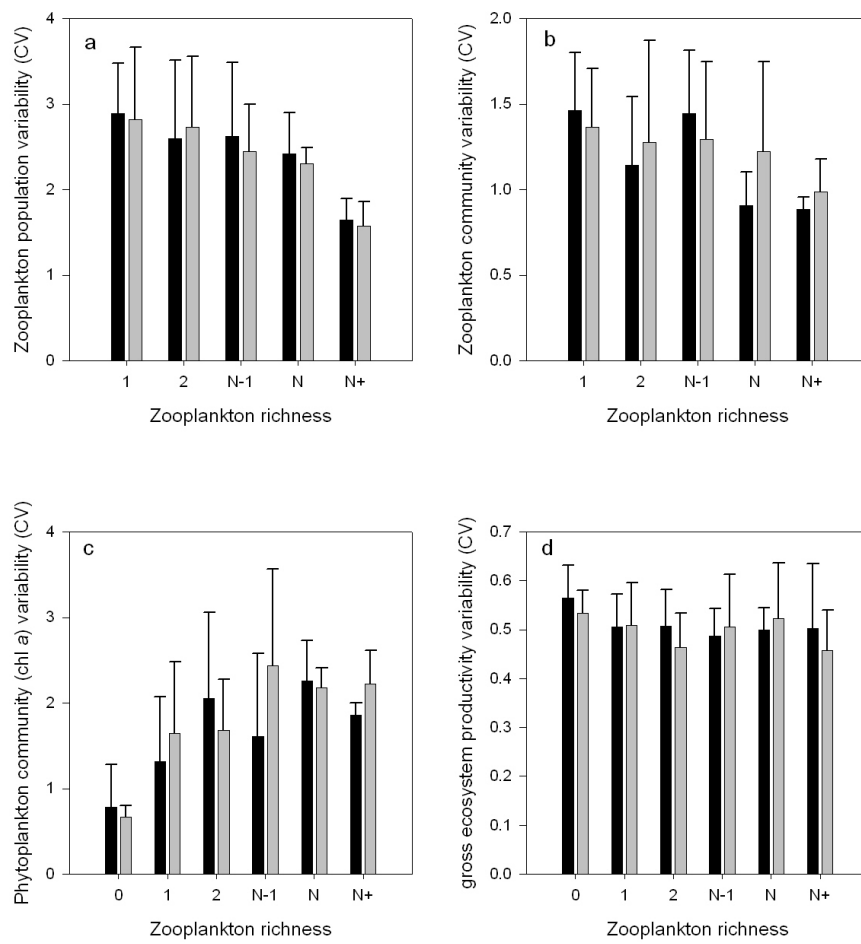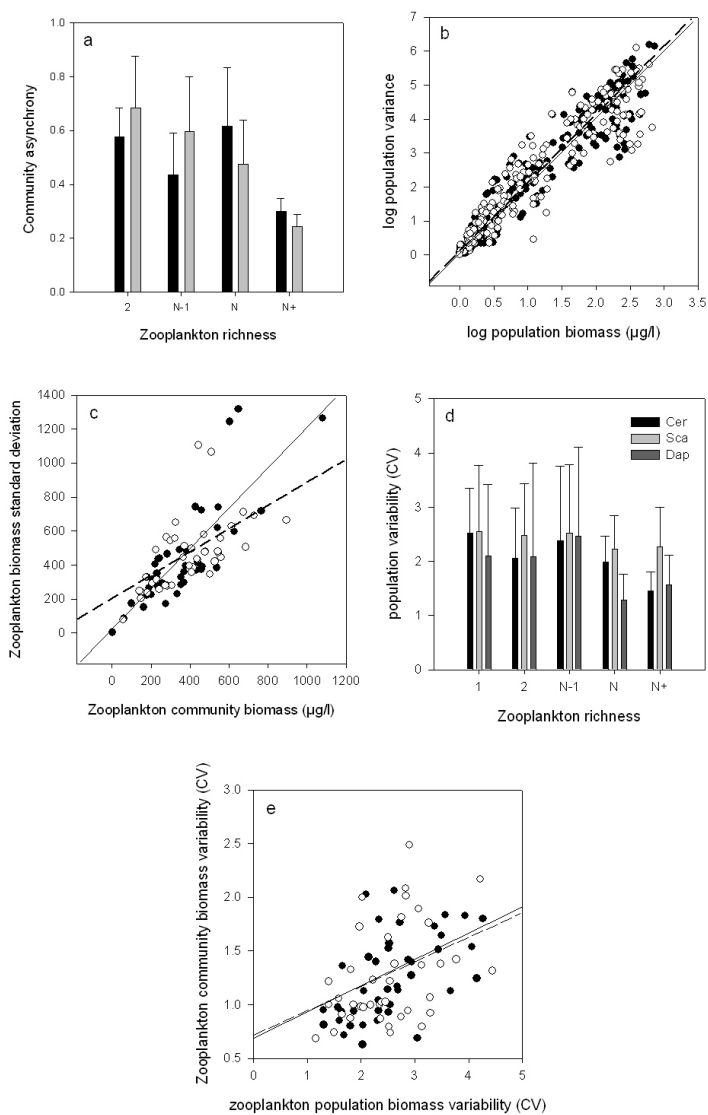
Figure 1

Figure 2

Figure 3

Appendix 1: Species-specific parameters for slope (z) of log (population biomass) vs log (population variance), rank abundance in N-1, N and N+ treatments for zooplankton species. 1 = most abundant. * = Minor cladocerans which are lumped together for some analyses.

Species	z	Rank abundance (µg/l)			population CV		
		N-1	N	N+	N-1	N	N+
Scapholebris	1.928	1	2	3	2.53	2.23	2.28
Ceriodaphnia	1.860	2	3	1	2.38	1.99	1.46*
Daphnia pulex	1.765	3	1	2	2.47	1.28	1.57
Chydorus	1.923	4	4	5	1.95	1.97	1.65*
Cyclopoids	1.790	5	5	6	2.83	2.77	1.29*
Nauplii	2.105	6	6	7	2.99	2.33	1.76*
Daphnia laevis	2.695	7	11	11	5.08	---	4.05*
Alona	1.953	12	7	12	3.11	4.22	2.32*
Copepods	2.250	8	8	13	2.98	2.95	1.95*
Bosmina	2.362	10	10	10	3.54	5.66	1.74*
Calanoids	2.386	9	11	14	2.87	---	2.77*
*Diaphanosoma**	1.990	11	11	8	2.40	---	1.95*
*Simocephalus**	2.117	14	11	4	4.26	---	1.79*
*Pleuroxus**	2.791	15	9	15	---	5.66	3.00*
*Macrocystis**	1.687	15	11	16	---	---	3.57
*Polyphemus**	2.379	13	11	9	4.63	---	2.46*
Minor Cladocerans	---	-	-	-	3.42	5.66	1.44*