Searching for Stability

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Understanding how multiple competing species can inhabit the same natural communities has been one of the most enduring challenges of ecology. Barring specific stabilizing mechanisms, diversity should erode over time as fitness differences lead to rapid competitive exclusion, although species with similar fitness might co-occur for longer periods during a random walk to extinction. Empirical studies addressing coexistence have lagged theoretical work, and this dissertation aims to address this knowledge gap. I focus on predation and some of the ways in which predation may contribute to stability, moving from laboratory microcosms to field enclosures, and from temporary ponds to the rocky intertidal. In chapter one, I show that larval salamanders tend to consume the most frequent prey in microcosms, consistent with frequency-dependent predation being a stabilizing force. However, some prey are always highly preferred, and would experience no refuge in rarity. In chapter two, I find that the overall effect of salamander predation on a zooplankton assemblage is to decrease diversity in pond enclosures, possibly due to small plankton population sizes in the enclosures. And in chapter three, I show that the rocky intertidal community can be quite robust to perturbation, with the predominant members each able to reinvade following experimental removals in the field, whereas the effects of predator exclusion on regrowth are minimal. Here, the provisioning of space through disturbance and the subsequent dynamics of recolonization are the likely drivers of coexistence. The evidence I accumulate shows that predation can have a strong influence on some communities, but it is not a singular and general driver of stable coexistence in multispecies communities.
Searching for Stability

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Searching for Stability

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Table of Contents

Introduction
Page 1

Chapter One: Larval salamanders exhibit frequency-dependence in their choice of zooplankton prey
Page 9

Chapter Two: Negative direct effects of salamander predation overwhelm positive indirect effects on diversity of a zooplankton assemblage
Page 55

Chapter Three: Evaluating reinvansion success as an indicator of coexistence in the rocky intertidal
Page 84
Introduction

Ecologists have known for a long time that multiple competing species should not be able to coexist indefinitely, because sooner or later the dominant competitor should exclude the rest (Grinnell 1904; Gause 1932). The niche differentiation paradigm that arose at the beginning of the last century (Grinnell 1917) and was refined several times over the following decades (Elton 1927; Hutchinson 1957) held that each species would have to carve out its own domain in space or season or resource specialization in order to persist (MacArthur 1958; Hutchinson 1959; Hardin 1960; Connell 1961; Schoener 1968).

To explain diversity patterns in hyper-diverse ecosystems like rainforests and coral reefs, Hubbell proposed in his neutral theory that species might be demographically identical and interchangeable, such that species’ relative abundances followed a zero-sum drift process (Hubbell 1997, 2001). By extension, ecological drift would cause species diversity to erode over time, just like evolutionary drift can reduce genetic diversity. Neutral theory was criticized for relying on invalid assumptions (Chave 2004), and for a poor match of its predictions to observed patterns (Dornelas et al. 2006; McGill et al. 2006; Ricklefs & Renner 2012) and experimental outcomes (Wootton 2005).

Peter Chesson’s (2000, 2003) formalization of modern coexistence theory has greatly helped to further ecologists’ understanding of diversity maintenance. The theory is based on invasion analysis: a coexisting community is one in which every member is able to recover from rarity (the “invader” state) when the other species (the “residents”) are at their stationary states (Chesson 2000; Barabás et al. 2018). Modern coexistence theory helps clarify how various processes and mechanisms can contribute to diversity in nature. One mode of interpretation of
the theory distinguishes between stabilizing and equalizing effects (Chesson 2000). Stabilizing effects allow species to have positive invader growth rates despite average fitness differences, meaning they tend to recover from rarity instead of becoming extirpated. Equalizing effects are those which reduce average fitness differences between species, decreasing the threshold for stabilizing effects to preserve diversity. In this way, the framework conveniently reconciles niche differentiation with neutrality: neutrality is the special case where all species have the same fitness (Adler et al. 2007).

Several classes of stabilizing effects are possible under modern coexistence theory. These include fluctuation-independent mechanisms, relative nonlinearities, storage effects, and growth-density covariances (Chesson 2000; Barabás et al. 2018). Fluctuation-independent mechanisms are independent of environmental variability, and they promote stable coexistence by ensuring that, just by becoming more frequent, a species experiences a disadvantage. The remaining mechanisms depend on environmental fluctuations.

The fluctuation-independent (a.k.a. equilibrium) effects consist of the classic resource partitioning processes and frequency-dependent predation. Resource partitioning includes niche differentiation, extending past environmental variables to encompass resources such as food, mutualistic partners, and even predator-free space. Frequency-dependent predation results in greater predator-induced mortality on any prey species that becomes especially abundant, and it can arise out of many specialized enemies (Janzen 1970; Connell 1971), or out of a single switching generalist predator (Roughgarden & Feldman 1975; Gleeson & Wilson 1986; Abrams & Matsuda 1996; Křivan 2003). Growth-density covariances might also be considered independent of environmental fluctuations, although they do require spatial heterogeneity across
a landscape. These arise if the invader tends to be concentrated in areas where it can grow faster (Barabás et al. 2018).

The fluctuation-dependent mechanisms – relative nonlinearity of competition and spatial or temporal storage effects – are more theoretically complex, but both classes of effects arise out of differential responses by species to environmental variability (Chesson & Huntly 1997; Chesson 2000, 2003). Relative nonlinearities occur when species have different nonlinear responses of growth rate to the availability of some common limiting factor, so environmental fluctuations are associated with fluctuations in competitive outcomes (Armstrong & Mcgehee 1980; Chesson 1994). A short-lived weedy species is more harmed by a year of poor recruitment than is a long-lived species with which it competes for space, for example. And storage effects occur when species have different responses to environmental conditions (e.g., different thermal optima for growth), there is covariance between environment and competition (concentrating intraspecific competition relative to interspecific competition), and there is buffered population growth (e.g. through a seed bank) (Chesson 1994, 2000).

The species making up any given community could therefore be experiencing a mixture of stabilizing mechanisms, each of which could interact synergistically or antagonistically (Chesson & Kuang 2010; Kuang & Chesson 2010; Stump & Chesson 2017). Likewise, any single mechanism could have both stabilizing and destabilizing effects (Chesson & Kuang 2010; Stump & Chesson 2017) or a stabilizing component and a competitive advantage-equalizing component (Stump & Chesson 2017). However, if stabilization is not strong enough to overcome competitive differences, then diversity will decay.

The chapters of this dissertation are each a step towards evaluating the empirical support for stabilizing or destabilizing forces that act in nature. In particular, I focus on frequency-dependent
predation. Frequency dependence is an attractive concept to evaluate because it can act independently of environmental conditions. Moreover, predation in general is widespread, and can have strong impacts on community structure and dynamics (Hairston et al. 1960; Paine 1966; Smith & Quin 1996).

Therefore the two themes I follow are stability and how it might be influenced by predation. Wherever possible, I use a multispecies approach because results from two-species systems do not generalize automatically to more diverse settings (Barabás et al. 2016; Levine et al. 2017). In Chapter 2, I investigate whether larval salamanders have a preference for frequent prey when encountering multiple types of zooplankton. In Chapter 3, I evaluate the potential for predation by larval salamanders to stabilize the diversity of a zooplankton assemblage when there is sufficient time for zooplankton to reproduce. And in Chapter 4, I test the ability of dominant members of a rocky intertidal community to each recover following targeted removals, providing a test of the invasibility criterion for coexistence. I pursue this work across three scales of inquiry and two different ecosystems, from lab microcosms to small in-pond enclosures to an open field experiment on the Atlantic shore. By using theory to guide the design and analysis of the experiments, I gain insight into the nuanced ways that one-on-one and one-on-many interactions between species can shape the community-level property of stability.

References


Chapter One:

Larval salamanders exhibit frequency-dependence in their choice of zooplankton prey

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Abstract

Species diversity can erode without a specific mechanism driving coexistence. Because species assemblages in nature often include multiple species with apparently overlapping niches, ecologists seek to identify the mechanisms that maintain diversity. Positive frequency-dependent prey switching is one such mechanism: by disadvantaging common prey species relative to rare species, predators might be able to stabilize prey species coexistence. We conducted lab-based trials to test for prey switching by a generalist apex predator in temporary ponds, the larval marbled salamander (*Ambystoma opacum*). In the experiment, we offered zooplankton prey to the salamanders, varying the frequencies of zooplankton taxa while keeping total prey abundance constant. This design allowed us to isolate the effect of prey frequencies on the salamanders’ preferences for those prey. We found that the frequency of each prey taxon altered the predator’s preference for, and feeding intensity on, that prey. Cladocerans and copepods were more preferred when frequent than when rare. In contrast, ostracods were most preferred by salamanders when rare, and salamanders became satiated even at moderate ostracod abundances. These results indicate that larval salamanders have distinct frequency-dependent responses to each prey taxon. The findings should be interpreted cautiously because of the limited habitat and community complexity in our trials. However, this study provides clear experimental evidence that a predator’s behavior can depend on the frequencies of multiple prey. In turn, this study helps resolve how predators might influence a diverse assemblage of species, potentially...
stabilizing some populations and destabilizing others through a functional response to prey frequency.

**Introduction**

Extensive theoretical and empirical work on multispecies coexistence (Hutchinson 1961; Chesson 2000; Adler *et al.* 2007; Levine *et al.* 2017) suggests that when niches overlap, diversity should erode as fitness differences lead to competitive exclusion (Connell 1961; Hutchinson 1961; Vellend 2016) or species gradually drift to extinction over time (Hubbell 2001; Vellend 2016). Mechanisms that reduce average fitness differences between species can delay, but not prevent, exclusion (Chesson 2000). Therefore, the high level of diversity we observe in many systems suggests the operation of stabilizing mechanisms. For two competing species, stabilizing mechanisms result in negative intraspecific effects of high population density exceeding negative interspecific effects (Adler *et al.* 2007), ultimately causing each species to limit itself more than it limits the other. Theorized stabilizing mechanisms include resource partitioning, frequency-dependent predation, the storage effect, and relative nonlinearity of competition (Chesson 2000). However, it is unclear how well coexistence theory developed largely on pairwise interactions can be generalized to multispecies assemblages (Barabás *et al.* 2016; Levine *et al.* 2017).

Dynamics that emerge between three or more species can overturn the two-species rules (Barabás *et al.* 2016), so the scarcity of empirical evidence currently limits our understanding of coexistence (Levine *et al.* 2017).

Frequency-dependent predation, one of the drivers of stable coexistence, is potentially general and widespread. For reasons including search image formation (Dawkins 1971), change in hunting mode (Lawton *et al.* 1974; Akre & Johnson 1979), and spatial segregation of prey
(Murdoch 1977), frequency-dependent predators consume multiple prey types and
disproportionately consume more common ones (Murdoch 1969). This behavior effectively
buffers rare species against extirpation (Abrams & Matsuda 1996; Chesson 2000), and can
potentially regulate a whole suite of prey species. The relationship between a single predator’s
rate of prey capture and prey density is called a functional response (Holling 1959; Denny 2014),
and positive frequency-dependence yields upward curvature in the functional response from low
to moderate prey density. Capture rate saturates at high prey density, yielding a sigmoid
relationship overall. In contrast, non-frequency-dependent generalist predators could drive
apparent competition (Holt 1977), and plausibly cause extinctions within communities, too
(Smith & Quin 1996; Roemer et al. 2001). Non-frequency-dependent generalist predation yields
a functional response curve that increases linearly, although saturation still occurs at high prey
density.

Historically, tests of frequency-dependent predation, or prey switching, have featured two
alternative prey (but see Smout and Lindstrøm 2007; Smout et al. 2010; Horst and Venable
2018). Experimentation has revealed prey switching behavior by consumers such as marine
snails (Murdoch 1969), larval damselflies (Akre & Johnson 1979), notonectid backswimmers
(Chesson 1989), and flounder (Mattila & Bonsdorff 1998). However, it is not clear how these
results from two-prey experiments generalize to more diverse prey assemblages. A predator that
switches between two prey types might never switch from a third, when given the option.
Furthermore, interactions between two species can be influenced both quantitatively and
qualitatively by the addition of a third species via indirect effects (Werner & Peacor 2003;
Levine et al. 2017), and the same can hold for additional species. Despite the apparent wealth of
studies of prey switching in simple systems, our understanding of predator choice with realistic prey diversity remains limited overall.

We designed the present study to test for frequency dependence in the presence of multiple prey types. We manipulated the relative proportions of three classes of aquatic zooplankton, spanning four predominant orders found in temporary ponds: Cladocera, Cyclopoida and Harpacticoida copepods, and Podocopida ostracods. This manipulation allowed us to evaluate the resulting feeding behavior of larval marbled salamanders (*Ambystoma opacum*), which are the typical apex predators in New England temporary ponds (Urban 2013). We predicted that the salamanders would behave as frequency-dependent generalist predators, such that they would incorporate disproportionately more of a given zooplankton type in their diet when it became more frequent, and consume disproportionately fewer when they were rare. We measured both the functional response of the predatory larval salamanders and their preference values (Chesson 1983) for each prey type to assess frequency-dependence. We expected to see an increase in *per capita* mortality with increasing frequency and an increase in preference as calculated by Chesson’s index. Consequently, we expected the functional response of the predator to increasing frequency of each prey to follow the Type 3 (sigmoid) form.

**Materials and Methods**

*Study Site, Focal Organisms, and Raising Animals*

Larval marbled salamanders are apex generalist predators found in temporary ponds throughout the eastern United States (Petranka 1998). Hatching out of eggs as soon as the ponds fill in late fall and early winter, they spend the winter feeding on zooplankton, predominantly cladocerans,
copepods, and ostracods (Petranka 1998). The larval salamanders are gape limited predators (Urban 2007), and as they grow larger, they start to incorporate larger prey into their diets, including macroinvertebrates and eventually other hatchling amphibians (Petranka & Petranka 1980; Petranka 1998; Wells 2007). However, even as the maximum size of prey increases, larval marbled salamanders continue to consume large numbers of zooplankton prey (Petranka 1998).

In May of 2014, we collected marbled salamander larvae and their zooplankton prey from Totoket Mountain in Northford, CT (41°23.6’ N, 72°46.2’ W) as a representative component of a temporary pond food web. Totoket, an isolated forested ridge approximately 20 km to the northwest of New Haven, CT, is in the northern part of the marbled salamander’s range (Petranka 1998). It features a network of perched temporary pond basins, and the land is closed to development and to most public access.

We raised the salamanders in 20 L buckets stocked with one individual per bucket and kept at ambient temperature under a 50% shade cloth in a fenced outdoor facility. Approximately every three days, we fed the salamanders with aliquots of natal pond water containing plankton concentrated by a tow-net. We supplemented this diet with larval spotted salamanders (A. maculatum) and gray tree frog (Hyla versicolor) tadpoles.

To obtain the composition of a real pond community for the feeding trials, we sampled zooplankton in the spring of 2013 and concurrently with salamander collection in 2014. We used a 10.2 cm diameter pipe sampler to capture a core of the water column from surface to benthos. We collected samples from the deepest point of the pond, as well as halfway between that location and the pond’s edge along each of the four cardinal directions. We pooled all five samples by pouring them through a 70 μm screen filter, and rinsing off and preserving the filtrate in 70% ethanol. We subsequently identified all zooplankton to genus (Thorp & Covich 2001),
except for harpacticoid copepods, which we did not identify beyond order. These zooplankton samples served as the basis for our feeding trial treatment compositions.

Experimental Procedure

We examined the feeding response of salamanders to different frequencies of three key zooplankton prey taxa. During June 2014, we staged feeding trials in 3.81L glass containers, filled with 1.5 L of water to approximate a shallow pond water column. We stocked each trial container with 450 individual zooplankton representative of predominant pond taxa (Table 1). We kept initial total density constant, and varied only the relative frequencies of the three specified prey types, in case salamander predation behavior varies with total prey density. The Pond Average treatment had similar composition and density to the average community composition observed in May 2013 and May 2014. The other treatments consisted of an approximately six-fold reduction in one taxon, with an equivalent increase allocated to the remaining taxa in proportion to their average abundances. We also experimental controls with every set of feeding trials, stocked similarly to the regular trials but without adding a predator. Controls were designed to detect changes in zooplankton numbers not due to predation, such as reproduction or mortality unrelated to salamander predation.

One experimental round consisted of a set of all four prey treatments, plus one control treatment. For three of five rounds, the experimental controls used the same composition as the “Pond Average” treatment. However, we altered the experimental controls over the course of the study to have enough zooplankton for the experimental treatments (Table 1). We used the control
treatments to estimate zooplankton losses due to factors other than salamander predation, such as equipment effects (becoming entrapped on a filter) or intraguild predation.

Before each trial, we transferred four randomly selected *A. opacum* individuals into containers without food. The salamanders underwent a 24-hour acclimation period without food in an incubator set for 13.2 degrees C and 12 hours of daylight, the average pond conditions in the middle of spring (Urban 2013). We counted live prey under a stereo microscope (Leica M125, Leica Microsystems, Germany) and photographed cladocerans and small ostracods (Leica DFC420 camera mounted on microscope). We added zooplankton to the trial containers and immediately added one *A. opacum* to each container. We then ran the feeding trial for 24 hours in the incubator. After each trial, we filtered container contents with a coarse mesh (1.6 mm) to extract the salamanders and then with a fine mesh (150 µm) to extract zooplankton. We immediately rinsed zooplankton with 70% ethanol and preserved them in vials. We weighed the salamanders (wet weight), euthanized them using a solution of MS-222, and preserved them in 70% ethanol. We counted preserved zooplankton under the stereo microscope, taking additional post-trial photographs of remaining cladocerans and ostracods. We used the photographs to adjust the post-trial leftover counts of *Scapholeberis* to account for reproduction that had occurred over the course of the trials (Supplement, *Image Analysis with ImageJ*).

**Measuring Preference**

We used Chesson’s $\alpha$, and its transformation $\epsilon$, to measure preference (Chesson 1983). This index compares diet composition against background levels of prey, allowing explicit comparisons of preference across different compositions of available prey. A shift in the index
indicates a behavioral shift towards consumption of a greater or lesser proportion of a given prey type. In the case where feeding depletes the number of available prey, as in our trials, the approximate moment estimator of $\alpha_i$ is

$$\hat{\alpha}_i = \frac{\ln\left(\frac{n_{i0} - r_i}{n_{i0}}\right)}{\sum_{j=1}^{k} \ln\left(\frac{n_{j0} - r_j}{n_{j0}}\right)} , i = 1, \ldots, k$$  \hspace{1cm} (1)

where $n_{i0}$ is the number of prey items of type $i$ present at the start of the experiment, $r_i$ is the number of prey items of type $i$ that are consumed during the experiment, and $k$ is the total number of prey types (Chesson 1983).

Because $\alpha$ ranges from 0 to 1, we then transformed preference values by the formula

$$\epsilon_i = \frac{k\alpha_i - 1}{(k-2)\alpha + 1} , i = 1, \ldots, k$$  \hspace{1cm} (2)

where $\epsilon$ ranges from -1 to +1. Negative values indicate that a prey item constitutes a smaller part of the diet than it does of the available prey base, and is thus non-preferred. Positive values indicate that a prey item constitutes a greater part of the diet than it does of the available prey base, and is thus preferred, and a value of 0 indicates that a prey item is consumed in direct proportion to its availability.

If the number of individuals left over in a trial is zero for a given taxon (i.e., $r_j = n_{j0}$), then $\ln\left(\frac{n_{j0} - r_j}{n_{j0}}\right)$ evaluates to $\ln(0)$ and direct computation of formula 1 fails. Analysis indicates that Chesson’s $\alpha$ reaches a limit of +1 for the preferred prey, and zero for the remaining prey, while $\epsilon$ approaches +1 for the preferred taxon and -1 for the remaining taxa. This outcome can mask any actual differences in preference between the remaining prey, for which $\epsilon$ values must all collapse to -1. We therefore analyzed our result as if a single ostracod had escaped consumption in the two cases (the low-ostracod treatments in rounds 3 and 4) when all ostracods were consumed.
We explored the effect that making this switch had on our analysis, relative to assigning $\epsilon = +1$ to ostracods and $\epsilon = -1$ to remaining prey, and found that the results were qualitatively similar (Supplement, Sensitivity Analysis). Unless otherwise noted, we present analyses in the main text after substituting one leftover ostracod.

**Linear Modeling and Model Selection**

To determine if salamanders acted as switching generalist predators, we used linear modeling in a model comparison framework to test whether preference for each prey type depended on the frequency of that prey. We conducted the statistical analyses in R version 3.3 (R Core Team 2018). Salamander feeding on all prey types occurred simultaneously, so we fit multivariate linear models (“lm” function in car package, version 2.1) (Fox & Weisberg 2011) to relate preference ($\epsilon$) to predictors (prey frequencies and salamander weight). We included salamander weight as a predictor variable because preliminary work indicated that preferences differ between larger and smaller salamanders, and because salamander size can influence risk for other kinds of prey (Urban 2007; Wells 2007).

We then compared models using Akaike’s Information Criterion with a correction for small sample size (AICc) (Burnham & Anderson 2002). In this and all subsequent analyses, we used a $\Delta$AICc value of 2 as the cutoff for determining that alternative models had substantial support relative to the minimum-AICc model in a set (Burnham & Anderson 2002). We used AICc to test the hypothesis that zooplankton frequencies were important predictors of feeding preference, by comparing values for models with and without initial zooplankton frequencies. Upon finding such support for a multivariate response of preference to the frequency of all prey types, we
further investigated the response of preference for a single prey type at a time. We used AICc to test the full set of models relating single-prey preference to all combinations of prey initial frequency and salamander weight, including two-way interactions and a quadratic term for initial frequency due to observed curvilinearity in preference plots (Supplement, *taxon-specific preference models*). Noting that the frequency of ostracods depended linearly on the frequencies of cladocerans and copepods, since there were always 450 total zooplankton per trial, we did not test models with all three taxa simultaneously.

**Predator Functional Responses**

We fit predator functional response curves to our data using maximum likelihood estimation (“mle2” function in *bbmle* package, version 1.0) (Bolker & R Development Core Team 2017), relating the number of individuals consumed per prey taxon, \( m \), to the initial frequency. For a Type 1 linear response,

\[
m = a \times N
\]

with mortality determined by attack rate \( a \), and abundance of prey \( N \).

For a Type 2 (hyperbolic, satiating) response,

\[
m = \frac{a}{1 + a \times h \times N}
\]

where \( h \) represents handling time (such that \( 1/h \) is the maximum number of prey consumed per unit time).

For a Type 3 (sigmoid, switching) response, the form
allows constraint of the attack rate to the closed interval \([0, 1]\).

For both the Type 2 and Type 3 functional responses, we solved for the logit transform of \(a\) and the logarithm of \(h\). This approach constrains optimization results to sensible ranges of \(a\) and \(h\) (\(0 \leq a \leq 1\) and \(h \geq 0\)) because attack rate must be a proportion between zero and one, and handling time cannot be negative. Such transformations were not necessary for optimization of a Type 1 response. This transformation introduced the problem whereby values of \(a\) close to 1 or values of \(h\) close to 0 were at the machine boundary of estimation, so confidence intervals could not be computed in such cases. Where estimated values of \(a\) were close to the upper limit of parameter space, we assumed that the upper confidence interval was 1. After obtaining the optimized parameters for each functional response type, we compared the three response types for each of the three prey taxa using AICc. We also calculated *per capita* mortality for each taxon to predict the effects of any switching behavior by salamanders on zooplankton populations.

### Results

Averaged across all trials, we found that *A. opacum* consumed more *Cypricercus* ostracods in relation to their initial frequencies, indicating positive preference for this prey taxon (Fig. 1A, B). The other ostracods, as well as the large cladoceran, *Simocephalus*, were consumed in proportion to their initial frequencies, indicating that *A. opacum* have neutral preference for them. All remaining zooplankton taxa, including the cladoceran *Scapholeberis* and the cyclopoid and harpacticoid copepods, were consumed rarely in relation to their initial frequencies, indicating avoidance.
Multi-taxon preferences

The model with the greatest statistical support (minimum AICc) included terms for cladoceran frequency, copepod frequency, salamander weight, and all two-way interactions, and had an adjusted multiple $R^2$ of 0.69 for the cladoceran preference response, 0.53 for the copepods, and 0.70 for the ostracods. A competing model ($\Delta$AICc $\leq$ 2) included cladoceran frequency, ostracod frequency, salamander weight, and two-way interactions of ostracod frequency with cladoceran frequency and with salamander weight. The same models were supported using both Chesson’s $\alpha$ and $\epsilon$. We present the models, AICc scores, and adjusted $R^2$ in the Supplement (Supplement, Multivariate Linear Modeling).

Using the strict preferences of +1/-1 for ostracods/other prey for the two instances when all ostracods were consumed resulted in AICc support for a different and broader set of models (Supplement, Sensitivity Analysis). Nonetheless, the frequency of at least one prey type always remained an important predictor of preference. Therefore, the overall finding of preference depending on prey frequency remained robust to the method of analysis.

Single-taxon preferences

For cladocerans, the model with the greatest support included salamander weight, cladoceran initial frequency, and a quadratic term for cladoceran frequency (Fig. 2), and had an adjusted $R^2$ of 0.53. The other supported model included a quadratic term for cladoceran initial frequency but did not include salamander weight. The best model indicates relatively similar preference for cladocerans at low and intermediate frequencies and increasing preference at high frequencies.
At pond-average and below-average cladoceran frequencies, salamanders neither preferred nor avoided cladocerans. At the highest cladoceran frequency, salamanders preferred cladocerans, consuming more of this taxon than would be expected due to its frequency alone.

For copepods, AICc supported two models of preference. The best model included salamander weight, copepod frequency, and a quadratic term for copepod frequency (Fig. 3), and had an adjusted $R^2$ of 0.48. The other supported model had copepod initial frequency with the quadratic term, but did not include salamander weight. The best model indicates similarly low preference (i.e., avoidance) for copepods at low and intermediate frequencies, and increased preference (no avoidance) for copepods at the highest frequency. The best fitting model indicates a dip in preference between the lowest and next-lowest copepod frequencies, but this likely is an artifact of the polynomial statistical function.

For ostracods, the full model had the best support. It included ostracod initial frequency, salamander weight, the interaction between weight and ostracod frequency, and a quadratic term for ostracod frequency (Fig. 4), and had an adjusted $R^2$ of 0.71. The model indicates that larger salamanders responded to ostracod abundance differently from smaller salamanders. The biggest salamanders consumed ostracods as a greater proportion of their diet relative to how common ostracods were in the available prey base, at all treatment levels. Preference values peaked at intermediate (pond average) ostracod frequencies, with a dip at both low and high ostracod frequencies. The smallest salamanders had highest preference for ostracods at the lowest ostracod abundance, and had negative values of preference (i.e., avoidance) for ostracods when ostracods were most abundant.
**Predator Functional Responses**

For each of the three prey taxa, the model with the lowest AICc supported a nonlinear response of predation to prey frequency. For cladocerans and copepods, the best-fitting functional response was the Type 3 (sigmoid, switching) response, indicating *per capita* predation risk decreased when these prey were rare (Table 2, Fig. 5, and Supplement, table S1). For ostracods the best fit was from a Type 2 (hyperbolic, satiating) response, indicating that *per capita* predation risk was lowest when ostracods were abundant.

Cladocerans experienced lower *per capita* predation at low frequencies than at intermediate frequencies, and predation risk stayed constant or dropped off slightly at elevated frequencies (Fig. 5a), yielding a sigmoid relationship between frequency and total mortality (Fig. 5d). Copepods experienced reduced *per capita* predation when they were below their typical abundance and much higher total and *per capita* predation when their frequency exceeded typical levels (Fig. 5b, 5e). Ostracods experienced the greatest *per capita* mortality when rare, and the lowest *per capita* mortality when abundant (Fig. 5c), although total mortality continued to increase slightly with increasing initial frequency (Fig. 5f).

**Discussion**

The multi-taxon preference analysis explicitly indicated that salamanders responded to prey frequency. The best-supported models included terms for the initial frequency of prey, which means that larval *A. opacum* altered their prey-specific capture rates based on prey composition. The single-taxon preference models indicated that a combination of prey frequency and salamander weight influenced preference. Larger salamanders had greater preference for
ostracods than did smaller salamanders, and consumed greater numbers of ostracods, particularly the large *Cypricercus*. Smaller salamanders had greater preference for cladocerans and copepods than did larger salamanders. Thus, over the salamanders’ ontogeny, the shift in functional responses to each prey species probably mirrors the effect of a prey species outgrowing predation risk (*sensu* Urban 2007, McCoy et al. 2011), with ever-larger prey becoming susceptible and preferred.

Within both the cladocerans and the ostracods, the subtaxon attaining greater body size (*Simocephalus* spp. and *Cypricercus* spp., respectively) was more highly preferred than the smaller taxon (*Scapholeberis* spp. and the various small ostracods, respectively) (Fig. 1a). Adult terrestrial salamanders are known to select large prey when prey density is high, in accordance with optimal foraging predictions (Wells 2007), and our findings contribute evidence that larval salamanders do, too. Another factor potentially influencing the profitability of prey is ease of capture, usually operationalized as handling time (Schoener 1971). Across our feeding trials, the fast-swimming cyclopoid copepods were the least preferred prey, whereas the slow-swimming ostracods were the most preferred prey, apparently in agreement with this prediction. Our experimental design did not allow for an explicit test of hypotheses regarding prey selection, however, and our functional response analysis indicated that copepods would yield the shortest handling time of all three zooplankton taxa if search time were not limiting.

Our study provided evidence of a Type 3 functional response of salamanders to cladoceran and copepod prey, but not to ostracods. In contrast, ostracods apparently had little to no refuge in rarity (Fig. 5c, Fig. 6), at least within the confines of the trial containers, consistent with a Type 2 functional response by the predator. This result does not translate automatically to natural settings, where structural refugia such as submerged macrophytes can provide shelter from some...
predators (Crowder & Cooper 1982; Meerhoff et al. 2007). Refugia were invoked to explain the disparity between experimental and observational results in a previous study by Holomuzki et al. (1994) with the closely related tiger salamander (*Ambystoma tigrinum*). Within experimental enclosures, salamanders reduced the abundance of large-bodied cladocerans, eliminated ostracods, and allowed cyclopoid copepod populations to grow; but in pond surveys there were no correlations between salamander abundance and prey diversity.

Increasing *per capita* predation risk for more abundant prey taxa is consistent with predation being a driver of stability in communities, because it would specifically limit common species and not rare ones. This scenario avoids the risk of apparent competition causing the extirpation of rare prey, as might otherwise happen when predation can strongly suppress prey populations (Holt 1984; Holt *et al.* 1994). Therefore, a single switching generalist predator could have a stabilizing effect on a diverse prey community just as would multiple specialized enemies (Janzen 1970; Connell 1971; Chesson 2000). Our results suggest predation by individual salamanders should be stabilizing for cladocerans and copepods, but strongly destabilizing for ostracods. If ostracods persist in ponds where there is heavy salamander predation, then some mechanism other than frequency-dependent predation would be responsible.

Aside from frequency-dependent predation, the other coexistence driver that is independent of environmental fluctuations is resource partitioning (Chesson 2000). Food partitioning is one possibility: ostracods typically consume algae and organic detritus, cladocerans primarily feed on small algae but also organisms such as bacteria and ciliates, and copepods are omnivorous, feeding on a wide variety of foods depending on species and life stage (Thorp & Covich 2001). Spatial partitioning is another possibility: zooplankton typically have patchy distributions across multiple scales (Pinel-Alloul 1995), and various species are known to segregate spatially (Heip...
& Engels 1977; Jakobsen & Johnsen 1987). Even within the small experimental arenas, we observed modest spatial segregation between bottom-tending harpacticoid copepods, pelagic cyclopoid copepods, surface-skimming *Scapholeberis*, and *Simocephalus* and ostracods that frequently rested on vertical surfaces. Therefore, it is possible that spatial segregation of prey encouraged frequency-dependent predation, as per Holt (1984). Our study did not address the behavioral mechanisms of frequency-dependent predation, but an explicit test of the various hypotheses for prey choice could help clarify the relative roles of spatial partitioning and prey choice *per sé*.

The other two drivers of stable coexistence, storage effect and relative nonlinearity of competition, are dependent on environmental fluctuations. While we lack evidence for or against relative nonlinearities in competition among zooplankton, the storage effect seems plausible in the pond community. Pond zooplankton, including ostracods, create resting eggs (Thorp & Covich 2001), enabling buffered population growth as required for the storage effect. Seasonality of pond conditions could enable the other two requisite conditions, differential responses to the environment by different species and covariance between environment and competition (Chesson 2000). If ostracods can take advantage of some temporal niche, they might be able to persist even despite elevated predation risk.

We explicitly investigated the effects of multiple prey types on predator behavior, and while a full consideration of possible trophic interactions exceeds the scope of this study, we note some limitations on inference to natural settings. For example, we tested feeding preferences of salamander larvae in isolation, whereas natural ponds tend to host many predators, both in number and kind (Sih *et al.* 1998; Relyea 2003). Both heterospecific and conspecific predators can enhance, interfere with, or otherwise modify the foraging behavior of a given individual (Sih
et al. 1998). In the presence of marbled salamanders that act as both predators and competitors, spotted salamander (*Ambystoma maculatum*) larvae deploy riskier active foraging strategies and more quickly deplete preferred prey (Urban 2013). Similarly, while our experiment included a broader selection of prey than other comparable studies, the selection of prey experienced by salamanders in real ponds is broader still. Future work might demonstrate

Predation might be an important component to species coexistence, and our findings illuminate how a generalist predator selects prey when many options are available. A more mechanistic framework for prey choice, one that accounts explicitly for both frequency and profitability of prey, would likely enable reasonable prediction of the impact of predation on an assemblage of prey. Experimentation in a more natural setting than a lab microcosm could then provide model validation, and thus more compelling evidence of a predators’ role in maintaining prey diversity. Teasing apart the emergent effects of multi-species interactions remains a major challenge for theory and experimental design in identifying coexistence and attributing mechanisms of stability in complex communities (Levine et al. 2017). Building on the fundamentals of these multispecies coexistence drivers in more complicated experimental designs is likely to reveal further how diversity persists in nature.

**Acknowledgments**

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Connecticut Department of Ecology and Evolutionary Biology. We declare no conflicts of interest. All applicable institutional and/or national guidelines for the care and use of animals were followed. This work was conducted under Institutional Animal Care and Use Committee permit A15-004. The South Central Connecticut Regional Water Authority granted permission to collect animals from their land. Data accessibility: data will be archived in Dryad.

References


community. Hydrobiologia, 300–301, 17–42.


Oecologia, 154, 571–580.


Table 1. Composition of experimental treatments. Total zooplankton per treatment was always 450.

<table>
<thead>
<tr>
<th>Pond Average</th>
<th>Low-cladoceran</th>
<th>Low-copepod</th>
<th>Low-ostracod</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladocera</td>
<td>20% cladocerans: 3% cladocerans:</td>
<td>40% cladocerans: 11 Scapholeberis</td>
<td>26% cladocerans: 29 Simocephalus, 136 Scapholeberis</td>
</tr>
<tr>
<td></td>
<td>22 Simocephalus,</td>
<td>4 Simocephalus,</td>
<td>45 Simocephalus,</td>
</tr>
<tr>
<td></td>
<td>68 Scapholeberis</td>
<td>11 Scapholeberis</td>
<td>86 Scapholeberis</td>
</tr>
<tr>
<td>Copepoda</td>
<td>55% copepods: 180 harpacticoid,</td>
<td>217 harpacticoid,</td>
<td>9% copepods: 11 cyclopoid</td>
</tr>
<tr>
<td></td>
<td>68 cyclopoida</td>
<td>82 cyclopoida</td>
<td>30 harpacticoid, 230 harpacticoid,</td>
</tr>
<tr>
<td>Ostracoda</td>
<td>25% ostracods: 90 Cypricercus,</td>
<td>109 Cypricercus,</td>
<td>51% ostracods: 182 Cypricercus,</td>
</tr>
<tr>
<td></td>
<td>22 otherb</td>
<td>27 otherb</td>
<td>46 otherb</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Controls 1, 2, &amp; 5</th>
<th>Control 3</th>
<th>Control 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladocera</td>
<td>20% cladocerans: 29% cladocerans:</td>
<td>16% cladocerans:</td>
</tr>
<tr>
<td></td>
<td>22 Simocephalus, 68 Scapholeberis</td>
<td>22 Simocephalus, 108 Scapholeberis</td>
</tr>
<tr>
<td></td>
<td>108 Scapholeberis</td>
<td>48 Scapholeberis</td>
</tr>
<tr>
<td>Copepoda</td>
<td>55% copepods: 180 harpacticoid, 68 cyclopoida</td>
<td>44% copepods: 0 harpacticoid, 200 cyclopoida</td>
</tr>
<tr>
<td></td>
<td>44% copepods: 0 harpacticoid, 200 cyclopoida</td>
<td></td>
</tr>
<tr>
<td>Ostracoda</td>
<td>25% ostracods: 90 Cypricercus, 22 otherb</td>
<td>40% ostracods: 90 Cypricercus, 30 otherb</td>
</tr>
<tr>
<td></td>
<td>27% ostracods: 90 Cypricercus, 90 Cypricercus,</td>
<td></td>
</tr>
</tbody>
</table>

a cyclopoid copepods: Microcyclops, Tropocyclops, and small Acanthocyclops
b other ostracods: primarily Cypridopsis, along with Cyprinotus, Cyclocypris, and Candona, approximately in the ratio 8:1:1:1
Table 2. Best-fitting functional responses, mean parameter estimates using maximum likelihood (95% confidence intervals in parentheses), and model \( R^2 \), by taxon. Confidence intervals that could not be computed directly are marked with an asterisk (*). The full set of fitted functional responses, parameter estimates, and associated AICc scores can be viewed in the supplementary materials (Tables S1 and S2).

<table>
<thead>
<tr>
<th>Functional Response</th>
<th>Parameter estimates</th>
<th>Model ( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladocerans</td>
<td>Type 3</td>
<td>0.96 (0.91, 1.0*) 9.4e-3 (7.9e-3, 1.1e-2)</td>
</tr>
<tr>
<td>Copepods</td>
<td>Type 3</td>
<td>1.0 (1.0*, 1.0*) 2.1e-3 (2.0e-3, 2.3e-3)</td>
</tr>
<tr>
<td>Ostracods</td>
<td>Type 2</td>
<td>1.0 (1.0*, 1.0*) 5.7e-3 (5.3e-3, 6.2e-3)</td>
</tr>
</tbody>
</table>
Figure 1. *Ambystoma opacum* preference (Chesson’s $\varepsilon$) for zooplankton taxa, both at the finest taxonomic resolution tracked (panel A) and pooled to the levels that we manipulated independently in feeding trials (panel B). Points represent mean preferences across all trials (n=20), and vertical bars indicate 95% confidence intervals. The dotted line indicates neutral preference, at which point the salamanders would consume a given prey taxon in proportion to its relative abundance. Cycl = cyclopoid copepods; Scaph = *Scapholeberis*; Harp = harpacticoid copepods; ostr = other ostracods; Simo = *Simocephalus*; Cypr = *Cypricercus*.
Figure 2. Actual preference (Chesson’s $\varepsilon$) for cladocerans (red circles) and predictions of the model with greatest AICc support (colored surface, with warmer colors indicating positive preference and cooler colors indicating avoidance). The model included salamander weight, cladoceran frequency, and a quadratic term for cladoceran frequency.
Figure 3. Actual preference (Chesson’s $\epsilon$) for copepods (red circles) and the predicted preferences (colored surface, with warmer colors indicating positive preference and cooler colors indicating avoidance) according to the model with the greatest AICc support. The model included salamander weight, copepod frequency, and a quadratic term for copepod frequency.
Figure 4. Actual preference (Chesson’s $\epsilon$) for ostracods (red circles) and predictions of the model with greatest AIC support (colored surface, with warmer colors indicating positive preference and cooler colors indicating avoidance). The model included salamander weight, initial frequency of ostracods, the interaction between weight and ostracod frequency, and a quadratic term for ostracod frequency.
Figure 5. The *per capita* and total mortality of each prey taxon. Panels A-C show *per capita* mortality as a function of taxon initial frequency. Panels D-F show the total number of individuals consumed of a given taxon in each trial relative to the initial frequency of that taxon. Points and vertical bars represent means and standard error (n = 5 trials at each frequency), respectively, with the best fitted functional response curve superimposed in each panel.
Figure 6. *Per capita* mortality of *Cypricercus* ostracods relative to initial frequency of *Cypricercus*. Vertical bars are standard errors.
Supplementary material

*Image Analysis with ImageJ*

We processed photographs of *Scapholeberis* in ImageJ to obtain a size distribution for all trials, including no-salamander controls. We visualized these distributions to identify a size cut-off, because reproduction among this species during the trials had inflated the post-trial leftover counts and decreased the calculated preference values. We examined results both from the *Ambystoma opacum* trials that are the subject of this manuscript and from an earlier pilot study with the salamander *Ambystoma maculatum* that followed a similar methodology. Since the size distribution was bimodal, with the small secondary peak in the smallest size range (Fig. S1), we were able to estimate a size threshold, 375 pixels, that agreed well with our visual estimates of true leftover individuals vs. newborn individuals. We adjusted *Scapholeberis* counts for our remaining analyses, excluding all individuals below the critical size. This procedure does not account for the possibility that a bimodal distribution could have resulted from the salamanders preferentially consuming intermediately-sized zooplankton. However, such preferential feeding on the basis of size could not result in leftover counts that exceeded initial counts. Therefore, we assume that the secondary peak in *Scapholeberis* abundance in the lowest size range is due to reproduction during the trial.
Figure S1. Post-trial size distributions (in pixels) of *Scapholeberis mucronata* following feeding trials with *Ambystoma opacum* (blue) and *A. maculatum* (pink). Vertical bar shows the size cutoff, 375 pixels, used for the analyses.
Functional response analysis

Table S1. Mean parameter estimates with 95% confidence intervals, and associated log-likelihoods, by prey taxon, for Type 1, Type 2, and Type 3 functional responses. Confidence intervals for parameter values near the boundaries (1 for $a$, 0 for $h$) could not be computed directly, and are marked with an asterisk (*).

<table>
<thead>
<tr>
<th>Prey Taxon</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladocerans</td>
<td>a=0.442, log-likelihood=-151.79 (0.420, 0.490)</td>
<td>a=0.442, h=6.74e-9 (0*, 0*)</td>
<td>a=0.958, log-likelihood=-151.79 (0.908, 1.00*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>log-likelihood=9.36e-3 (7.94e-3, 1.09e-2)</td>
</tr>
<tr>
<td>Copepods</td>
<td>a=0.447, log-likelihood=-942.7 (0.433, 0.462)</td>
<td>a=0.447, h=2.24e-10 (0*, 0*)</td>
<td>a=1.00, log-likelihood=-942.7 (1.00*, 1.00*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>log-likelihood=2.14e-3 (2.01e-3, 2.29e-3)</td>
</tr>
<tr>
<td>Ostracods</td>
<td>a=0.527, log-likelihood=-360.58 (1.00*, 1.00*)</td>
<td>a=1.00, h=5.71e-3 (5.27e-3, 6.19e-3)</td>
<td>a=1.00, log-likelihood=-219.74 (1.00*, 1.00*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>log-likelihood=1.05e-2 (9.83e-3, 1.13e-2)</td>
</tr>
</tbody>
</table>
**Functional response AICc tables**

Table S2. We report AICc and ΔAICc values for the maximum likelihood estimated parameterizations of Type 1, Type 2, and Type 3 functional response curves for cladocerans, copepods, and ostracods. Models are arranged in descending order of AICc support. Boldface font indicates all models with ΔAICc ≤ 2 within a set. Asterisks (*) designate the single lowest scoring model in a set.

<table>
<thead>
<tr>
<th>Cladocerans</th>
<th>AICc</th>
<th>ΔAICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 3 *</td>
<td>302.6</td>
<td>-</td>
</tr>
<tr>
<td>Type 1</td>
<td>305.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Type 2</td>
<td>308.3</td>
<td>5.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Copepods</th>
<th>AICc</th>
<th>ΔAICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 3 *</td>
<td>1724.2</td>
<td>-</td>
</tr>
<tr>
<td>Type 1</td>
<td>1887.6</td>
<td>163.5</td>
</tr>
<tr>
<td>Type 2</td>
<td>1890.1</td>
<td>165.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ostracods</th>
<th>AICc</th>
<th>ΔAICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2 *</td>
<td>444.2</td>
<td>-</td>
</tr>
<tr>
<td>Type 1</td>
<td>723.4</td>
<td>279.2</td>
</tr>
<tr>
<td>Type 3</td>
<td>817.1</td>
<td>372.9</td>
</tr>
</tbody>
</table>
Multivariate linear modeling – AIC tables

Table S3. We show all of the models without zooplankton frequency and a selection of models that do include zooplankton frequency. We show a representative set of models, because as long as any models with frequency perform better than all models without frequency, our hypothesis is supported. For comparison, we include models for both Chesson’s $\epsilon$ and Chesson’s $\alpha$. We also show adjusted $R^2$ for each response variable, including cladocerans, copepods, and ostracods.

Boldface font indicates all models with $\Delta$AICc $\leq$ 2.0 within a set. Cladoceran frequency is abbreviated as “clado”, copepod frequency as “cope”, and ostracod frequency as “ostr”.

“Weight” is the term for salamander weight.

<table>
<thead>
<tr>
<th>Chesson’s $\epsilon$: (clado.$\epsilon$,cope.$\epsilon$,ostr.$\epsilon$) ~ Model Terms</th>
<th>AICc</th>
<th>$\Delta$AICc</th>
<th>Adjusted $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Clado</td>
<td>Cope</td>
</tr>
<tr>
<td>weight+clado+cope+weight:clado+weight:cope+clado:cope</td>
<td>105.8</td>
<td>0.69</td>
<td>0.53</td>
</tr>
<tr>
<td>weight+clado+ostr+weight:oyal +clado:ostr</td>
<td>105.2</td>
<td>0.6</td>
<td>0.64</td>
</tr>
<tr>
<td>weight+clado+cope+weight:clado+weight:cope</td>
<td>-90.0</td>
<td>15.8</td>
<td>0.68</td>
</tr>
<tr>
<td>weight+clado+cope</td>
<td>-86.4</td>
<td>19.4</td>
<td>0.65</td>
</tr>
<tr>
<td>weight+cope</td>
<td>-81.9</td>
<td>23.9</td>
<td>0.61</td>
</tr>
<tr>
<td>weight+cope+weight:cope</td>
<td>-81.4</td>
<td>24.4</td>
<td>0.59</td>
</tr>
<tr>
<td>weight+ostr</td>
<td>-80.9</td>
<td>24.9</td>
<td>0.62</td>
</tr>
<tr>
<td>weight+ostr+weight:ostr</td>
<td>-80.8</td>
<td>25.0</td>
<td>0.60</td>
</tr>
<tr>
<td>clado+cope+clado:cope</td>
<td>-70.9</td>
<td>34.9</td>
<td>0.48</td>
</tr>
<tr>
<td>clado+cope</td>
<td>-65.4</td>
<td>40.4</td>
<td>0.50</td>
</tr>
<tr>
<td>ostr</td>
<td>-62.9</td>
<td>42.9</td>
<td>0.48</td>
</tr>
<tr>
<td>cope</td>
<td>-62.5</td>
<td>43.3</td>
<td>0.49</td>
</tr>
<tr>
<td>weight+clado</td>
<td>-57.9</td>
<td>47.9</td>
<td>0.14</td>
</tr>
<tr>
<td>weight+clado+weight:clado</td>
<td>-56.3</td>
<td>49.5</td>
<td>0.14</td>
</tr>
<tr>
<td>weight</td>
<td>-48.4</td>
<td>57.4</td>
<td>-0.03</td>
</tr>
<tr>
<td>clado</td>
<td>-48.3</td>
<td>57.5</td>
<td>0.15</td>
</tr>
<tr>
<td>1</td>
<td>-42.1</td>
<td>63.7</td>
<td>-</td>
</tr>
</tbody>
</table>
Chesson’s $\alpha$: (clado.$\alpha$, cope.$\alpha$, ostr.$\alpha$) ~

<table>
<thead>
<tr>
<th>Model Terms</th>
<th>AICc</th>
<th>$\Delta$AI</th>
<th>Adjusted $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clado</td>
<td>Cop</td>
<td>Ostr</td>
</tr>
<tr>
<td>weight+clado+cope+weight:clado+weight:cope+clado:cope</td>
<td>-197.9</td>
<td>-0.75</td>
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<tr>
<td>weight+clado+ostr+weight:ostr+clado:ostr</td>
<td>-196.4</td>
<td>0.5</td>
<td>0.74</td>
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<td>weight+clado+cope+weight::clamp+weight:cope</td>
<td>-180.9</td>
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<tr>
<td>weight+cope+clado</td>
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<td>-170.4</td>
<td>27.5</td>
<td>0.66</td>
</tr>
<tr>
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<td>-162.1</td>
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<td>0.51</td>
</tr>
<tr>
<td>clado+cope</td>
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</tr>
<tr>
<td>cope</td>
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<td>weight+clado</td>
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<tr>
<td>ostr</td>
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<td>45.0</td>
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<tr>
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<td>-150.8</td>
<td>47.1</td>
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<td>1</td>
<td>-136.0</td>
<td>61.9</td>
<td>-</td>
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</table>
**Taxon-specific preference models**

To relate single-taxon preference to initial frequency of that taxon and to salamander weight, we tested eight models for each prey taxon with AICc. The most complex model included the two-way interaction of weight and frequency, plus a quadratic term for frequency to account for curvilinearity. The models were the following:

1. Preference ~ Weight + frequency + weight:frequency + frequency^2
2. Preference ~ Weight + frequency + frequency^2
3. Preference ~ Frequency + frequency^2
4. Preference ~ Weight + frequency + weight:frequency
5. Preference ~ Weight + frequency
6. Preference ~ Frequency
7. Preference ~ Weight
8. Preference ~ 1 (constant)
Sensitivity Analysis

In two of the trials (the low-ostracod treatment in rounds 3 and 4, which was “treatment 3” in each round), zero ostracods remained at the end of the trials, introducing \( \log(0) \) in the formula for Chesson’s \( \alpha \). We therefore analyzed preference data as if one ostracod had remained, under the assumption that a change of one less individual consumed should not greatly impact the preference index. To investigate this assumption, we recalculated preferences after replacing the number of leftover ostracods with 3, 2, 1, 0.1, 0.01, 0.001, 0.0001, or \( 1 \times 10^{-5} \) individuals, as well as 0. Results are shown below (Fig. S2). Preference values remained level across substitutions in the neighborhood of +0.1 to +3, but changed rapidly from +0.1 down to 0. This analysis of the preference index sensitivity to different substituted values shows that a small proportional change in total consumption (i.e., one individual out of 407 consumed in round 3, and one out of 271 in round 4) can result in substantial shifts to all calculated preferences. Since a single individual consumed out of several hundred should not imply a drastic change in behavior, we interpreted this pattern as an indication of unreliability of the index at the boundary of 100% consumption.
Figure S2. Calculated preference for prey taxa under different substitutions for leftover ostracods, when all ostracods had actually been consumed. Substitutions for zero include 3, 2, 1, 0.1, 0.01, 0.001, 0.0001, or 1e(-5) individuals, as well as 0. Red points and linear interpolations show preference for ostracods, green points and linear interpolations show preference for copepods, and blue points and linear interpolations show preference for cladocerans.
Analytically, strict application of Chesson’s preference indices in these two instances should result in a value of +1 for the ostracods, and -1 for both copepods and cladocerans. Therefore we also recalculate all preferences (Fig. S3), and we present multivariate AIC tables based on the strictly interpreted preference values, without the substitution of one ostracod remaining (Table S4).
Figure S3. Comparison of averaged preferences for prey at the finest resolution tracked (left panels) and at the resolution that we manipulated independently for the different feeding treatments (right panels). The top panel (“Substitution”) repeats the figures shown in the main text (for comparison), and the bottom panel (“Strict”) presents results using the strict
interpretation of preference = +1 for ostracods and -1 for other prey for the trials where all ostracods were consumed. Error bars are standard error. Key to abbreviations: Cycl = cyclopoid copepods; Scaph = Scapholeberis; Harp = harpacticoid copepods; ostr = other (small) ostracods; Simo = Simocephalus; Cypr = Cypricercus.
Multivariate linear modeling – AIC tables (Strict interpretation of preference values)

Table S4. AICc tables for Chesson’s $\epsilon$ and Chesson’s $\alpha$ using the strictly interpreted values of preference. For low-ostracod treatments in rounds 3 and 4, this meant setting ostracod preference to 1 and both cladoceran and copepod preferences to -1. A different set of models have the lowest AICc scores than under the original methodology, but models incorporating zooplankton frequency continue to outperform models without zooplankton frequency. Boldface font indicates all models with $\Delta$AICc $\leq$ 2.0 within a set.

Chesson’s $\epsilon$: (clado.$\epsilon$, cope.$\epsilon$, ostr.$\epsilon$) ~

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
<th>$\Delta$AICc</th>
<th>Adjusted $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Cope</td>
<td>Ostr</td>
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Chesson’s $\alpha$: (clado.$\alpha$, cope.$\alpha$, ostr.$\alpha$) ~

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Chapter Two:

Negative direct effects of salamander predation overwhelm positive indirect effects on diversity of a zooplankton assemblage

Michael S. Hutson

Abstract

Complete competitors should not be able to coexist, according to the competitive exclusion principle, yet nature is full of diverse and competing species. Modern coexistence theory explains the persistence of diversity via equalizing forces, which reduce average fitness differences between species, and stabilizing forces, which cause species to limit themselves more than they limit others. Frequency-dependent predation is a potentially general and widespread example of a stabilizing force, and it has been documented in larval marbled salamanders (Ambystoma opacum) feeding on zooplankton. I tested the prediction that frequency-dependent predation by salamanders would counteract competitive exclusion among zooplankton and maintain diversity. I conducted the work using replicated pond enclosures and included both no-predator and nonselective thinning treatments. I found that salamander predation reduced richness and evenness of zooplankton assemblages relative to control treatments, counter to my predictions. The temporal variability of diversity was lower in predation treatment enclosures as well. The populations of large cladoceran zooplankton did not compensate for the population growth of smaller zooplankton in predation enclosures as they did in the other treatments. Frequency-dependent predation can stabilize a prey assemblage in theory, but in practice, the negative direct effect of predation on populations can overwhelm the positive indirect effects. Diversity maintenance in this system must depend on factors beyond frequency-dependent predation alone.
Introduction

Community composition and diversity can vary widely over space and time in nature. To explain how diversity is regulated in communities, modern coexistence theory distinguishes between equalizing and stabilizing mechanisms (Chesson 2000). In this framework, equalizing mechanisms are processes that reduce average fitness differences between species, and stabilizing forces are those that cause species to limit themselves more than they limit others. Both processes can weaken the tendency toward competitive exclusion, but only stabilizing mechanisms can counteract it entirely (Levine et al. 2017). The prevalence of stabilizing forces in nature – and therefore the degree to which communities in nature consist of coexisting species versus simply co-occurring ones – is still largely unknown (HilleRisLambers et al. 2012). This uncertainty poses a challenge for predicting community responses to disturbances and long-term changes (Gilman et al. 2010; Valladares et al. 2015).

Predation, one possible stabilizing mechanism, can have opposing effects on diversity. Theory alternately predicts that predators can decrease diversity of prey in communities (Holt 1977; Abrams & Kawecki 1999; Chase et al. 2002a; Chesson & Kuang 2008; Holt & Bonsall 2017) or increase it (Paine 1966; Abrams & Matsuda 1996; Chesson 2000; Chase et al. 2002a; Chesson & Kuang 2008; Socolar & Washburne 2015; Holt & Bonsall 2017; Stump & Chesson 2017). One way that predation can act as a stabilizing mechanism and promote diversity is when prey selection by the predator is frequency-dependent (Abrams & Matsuda 1996; Chesson 2000; Socolar & Washburne 2015). A tendency to switch over to consuming more frequent prey (sensu Murdoch, 1969), potentially arising from optimal foraging behavior (MacArthur & Pianka 1966; Stump & Chesson 2017), could provide a demographic advantage to rare prey, thus promoting coexistence (Chesson 2000). Frequency-dependent predation has been documented broadly in nature in consumers as small as protozoans and as large as whales (Jeschke et al. 2004; Smout & Lindstrøm 2007). However, the community-level effects of frequency-dependent predation remain poorly understood, especially since the majority of evidence comes from laboratory experiments or studies without alternative prey available (Jeschke et al. 2004).

Here, I examine if frequency-dependent predation can promote prey diversity in field enclosure experiments. A prior study suggests that larval marbled salamanders (Ambystoma opacum), the top predators in many temporary ponds throughout the eastern United States (Petranka 1998;
Urban 2013), exhibit frequency-dependent predation when encountering multiple zooplankton prey types (Hutson et al. n.d.). That study shows elevated per capita predation risk for copepod and cladoceran prey when such prey are at elevated frequency, although a third and highly preferred prey type, ostracods, experiences declining per capita risk with increasing frequency consistent with satiation. These findings suggest that frequency-dependent predation could contribute to diversity maintenance in temporary ponds. However, the limited spatial and temporal scope of the lab study precludes broader conclusions about the role of predation in structuring the community. Establishing a link between predator behavior and community dynamics, and especially the effect of predation on prey diversity, requires tests under more realistic conditions and at broader spatial and temporal scales.

I conducted a six-week pond enclosure experiment, manipulating the predation regime experienced by a zooplankton assemblage. After stocking identical initial assemblages, I allowed each enclosure community to develop under no predation, selective (frequency-dependent) salamander predation, or non-selective (frequency-independent) thinning to simulate frequency-independent predation. This design allowed partitioning of the effects of selective frequency-dependent predation relative to non-selective generalist predation. Since frequency-dependent predation is a potential stabilizing mechanism, I predicted that zooplankton assemblages experiencing salamander predation would retain greater diversity than would the assemblages experiencing non-selective thinning or no predation. While I expected the rapid elimination of ostracods in the predation treatment, since they are highly preferred prey of salamanders (Hutson et al. n.d.), I expected competitive exclusion would gradually deplete richness even further in the remaining treatments. One potential outcome of stability in nature, assuming non-cyclical dynamics, is lower variability over time (McCann 2000, 2011), so I further predicted that the prey taxa experiencing selective predation in the microcosm experiment would have the least variation in population sizes in the pond enclosures. I predicted that non-selective thinning would reduce all species abundances proportionally, reducing the strength of competition for resources (Connell 1978; Thorp & Cothran 1984), and thus having a diversity preserving effect, albeit weaker than that of frequency-dependent predation. Lastly, I predicted that populations experiencing selective predation would be less prone to large fluctuations than would populations experiencing non-selective mass removal or no predation, due to the salamanders’ prey-switching response (Hutson et al. n.d.).
Methods

Marbled salamander larvae are widespread apex predators in temporary ponds across the eastern United States, with a range extending from eastern Texas and the Florida panhandle to southern New Hampshire (Petranka 1998). Adults have high natal pond fidelity, and lay eggs in dry temporary pond basins in late summer and early fall (Gamble et al. 2007). The larvae hatch upon pond re-inundation in late fall and early winter, and feed primarily on cladoceran, copepod, and ostracod zooplankton while they overwinter in their natal ponds (Petranka 1998; Urban 2013). In spring, the larvae are large enough to feed on macroinvertebrates and the hatchlings of other amphibians (Petranka 1998; Urban 2007), but they continue to consume zooplankton as well (Petranka 1998). Long-term presence of marbled salamanders in ponds is associated with greater diversity of zooplankton, even though marbled salamanders reduce the density, biomass, and diversity of prey in mesocosms (Urban 2013).

I conducted my study in a temporary pond on Totoket Mountain in Northford, CT, approximately 20 km northwest of New Haven, CT. Totoket is a forested basaltic massif that supports a network of perched temporary ponds. The land where I conducted the study is protected as a public watershed, with restricted public access. I selected B-7, a pond located at 41°23.6’ N, 72°46.2’ W, where I had previously collected A. opacum salamander larvae for a related study on feeding preferences (Hutson et al. n.d.).

I constructed enclosures using 20 L plastic containers, drilling four 3.8 cm diameter holes centered 5.1 cm above the bottom of each bucket. I glued 50 μm mesh – fine enough to exclude all adult zooplankton previously observed in the pond, and most zooplankton eggs – over the holes on the inside of the buckets, and window screen mesh on the outside to protect the windows against large debris. I used the same window screen mesh to cover the tops of the buckets. I collected American beech (Fagus grandifolia) and chestnut oak (Quercus montana) leaves from the leaf litter surrounding the pond, sterilized the leaves by boiling for fifteen minutes, and then added one of each leaf type to every enclosure. Three enclosures, floated on a piece of foam around a central stake, constituted a single block (Figure 1), and I installed ten replicate blocks. Once the enclosures were in place, I randomly assigned one of each treatment within every block.
I installed enclosures and began stocking them with zooplankton in March of 2016, introducing identical numbers of plankton from across six taxa that were the most abundant in plankton tows at that time: the cladocerans *Chydorus* and *Simocephalus*, cyclopoid and harpacticoid copepods, and the ostracods *Cyclocypris* and *Cypricercus*. I collected live plankton from the experimental pond with a tow net and sorted under a stereo microscope (Leica M125, Leica Microsystems, Germany) in the lab. I identified cladocerans and ostracods by genus and copepods by order, using the keys from Thorp & Covich (2001). I then assembled replicate sets of zooplankton (Table 1) in 230 mL glass mason jars and transported them back to the experimental pond for release into each enclosure, introducing 111 total zooplankton per enclosure over the span of one month. Following the first round of zooplankton stocking, I discovered two *Cypricercus* ostracod still inside otherwise empty glass jars. I could not attribute these individuals to any particular enclosure because the jars were presumed to be identical, and thus lacked labels. Even if both had been lost from a single enclosure, they would represent a difference in *Cypricercus* stocking abundance of less than five percent.

I introduced one larval salamander into each predation treatment enclosure on April 30, selecting individuals that were similar in size. I collected zooplankton samples from the enclosures on May 5, May 15, May 27, and June 9, using a PVC pipe (7.6 cm diameter) sampler, and taking three pooled dips per enclosure. These samples constituted 11-16% of total water volume in the enclosures. From the thinning treatment, I took an additional three dips, for six dips total, constituting 21-29% of water volume in the enclosures. On May 13, I took a further eight pooled dips from each of the thinning enclosures. I poured each pooled sample through a 150 µm mesh filter, and immediately rinsed off and preserved the filtrate in 70% ethanol. I collected salamanders on June 15, then euthanized them in a solution of 50 mg/L benzocaine (Orajel).

I counted zooplankton and aquatic macroinvertebrates in each sample under a stereo-microscope (Leica M125) at 16x-40x magnification, once again identifying cladocerans and ostracods to genus and copepods to order. Zooplankton became very abundant by June 9, so I split most samples that appeared to have 500 individuals or more upon preliminary visual examination (Control blocks 2, 8, and 10; Thinning blocks 1, 3, 4, 5, 6, 7, 8, 9, and 10; Predation blocks 1, 4, 7, 8, 9, 10) using a Folsom plankton splitter, and randomly selected a half-sample to sort. The minimum number of zooplankton identified in such a split sample was 221. Since the interior of
the pipe sampler was irregularly shaped, I determined sample volume empirically based on measured water depth. Prior to field use, I obtained a calibration curve by measuring both the depth of water (replicates at 10.5, 16, and 20 cm) in a container and the volume of water removed by the pipe sampler. For a water depth of \( d \) centimeters, I therefore estimated a sample volume of \( 44.7^*d-193 \) mL (slope SE = 0.41, intercept SE = 6.5, adjusted R-squared = 0.998) per dip, calculated the water depth for the reduced volume of water (since the level rose very slowly), and repeated for the appropriate total number of pooled dips.

I analyzed the effect of predation regime on zooplankton assemblage in R version 3.4.3 (R Core Team 2018). To evaluate population variability, I measured the coefficient of variation of target zooplankton populations, and tested for differences with a MANOVA using the car package version 2.1 (Fox & Weisberg 2011). To evaluate diversity, I analyzed the Hill numbers of species richness and Simpson’s Diversity, as well as Simpson’s Evenness. Hill numbers are desirable diversity metrics because they correspond to the effective number of equally abundant species that would result in a given value of the diversity index (Hill 1973; Chao et al. 2014). Richness is particularly sensitive to sample size, so I also tested rarefied richness. The results were qualitatively similar to those using absolute richness, so I present the analysis of absolute richness in the main text and rarefied richness in the Supplement. Simpson’s Diversity is calculated as

\[
D = \frac{1}{\sum_{i=1}^{S} p_i^2}
\]

(1)

Where \( S \) is richness and \( p_i \) is the frequency of species \( i \). Simpson’s Evenness is Simpson’s Diversity divided by richness,

\[
E = \frac{D}{S} = \frac{1}{\sum_{i=1}^{S} p_i^2} * \frac{1}{S}
\]

(2)

I restricted my analysis to zooplankton only, including cladocerans and rotifers that invaded into the enclosures. This meant excluding the sporadic individuals from orders Coleoptera, Collembola, Diptera, Hemiptera, Thysanoptera, Trombidiformes, class Turbellaria, and phyla Nematoda and Tardigrada that also invaded the enclosures. I fitted linear mixed effects models using the LME4 package version 1.1 (Bates et al. 2015), with treatment, time, and the interaction of the two as fixed effects, and block and time as random effects reflecting the structure of my experiment. I then performed backward elimination of non-significant model effects using the
Kenward-Roger approximation for degrees of freedom in an F test in the pbkrtest package version 0.4 (Halekoh & Højsgaard 2014).

After determining that predation regime was an important predictor of assemblage diversity, I sequentially excluded each zooplankton taxon, and repeated the model analysis. When the exclusion of a taxon resulted in a model term losing statistical support (relative to the full analysis), I interpreted this post hoc result to mean that the individual taxon was one of the key components of the treatment effect.

Results

Taxonomic richness varied only by treatment ($F_{2,105} = 7.50, P = 9.0e-4$), because neither the interaction of treatment:time ($F_{2,103} = 0.25, P = 0.78$) nor the main fixed effect of time ($F_{1,2} = 6.54, P = 0.12$) was significant. Zooplankton assemblages in the salamander predation treatment were on average 0.5 taxa poorer than in the control treatments, and 1.0 taxa poorer than in the thinning treatments (Figure 2). This pattern persisted when I restricted my analysis to stocked taxa only, with the larger zooplankton (*Cypricercus* and *Simocephalus*) more likely to be absent in the predation enclosures than in the control or thinning enclosures. Results were qualitatively similar for rarefied richness, except that the thinning treatments were not richer than the control treatments after correcting for the differences in sample size (Supplementary Information: Rarefied Richness).

The effect of treatment on Simpson’s Diversity varied by time (treatment:time interaction $F_{2,103} = 4.43, P = 0.014$), and time was also significant as a random effect. Diversity was initially similar across all treatments but increased in the control and thinning treatments in the May 27 samples before returning to the initial range for June 9, whereas the salamander predation treatments registered a steady decline across all time points (Figure 3).

Simpson’s Evenness followed a broadly similar pattern (Figure 4) to that of Simpson’s Diversity, with control and thinning treatments both experiencing a peak in evenness on May 27 followed by a sharp decline on June 9, while the salamander predation treatments experienced gradually declining evenness over time. The most parsimonious model indicated that the effects of treatment depended on time (treatment:time interaction $F_{2,103} = 3.45, P = 0.035$).
The trajectories of the Simpson’s Diversity and Simpson’s Evenness indices can largely be attributed to the cladoceran Simocephalus. While the small cladoceran Chydorus was always more abundant than Simocephalus, Simocephalus grew quickly enough in the control and thinning treatments through the first four weeks of the experiment that assemblage evenness increased for this period. By mid-June, Simocephalus remained at around the same or slightly lower density it had attained in late May, whereas Chydorus continued its exponential growth, so evenness declined in the control and thinning treatments in June. In terms of frequency, Simocephalus increased from 2.5% and 2.7% of sample individuals in May 5 in control and thinning treatments, respectively, to 23% and 19% respectively on May 27, even though it was consistently rare or absent in the predation treatments. The increase was followed by a decline to 11% and 14% respectively for June 9. When I excluded Simocephalus from the data and repeated all the analyses, I found that Simpson’s Diversity varied only by treatment, and none of the fixed effects were predictive of Simpson’s Evenness.

Keratella also diverged between the predation treatment and the other two treatments. While Keratella invaded every enclosure, its density remained low in the control and thinning treatments, and its frequency declined as other taxa became more abundant. In contrast, in the predation treatments its density grew exponentially, increasing over 100-fold from May 5 to June 9 until it constituted around 80% of sampled individuals. Excluding Keratella from the data resulted in a consistent effect of treatment on Simpson’s Diversity at all times, indicating that Keratella was another driver of the diversity pattern. This pattern was mirrored by at least one other rotifer, Lepadella. Much rarer than Keratella, and absent entirely from most control and thinning enclosures, it nonetheless established small populations in most predation enclosures by late May. Lastly, excluding either Chydorus or cyclopoid copepods resulted in a consistent effect of treatment on Simpson’s Evenness across all times, indicating they were important drivers of the evenness pattern.

Salamander predation did not cause zooplankton populations to be more stable over time relative to non-selective mass removal or no predation (Figure 5). Coefficient of variation of abundance by taxon did not change systematically across treatments for the six zooplankton taxa that were introduced to the enclosures (MANOVA: Pillai trace = 1.14, $F_{12,14} = 1.56$, $P = 0.21$). However, the CVs of the set of diversity values (rarefied richness, Simpson’s Diversity, and Simpson’s
Evenness) did vary by treatment (Manova: Pillai trace = 0.694, F_{6,52} = 4.60, P < 0.001). Univariate tests of CVs indicated an effect of treatment on Simpson’s Diversity (P = 0.006) and Simpson’s Evenness (P = 0.030), and a marginally significant effect on rarefied richness (P = 0.068). Post hoc treatment contrasts by Tukey’s HSD indicated that CV of Simpson’s Diversity was lower in the predation treatments than in the control treatments (mean difference = 0.11, adjusted P = 0.005), and marginally lower in the predation treatments than in the thinning treatments (mean difference = 0.067, adjusted P = 0.099) (Figure 6). For evenness, Tukey’s HSD indicated that the control treatments had higher CVs than the thinning treatments (mean difference = 0.11, adjusted P = 0.037), and marginally higher CVs than the predation treatments (mean difference = 0.095, adjusted P = 0.081) (Figure 7).

**Discussion**

The aim of this study was to investigate whether frequency-dependent predation would translate into preservation of diversity in a prey assemblage. Frequency-dependent predation is a potential stabilizing mechanism in nature (Chesson 2000), and a lab study indicated that larval marbled salamanders selected some prey species in proportion to their frequency (Hutson *et al.* n.d.). However, it is unknown whether this behavior persists in more natural settings, and how it can influence community dynamics. I therefore evaluated the effect of selective predation by larval salamanders on an assemblage of zooplankton prey, to test whether predation increased diversity and reduced its variability. I contrasted selective predation by salamanders against nonselective thinning to evaluate the effect of salamander prey choice versus density reductions on the zooplankton assemblage.

I found that zooplankton assemblages experiencing salamander predation had 0.5 fewer taxa on average than did the corresponding assemblages in the control treatments, and one taxon less than in the thinning treatments (Figure 2) and this result was robust to analyses controlling for sample size (Figure S2). Simpson’s Diversity and Evenness had a more complicated temporal pattern, but these indices also generally had lower values in the predation treatments than in the control or thinning treatments (Figures 3, 4). Thus, selective predation led to reduced, not increased, diversity. In this regard, my results are consistent with individual predator species
directly reducing prey diversity (Schoener & Spiller 1996; Shurin 2001; Nordström & Korpimäki 2004; Urban 2013).

I also predicted that the zooplankton taxa experiencing selective predation would have the least variable populations and the greatest diversity, treatments with no predation would experience strongest competition and decreased diversity, and nonselective thinning treatments would fall in between. Instead, I found largely the opposite pattern, with the lowest diversity in the predation treatments and little difference between control and thinning treatments. Salamander preferences, while responsive to prey frequency, were not flexible enough to prevent exclusion of highly preferred prey, at least not in this simple system. Overall, assemblages experiencing salamander predation were less diverse, consistently so in terms of richness, and occasionally so in terms of evenness. Since there were no clear temporal trends in richness, the divergence – and subsequent convergence – must have been driven by changes in evenness, and specifically by population growth of Simocephalus. Assemblage diversity, particularly as measured in terms of Simpson’s Diversity, fluctuated the least in the predation treatments. This indicates that salamander predation stabilized diversity at a low level, largely by limiting Simocephalus. Other studies have also indicated that salamanders are capable of depressing or even excluding certain zooplankton – and especially large cladocerans and ostracods – in pond communities (Sprules 1972; Holomuzki et al. 1994; Urban 2013). While I did not track zooplankton biomass, I also noticed incidentally that when Simocephalus was present in predation enclosures, large individuals were rarer than in the control and thinning enclosures, also consistent with the results of Holomuzki et al. (1994).

There were no consistent trends for variability at the population level, but at the community level the variability of Simpson’s Diversity was lower in the predation treatments than in the no-predation controls, and marginally lower than in the thinning treatments. Therefore, to the extent that stability connotes low variability over time (McCann 2000, 2011), predation stabilized the zooplankton community, albeit at a decreased level of diversity compared to the control treatments, and it did not reduce variability of individual prey populations over time.

Theory indicates that a generalist predator that preferentially consumes frequent prey should promote prey coexistence (Chesson 2000). Frequency dependence can be explicit (Abrams & Matsuda 1996) or implicit, as when an optimally foraging predator selects between spatially
segregated prey patches based on total prey density (Holt 1984). At the same time, stochastic deviations from perfect frequency dependence, whether caused by predator behavior or by demographic noise, can reduce the effect of switching or overwhelm it entirely. Lagged switching in response to prey frequency (Abrams & Matsuda 2004) or reduced sensitivity to frequency altogether at low prey abundance (Abrams & Matsuda 2003) can alter the relative advantage experienced by rare prey. Socolar and Washburne (2015) show that when prey carrying capacity is low, even a strongly switching predator will tend to reduce diversity, since real populations are finite and discrete and therefore can be tipped stochastically toward extirpation as they grow smaller. This dynamic could have played out in my enclosures, since zooplankton did not attain typical pond densities until the second half of the experiment.

Therefore it is possible that the experimental outcome would have differed if the enclosures were larger, and the zooplankton assemblages had more time to develop prior to salamander introduction. Zooplankton densities increased over time in all treatments, potentially influencing population dynamics. My enclosures had a surface area of roughly 0.057 m$^2$ at 15 cm water depth, so the effective predator density was about 18 salamander larvae/m$^2$. This value is between what has been considered low density (4-8 larvae/m$^2$) and high (22-41 larvae/m$^2$) for A. opacum (Scott 1990), although those reported values are for hatchlings whereas I used older (overwintered) larvae. Taylor et al. (1988) reported springtime A. opacum densities of 0-7.5 larvae/m$^2$ from a South Carolina pond, although this species was rare relative to Eurycea quadridigitata, A. talpoideum, and Notopthalmus viridescens, which reached maximum densities of 27.5, 40, and 35 larvae/m$^2$, respectively, at the same site. Most recently, Urban (2013) reported A. opacum densities of 0-6.1 larvae/m$^2$ over five years in a set of ponds on Totoket Mountain, including B7, and stocked mesocosms to a density of 1.8 larvae/m$^2$, although those figures do not include larvae of other salamander species. Thus, prey-switching might have been weaker in the enclosures than it would be at lower predator densities.

Modeling highlights some of the ways in which predator behavior can interact with other drivers of coexistence, for example supporting or reducing the storage effect, which has previously been implicated as a stabilizing mechanism for certain zooplankton (Cáceres 1997). The storage effect enables coexistence when (1) competing species vary in their responses to a fluctuating environment, (2) there is covariance between inter- and intraspecific competition and the
environment, and (3) competitors have buffered population growth (Chesson 1994, 2000), as is the case with zooplankton resting eggs (Hairston 1996). Predation can lead to a competition-predation trade-off, an equalizing effect that reduces fitness differences between species and better positions the storage effect to stabilize the assemblage (Stump & Chesson 2017). Yet frequency-dependence arising out of optimal foraging behavior could undermine the storage effect, particularly if the trade-off between predation and competitive ability is not perfectly aligned (Stump & Chesson 2017). In other words, if a weaker competitor also happens to be highly preferred as prey, and thus experience no reprieve from predation in rarity, it could be excluded. *A. opacum* has distinct prey preference hierarchies (Hutson et al. n.d.; Urban 2013), and the literature reveals asymmetries in competitive abilities among zooplankton (Neill 1974; Gilbert 1985, 2012; Wallace et al. 2015), though competitive performance is still unavailable for many of the taxa in this study. Therefore predation by salamanders could be both stabilizing *via* frequency dependence and destabilizing *via* interference with the storage effect. Or, more simply, predation could be a stabilizing force for less preferred prey but destabilizing for highly preferred prey (Hutson et al. n.d.; Stump & Chesson 2017), driving them toward extirpation.

As a side note, while I attempted to minimize dispersal into and out of enclosures, enough connectivity remained that an increasing number of species invaded into my enclosures over time, including rotifers such as *Keratella* and *Lepadella*. Fortuitously, the unexpected invaders revealed a possible food web interaction mediated by salamander predation. Small rotifers are inferior competitors of larger microcrustacean zooplankton (Gilbert 2012; Wallace et al. 2015), and are typically rare or absent in pond samples from Northford. But in the predation treatment enclosures, where large zooplankton populations were suppressed, rotifers became abundant to the point of numerically dominating the assemblages, consistent with competitive release. In fact, the rotifers (and *Keratella* in particular) grew so abundant that the total zooplankton density in the predation treatment enclosures was on average 40% higher than in the control treatment enclosures (Supplementary Information: Figure S1), a result counter to that of Urban (2013). This result is consistent with that of Shurin (2001), who found that predaceous fish facilitated invasion of mesocosms by zooplankton from the regional species pool when dispersal was high.

Since evaluation of predator-prey dynamics and competition requires timespans of multiple generations (e.g., Huffaker, 1958; Krebs et al., 1995; Turchin, 2003; Stomp et al., 2004; Jiang &
Morin, 2007), it was encouraging to observe population growth among the cladocerans and cyclopoid copepods. However, I found very few juvenile ostracods or harpacticoid copepodites across all samples. It is not clear whether this was due to differences in reproductive strategies – e.g., ostracods of the genus Cypricercus tend to be univoltine (Turgeon & Hebert 1995) – or unsuitability of the enclosure habitat. A more thorough evaluation of the role of frequency dependence in stabilizing pond zooplankton assemblages would follow multiple generations of all prey, and ideally of the predator, too. Furthermore, my decision to stock one salamander larva per enclosure for the predation treatment precluded inter- and intraspecific interactions with other predators. Multiple predators foraging together can combine to deplete prey independently (additively) or nonlinearly (synergistically or antagonistically), so tests of individual predators might be poor predictors of multiple predator effects (McCoy et al. 2012). Indeed, in ponds where spotted salamanders (A. maculatum) are locally adapted to A. opacum, the former has a stronger tendency to consume prey that the latter avoids (Urban 2013). Lastly, enclosures sometimes can distort biological processes and yield results that do not translate directly to real ecosystems (Scott 1990; Holomuzki et al. 1994; Schindler 1998). This provides an argument for whole-ecosystem manipulations for greater inferential strength, e.g. to remove A. opacum where it is present and introduce it where it is absent. However, whole-ecosystem experiments come with attendant challenges of control and replication (Schindler 1998). Therefore small-scale enclosures have an important role insofar as they can yield valuable and surprising insights into biological processes that are challenging to isolate on a larger scale (Srivastava et al. 2004; Stewart et al. 2013) and help generate nuanced predictions for larger-scale tests to follow.

Overall then, this study helps to narrow the envelope of uncertainty around predator influence on diversity. Frequency-dependent predation can stabilize a prey assemblage in theory, but in practice, the negative direct effect of predation on populations can overwhelm any positive indirect effects, at least under the constraints discussed above. Future work should improve on the realism of experimental conditions, relying on theory for guidance as to which conditions to manipulate.

Recent theoretical developments suggest that higher-order competitive interactions between three or more species might be decomposable into constituent pairwise interactions (Grilli et al. 2017). Insofar as predation can drive apparent competition (Holt 1977; Chase et al. 2002b; Holt
& Bonsall 2017) that resembles pairwise interaction, it is tempting to speculate that predation — including the frequency-dependent variant — will eventually inform similar higher-order interactions. More immediately, mechanistic models of adaptive prey choice could better predict predators’ switching responses to diverse prey, including features like high attack rates on favored prey even at low frequencies (Hutson et al. n.d.; Charnov 1976; Stump & Chesson 2017). In turn, such models would help generate better predictions of community responses to predation, and ultimately improve our understanding of how diverse communities persist in nature.

Acknowledgments

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References


Table 1. Zooplankton introductions by date.

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<tr>
<th></th>
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<th>April 9, 2016</th>
<th>April 28, 2016</th>
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<td>1</td>
<td>4</td>
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<tr>
<td><em>Cypricercus</em></td>
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<td>43</td>
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<td>27</td>
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</tr>
</tbody>
</table>
Figure 1. Three experimental treatments arranged in a block, floated around a central stake. A salamander is visible at the five o’clock position in the bottom-left enclosure, and fine mesh windows are visible at the 12- and 3 o’clock positions in the right-most enclosure.
Figure 2. Zooplankton richness by treatment (n=30 measurements per treatment). Points show means, vertical bars show standard error.
Figure 3. Simpson’s Diversity by treatment over time. Points show treatment means (n=10 enclosures), vertical bars show standard error.
Figure 4. Simpson’s Evenness by treatment and time. Points show treatment means (n=10 enclosures) and vertical bars show standard error.
Figure 5. Coefficients of variation of abundances by predation treatment, for introduced zooplankton taxa. Each point corresponds to the mean CV (across n=10 enclosures per treatment) of a given taxon.
Figure 6. Coefficient of variation of Simpson’s Diversity. Figure shows treatment means (n=10) and standard errors.
Figure 7. Coefficient of variation of Simpson’s Evenness. Figure shows treatment means (n=10) and standard errors.
Supplementary Information

Density analysis:

Zooplankton density (evaluated as the logarithm of density) increased over time in all treatments (Figure 5). Backward elimination of non-significant model effects indicated that the interaction of treatment and time was non-significant ($F_{2,103} = 1.66$, $P = 0.20$), whereas treatment and time were both supported as main effects (treatment: $F_{2,105} = 6.21$, $P = 0.0028$ and time: $F_{1,2} = 178.5$, $P = 0.0056$).

Figure S1. The logarithm of sampled zooplankton density by treatment and time ($n=10$ enclosures per treatment). Vertical bars show standard error.
Rarefied richness:

Rarefaction to the minimum sample size across all times would be very conservative, especially because zooplankton population density increased through multiple orders of magnitude throughout the experiment (Figure S1). Therefore, I rarefied all samples within a single time step to the minimum sample size for that time, even though that would hamper comparisons over time. The rarefied sample sizes were 33 for May 5, 117 for May 15, 82 for May 27, and 187 for June 9. I conducted rarefaction using the package vegan version 2.4 (Oksanen et al. 2018).

Rarefied taxonomic richness varied only by treatment ($F_{2,105} = 40.01, P = 1.2e-13$), because neither the interaction of treatment:time ($F_{2,103} = 1.51, P = 0.23$) nor the main fixed effect of time ($F_{1,2} = 0.19, P = 0.71$) was significant. Zooplankton assemblages in the salamander predation treatment were on average one taxon poorer than were the corresponding assemblages in the control and thinning treatments (Figure S2). This pattern persisted when I restricted my analysis to stocked taxa only, with the larger zooplankton (Cypricercus and Simocephalus) more likely to be absent in the predation enclosures than in the control or thinning enclosures.

Figure S2. Rarefied zooplankton richness by treatment (n=30 measurements per treatment). Points show means, vertical bars show standard error.
Chapter Three:
Evaluating reinvasion success as an indicator of coexistence in the rocky intertidal

Abstract

Coexistence in nature is frequently assumed but rarely tested. Consequently, it is challenging to predict how communities will respond to disturbance without understanding the underlying mechanisms. I conducted an empirical test of the invasibility criterion for coexistence, evaluating whether three predominant space competitors in a rocky intertidal community could increase from experimentally induced rarity. I further manipulated predator access to manipulated plots via caged exclusion, because predation is a strong driver of community dynamics in the New England rocky intertidal system. I found that fucoid algae, barnacles, and mussels all increased from rarity, and with great consistency, suggesting that invasibility is likely in this system. Predator exclusion had a marginal influence on community compositional changes over a half year of plot recovery following targeted removals but did not affect reinvasion success. The rocky intertidal community is exemplary of a community that is robust to small-scale disturbance, and this study shows how the predominant members of a community could persist together over long periods of time despite – or even due to – such disturbance.

Introduction

The extent to which species in communities truly coexist (sensu Chesson, 2000), rather than their constituent populations simply co-occurring for long periods while drifting toward extirpation, is largely unknown (Siepielski & McPeek 2010; Levine et al. 2017). Historically, researchers often
assumed that all the members of a community coexisted through a mixture of positive and negative associations, without testing these assumptions (Clements 1916, 1920). Even in modern times, ecologists have made unsubstantiated claims about coexistence (Siepielski & McPeek 2010; Barabás et al. 2018), perhaps because of confusion about what coexistence really entails. Although there is no single definitive measure of equilibrium in nature (Mccann 2011), evaluating community robustness and stability can improve our understanding of the dynamics of community assembly and persistence, as well as resilience to environmental change. In turn, such knowledge is crucial for navigating the ongoing biodiversity crisis (Barnosky et al. 2012; Kolbert 2014; Pimm et al. 2014; Ceballos et al. 2015). The entire field of biodiversity and ecosystem multifunctionality is premised on the persistence of biological diversity for the maintenance of diverse functions (Hector & Bagchi 2007; Zavaleta et al. 2010; Maestre et al. 2012), yet there is much less attention given to how diversity can persist.

Coexistence occurs when the members of an assemblage can increase from rarity instead of becoming extirpated (Chesson 2000; Barabás et al. 2018). Coexistence can therefore be evaluated with the invasibility criterion (Chesson 2000; Siepielski & McPeek 2010; Levine et al. 2017; Barabás et al. 2018). This criterion states that members of an assemblage must invade and increase from rarity when the other species are at equilibrium. In a review of empirical studies addressing local coexistence, Siepielski and McPeek (2010) identified just seven studies that tested the invasibility criterion out of 323 that made statements about coexistence. Since Siepielski and McPeek’s critique, the outlook has improved, with researchers combining field and lab tests with observation and theory to evaluate mutual invasibility by co-occurring species (Hooper & Dukes 2010; Hart & Marshall 2012, 2013; Hart et al. 2012; Yi & Dean 2013; Zarnetske et al. 2013; Burns & Brandt 2014; Lamb et al. 2014; Godoy et al. 2014; Holt &
Much of this work has been limited to paired competitors, however, whereas communities in nature can consist of far more than two species, and they can experience emergent (Levine et al. 2017) and potentially interacting (Kuang & Chesson 2010; Stump & Chesson 2017; Barabás et al. 2018) mechanisms that affect diversity. Coexistence is a property of the entire community (Chesson 2003; Barabás et al. 2018), so community stability might not be predicted by whether or not any particular species pair meets the criteria for coexistence (Barabás et al. 2016, 2018; Levine et al. 2017). Thus, the study of coexistence requires a multispecies framework. Yet, of the aforementioned studies, only four tested mutual invasibility for three or more species at once, including two with plants (Hooper & Dukes 2010; Stein et al. 2016) and two with invertebrates (Hart & Marshall 2012; Cothran et al. 2015). A fifth study parameterized a multispecies model after empirically evaluating single-species germination rates in plants (Holt & Chesson 2014). And few of these studies have been conducted as field experimental manipulations of naturally occurring communities (but see Hart & Marshall 2012), limiting realism.

The rocky intertidal zone is a promising venue for empirical study of coexistence. Rocky intertidal communities have featured prominently in community ecology (Connell 1961; Paine 1966; Sousa 1979; Berlow 1999; Petraitis & Dudgeon 1999) because they are conducive to experimental manipulation of multiple species. To date, for example, one of the more comprehensive field tests of species coexistence was conducted on a pair of barnacle species (*Jehlius cirratus* and *Notochthalamus scabrosus*) growing in the high intertidal zone of the Chilean coast (Shinen & Navarrete 2014). This study revealed that the barnacles appear to be relatively neutral with respect to one another, without any indication of niche partitioning, and
thus probably persist via a lottery for space. Both species increased from rarity, satisfying the invasibility criterion for coexistence, but neither displayed stronger intraspecific density dependence than interspecific density dependence. The barnacles’ coexistence was thus probably dependent on environmental fluctuations (sensu Chesson 2000), more likely through the storage effect than relative nonlinearity of competition (Shinen & Navarrete 2014). For the storage effect, differential responses to the environment in combination with covariance between environment and competitive effects and a buffered population growth stage can allow coexistence in a variable environment (Chesson 2000). Shinen and Navarrete’s (2014) barnacles appeared to meet all three criteria, although there was insufficient information to make a conclusive determination. The authors dismissed nonlinearity of competition, where species differ in their population responses to a variable limiting factor (Chesson 1994, 2000), because there were no significant differences in mortality between the species. However, the authors acknowledged that some mortality due to competition could have occurred within the first few weeks after recruitment, before the animals were developed enough to allow differentiation between species in photographs. Another possible driver of coexistence, predation, was not considered in the study. Predation (and, more broadly, natural enemies) might drive stable coexistence directly when all prey have their own specialized predators (Janzen 1970; Connell 1971) or share a single frequency-dependent switching predator (Murdoch 1969; Chesson 2000). Alternatively, predation might be an agent of patch creation, enabling the storage effect, or a limiting factor driving nonlinearity (Chesson 2000).

While predation has not been tested as a driver of coexistence in the rocky intertidal zone, ample evidence shows that it influences the structure of rocky intertidal communities (Paine 1966; Lubchenco 1983; Menge 1991, 2000; Underwood 2000). Barnacles and mussels share a common
predator in whelks (*Nucella lapillus*), and a foundational study of prey switching showed that predatory snails can select between barnacle and mussel prey based on the relative frequency of each (Murdoch 1969). Another snail, the herbivorous common periwinkle (*Littorina littorea*), mostly prefers ephemeral algae but also plays a role in limiting *Fucus*, especially young germlings (Lubchenco 1978, 1980, 1983). Therefore, intertidal predators and consumers together might act in a frequency-dependent manner to promote invasibility by rare species. Alternatively, predation might not be frequency-dependent, in which case it could impede reinvasion, or even undermine other stabilizing mechanisms, such as by reducing the effect of environment-competition covariance (Kuang & Chesson 2009).

I therefore evaluated invasibility, with and without predation, for the three dominant and co-occurring space-holding organisms in the rocky intertidal zone of New England, the fucoid algae (*Fucus spp.*), barnacles (*Semibalanus balanoides*), and blue mussels (*Mytilus edulis*). All species are jointly limited by available space (Connell 1961; Bertness 2007), and their persistence suggests – but does not guarantee – that they coexist (Siepielski & McPeek 2010). I reduced the abundance of one focal species in each plot, in replicate plots, and included unmanipulated controls to track natural community dynamics. I then monitored reinvasion of the plot, simultaneously manipulating mobile consumer access *via* caged exclusion as a factor influencing reinvasion.

In sum, I sought primarily to determine whether the dominant members of the rocky intertidal community can invade from rarity, as a necessary precondition for coexistence. Secondarily, I evaluated support for predation as a driver of coexistence by manipulating consumer access with cages. If predation is frequency-dependent, then reinvasion should be most successful when predators are allowed free access, and least successful when predators are excluded. If predation
is not frequency-dependent, reinvasion might be more successful where predators are excluded, reducing pressure on rare species. I further predicted that if within-plot frequency-dependent dynamics govern diversity, then the local (patch/plot) species assemblage would return to its initial state following disturbance, no matter which species was removed.

Methods:

Natural History

In the rocky intertidal of New England, the common limiting resource of space defines a guild of sessile species that span both invertebrate filter feeders and macroalgal primary producers. The dominant species in the guild – fucoid algae, barnacles, and mussels – all reproduce sexually, develop through a planktonic phase prior to recruitment, and then compete for limited space as juveniles and adults (Menge 1976; Lubchenco & Menge 1978; Bertness 2007). As such, these sessile space-holding organisms in the mid intertidal constitute a reasonable assemblage (sensu Vellend 2016) to investigate.

The northern acorn barnacle (*Semibalanus balanoides*) is the most common species of barnacle found in the mid-intertidal (Bertness 2007), and it competes for space with the other filter feeders and primary producers. Individuals of *S. balanoides* also compete with conspecifics, both by exploiting resources (Bertness 1989) and by interfering with recruitment (Young & Gotelli 1988; Navarrete & Wieters 2000). Mussels of the genus *Mytilus* are competitively dominant over and facilitated by *S. balanoides* (Menge 1976; Menge et al. 2011), so they can form dense beds when not limited by predators (Menge & Sutherland 1976). Among the macroalgae that also compete for space with *S. balanoides* in the mid-intertidal, the fucoids *Fucus vesiculosus*, *Fucus distichus*,
and _Ascophyllum nodosum_ are the most abundant (Menge 1976). Experimental evidence from the Gulf of Maine suggests that juvenile barnacles inhibit fucoid growth, whereas adult barnacles facilitate it (Kordas & Dudgeon 2011). Fucoid algae are competitively dominant over barnacles when predation is minimal (Menge 1991), and they can inhibit settlement and establishment of barnacles by sweeping across the surface (Jenkins _et al._ 1999). Other species of algae are present, and among them, ephemeral algae (including _Ulva, Porphyra_) can inhibit recruitment of _Fucus_ if not grazed by snails (Lubchenco 1983). Mobile consumers include those that are active at low tide, such as birds (Ellis _et al._ 2005); high tide, such as crabs and fish (Edwards _et al._ 1982; Hunter & Naylor 1993; Rilov & Schiel 2006; Perez _et al._ 2009); or both, such as predatory dog whelks (_Nucella_), littorine snails, and limpets (Menge 1976; Menge & Sutherland 1976).

Recruitment of barnacles, mussels, and fucoid algae are strongly seasonal in New England, overlapping broadly, but also somewhat sequential. Barnacle recruitment at wave-exposed sites in Narragansett Bay occurs in late winter and early spring, starting in late February or early March, peaking mid-March, and tapering out by mid-April (Bertness _et al._ 1992; Leonard 2000). Mussel recruitment in New England varies from April to September in the Gulf of Maine (Leonard _et al._ 1998; Dudgeon & Petraitis 2001) to June to December in the Thames River estuary on Long Island Sound in Connecticut (Fell & Balsamo 1985). And fucoid algae recruitment has a peak in April and persists through much of the summer in the Gulf of Maine (Dudgeon & Petraitis 2001), the closest region for which data were available.

Study Site and Experimental Methods

I conducted my study at Beavertail State Park on the southern end of Conanicut Island in Rhode Island’s Narragansett Bay. I established plots across three different sets of rock platforms around
the park, several hundred meters apart (Figure 1), to reduce the risk of the entire experiment being disrupted by natural or human causes. I selected locations where the three focal species were abundant throughout, and where sufficient space was available with surfaces suitable for cage installation. Thus, I defined the mid-intertidal largely by the extent of the fucoid algae zone as done in previous studies (Menge 1976; Lubchenco 1980).

I monitored recruitment by installing artificial surfaces, then swapping the surfaces and counting new recruits. I used the recruitment surfaces primarily as positive controls, so that in case of a low rate of reinvasion I would know whether to attribute such an outcome to within-plot processes or to lack of regional recruitment. The recruitment surfaces included 10 x 10 cm Lexan squares covered with 3M Safety-Walk tape for barnacles (Farrell et al. 1991; Menge et al. 1999, 2003; Shinen & Navarrete 2014), Clorox SOS Tuffy sponges (Menge et al. 1999, 2003) and shag carpet squares (Menge 1991) for mussels, and travertine stone tiles for algae. The mussel recruitment surfaces were initially all SOS Tuffy sponges, but this product was discontinued mid-study. Thus, starting with the July 21 recruitment surface swap date, I switched to using carpet squares only, after having used only Tuffy sponges prior to then. Due to the lack of temporal overlap between recruitment surface types, quantitative comparisons were not possible across this division. I stratified the shoreline lengthwise into three sections at each site, counting along established plot locations (details below). For each recruitment surface within a stratum, I chose a random horizontal percentage between the first and last plot within the stratum, and then a vertical percentage from the lower to the upper extent of the mid-intertidal at that location. I installed one of each recruitment surface within each stratum, for three replicates per site and nine of each type overall.
For the experimental plots, I haphazardly chose plot locations across each site, although the spatial constraints caused the spatial distribution of plots to be somewhat uniform along the mid-intertidal contour line overall. If one or more focal species was absent at a chosen location, or if the substrate was too rugged or impenetrable for bolt installation, I repositioned the assigned location to the nearest suitable location. After marking two corners across the diagonal for each plot by installing stainless steel hex bolts, I assigned experimental treatments using a random number generator. For the removal treatment (4 levels), I removed all the fucoid algae, or barnacles, or mussels, or no organisms, from a 20 x 20 cm plot where all three had initially been present. Removing mussels frequently resulted in unintentional barnacle removals, whereas removing barnacles often resulted in the loss of fucoid algae. Especially in the latter case I attempted to leave in place individual barnacles with algal growth on them – though I was not always successful. Likewise, fucoid algae removals sometimes resulted in minor damage or outright destruction of individual barnacles, although this was a proportionally smaller impact than the incidental removal of a mature fucoid. *Fucus* regenerates readily from even 0.5 mm of holdfast (Malm *et al.* 1999), so I erred on the side of thoroughness for removals. For the predation treatment (3 levels), I installed either 20 x 20 x 4 cm predator exclusion cages (Menge 1976; Miller 2006; Miller & Gaylord 2007) (Type 304 stainless steel welded cloth from McMaster-Carr, with 0.55 x 0.55 cm openings, 0.81 mm wire diameter, 76% open area), open cage controls (same dimensions, with two sides cut away) hereafter termed “roof”, or no structure at all for an open control. I crossed removals and predation manipulations for 12 treatment combinations, and I replicated each combination three times per site for all three sites, for 108 total plots.
I conducted quarterly photographic surveys of the plots to measure growth rates of all species, including but not limited to the focal species. I primarily used a Nikon Coolpix S33 digital camera (13 MP, 30 mm focal length equivalent) for the photographs, although some photographs were taken with an Apple iPhone SE (12 MP, 30 mm focal length equivalent) with no obvious difference in clarity. For each photograph, I aligned a 0.25 m quadrat with the corner bolts of a plot, then aligned and centered the camera approximately 1 m above the plot so the quadrat walls overlapped with the top and bottom edges of the viewfinder. The initial set of pre-treatment plot photographs occurred between 27 December 2016 and 25 February 2017, progressing roughly linearly through sites L2, L3, and finally L1. Shortly after taking pre-treatment photographs – between 0 and 29 days later, and 9 days on average – I applied the experimental treatments and took post-treatment photographs. Consequently, the post-treatment photographs occurred between 28 December 2016 and 12 March 2017. I completed each of the remaining photographic surveys within less than a week, with the last survey running from 21 August to 23 August 2017. Whenever I encountered mobile consumers inside a caged exclusion plot, I recorded their identity and approximate size, and removed them from the plot prior to reattaching the cage. I assumed the no-removal treatment happened instantaneously, and therefore used the same photograph for both Initial (pre-treatment) and Treatment time steps. This decision could have influenced variability across time for the no-removal plots, but I did not do any comparisons over time with these plots so bias in variance over time was not problematic per sé.

Four plots – L2-19, L2-23, L2-24, and L2-26 – were accidentally scraped (barnacles removed from L2-26 and Fucus from the rest) before I was able to take Initial photographs. This oversight did not impact the reinvasion component of the experiment (details below), because I only compared Treatment to Recovery coverages. However, it could have affected ordination results
(details below). I estimated barnacle cover for L2-26 based on removal scars visible on the rock in the post-treatment photographs, and assumed negligible mussel and algae cover based on my notes of volume of material removed (a given coverage of barnacles fills less pace than comparable coverage of Fucus or Mytilus). For the other three plots, I estimated Fucus coverage based on field notes and surrounding area coverage, and then proportionally adjusted post-removal counts of understory organisms to add to 100%. Repeating ordination analyses with the four missing plots excluded did not affect my findings, so I show results based on including estimates in the main text and results with missing plots excluded in the Supplement (Sensitivity of ordinations to estimates of missing plot coverages).

Analysis

In multispecies communities, the invasibility criterion can be cumbersome to test. An equilibrium state with one fewer species might not even exist if removal of one species triggers secondary extinctions of other species (Barabás et al. 2016). In practice, the equilibrium state has been approximated by treating the remaining species as if they were at equilibrium from the moment of completion of targeted removals (Germain et al. 2018), including in the rocky intertidal (Shinen & Navarrete 2014). I adopted this approach and used both the ability of species to re-invade after experimental exclusion (Chesson 2000; Shinen & Navarrete 2014) and the tendency for communities on experimentally disturbed plots to converge with those on control plots to assess support for my hypotheses (Siepielski & McPeek 2010).

For coverage analysis, I picked the single best whole-plot photograph based on clarity. I used the Interactive Perspective Tool in ImageJ (Schindelin et al. 2012) to make minor perspective
adjustments to the photograph until the inner border of the quadrat had all 90° angles, as confirmed by making a rectangular selection. I then cropped the field of view to the square delimited by the two anchor bolts in the plot and rescaled the image to 2000 x 2000 pixels. I traced outlines of all identifiable organisms of a given type, then measured the area covered in pixels, and converted area to a percentage of the whole plot. Digitization of images offers good precision when species cover 30% of available space or more (Meese & Tomich 1992), as was often the case for the focal species in this study. I pooled coverage of Ulva, Porphyra, and other ephemeral algae into a single “ephemeral algae” group after initially counting as three separate groups, since they are functionally similar in their interactions with my focal taxa and with herbivores (Lubchenko 1978, 1986; Lubchenko & Menge 1978). I counted both Ralfsia and Hildenbrandia as “crustose brown/red algae” because they were difficult to distinguish in some photographs. I did distinguish between living and dead barnacles, since the latter could serve as a substrate for recruitment distinct from bare rock. The eleven groups I recognized were thus Fucus, Mytilus, barnacles (live Semibalanus), dead barnacles, Ascophyllum, coraline algae, crustose brown/red algae, ephemeral algae, hydroid, bare space, and debris, and together they accounted for 100% of cover in all plots.

I conducted all statistical analyses in R version 3.4.4 (R Core Team 2018). To evaluate the invasibility criterion, I evaluated whether each species was able to increase from rarity (Siepielski & McPeek 2010), and specifically whether each species increased its coverage after being removed. I also tested for the effect of predator exclusion on invasibility. I modeled increase from rarity as a binomial distribution to obtain a point estimate and confidence intervals, then evaluated whether the confidence intervals overlapped zero. I evaluated whether the proportion of plots showing an increase was greater than 50% using a one-sided proportion test.
with Yates’ continuity correction. Rejecting the null hypothesis that the proportion of plots showing reinvasion was less than or equal to 50% would indicate that a given species is more likely than not to reinvade following removal. I adjusted p-values using the Holm-Bonferroni correction (Holm 1979) to control the family-wise error rate for each set of comparisons. I also used a two-sided chi-squared test for equality of proportions to evaluate the effect of predator exclusion treatment on reinvasion success.

I evaluated whether the community recovered to its original state following disturbance by using non-metric multidimensional scaling (nMDS). I calculated Bray-Curtis dissimilarities for my community matrix after applying a square root transformation and Wisconsin double standardization, and I used $k = 3$ dimensions for ordination because stress exceeded 0.2 at $k = 2$ dimensions. I conducted ordination using the vegan package (Oksanen et al. 2018) and visualized results using ggplot2 (Wickham 2016). I ordinated the entire community dataset as a whole so that each timestep could be compared on the same MDS axes. nMDS is not a statistical technique, so I also statistically tested treatment differences with a permutational multivariate analysis of variance (Anderson 2001; Anderson & Walsh 2013), or PERMANOVA, on the dissimilarity matrix, also using vegan. For each timestep I used the model formula $Y \sim$ predation + removal + predation:removal (interaction), where $Y$ is the dissimilarity matrix. I ran 9999 permutations stratified by site to reflect real structure. Sums of squares were calculated sequentially, and I evaluated the effect of removal treatment after the effect of predator treatment to set a more stringent criterion for evaluating removal effects. Given the support for a predation:removal interaction, I display nMDS results grouped by both removal and predation treatments in the Supplement. I display groupings by removal treatment alone in the main text
for clarity, since the results are qualitatively similar. I also visualized the coverages of each focal species over the three time points to aid interpretation of compositional changes.

Results

Barnacle recruitment occurred during February-April 2016. The first cyprids began appearing on exposed natural surfaces at site L1 in late February, before I had installed recruitment surfaces. Recruitment to the plates both peaked and concluded during the second period, with barnacle cyprids settling even directly underneath the recruitment surfaces. From the third period onward, I did not find a single new recruit on any artificial surface through the end of the study. Fucal recruitment surfaces failed to show any recruitment. *Fucus* did appear on top of freshly recruited barnacles, indicating that recruitment was occurring on natural substrates. Mussel recruitment continued through the duration of the experiment, with a range of 66-533 recruits per artificial substrate unit per survey period. In addition to larval recruitment, the artificial recruitment surfaces revealed a second source of mussels. Between 0 and 15% of mussels found in any single recruitment substrate were 3 mm or larger (and as large as 9 mm), a size they were unlikely to have reached in one month (Kautsky 1982). These mussels were probably dislodged from their initial sites of recruitment and transported along the shore prior to (re)attachment (Paine & Levin 1981; Wootton 1993).

All focal species were more likely than not to regrow following removal: no confidence intervals overlapped 0, and there was evidence at $\alpha = 0.05$ to reject $H_0$ that the proportion of plots with regrowth was less than or equal to 50% (Table 1, Figure 2a). The amount of regrowth was substantial, on average 20% or more (Figure 2b). Part of this regrowth might be attributable to
the increased bare space created by removals (Figures 3 and S1), but each focal species increased in coverage by a greater amount when invading than when it was a resident (Figures 4 and S2), indicating that reinvasion was not simply a matter of bare space provisioning. Regrowth happened across all predator exclusion treatments, so there were no significant differences by exclusion treatment even before applying a Holm-Bonferroni correction (Table 2).

The PERMANOVA revealed a significant interactive effect of predation and removal ($R^2 = 0.11$, $P=0.007$) (Table S1), indicating differences between plots at the start of the experiment even though I completely randomized treatment assignments (Figures 5, 6, S4, S7). This appears to be due at least in part to the tendency for higher *Fucus* and lower *Mytilus* and barnacle cover in the plots assigned to the no-removal and roofed treatment combination compared to all other treatment combinations (Figures S4, S7). For example, excluding plots L1-1, L1-25, L2-22, L2-6, L3-11, and L3-12 from the data reduced the interactive effect to marginal significance ($R^2 = 0.088$, $P=0.059$). The effect was apparently only detectable in multivariate space, since I could not determine any single taxon or treatment combination as the sole driver. Upon application of treatments, all three treatment types diverged in separate directions of ordination space whereas untreated plots remained little changed. Examination of individual species showed that removal treatments successfully reduced coverages of target species while leaving non-target species unchanged, except for fucoid removal exposing barnacle cover that had previously been covered by the algal canopy (Figures 5, 6, S5, S7). PERMANOVA indicated an effect of removal conditional on predator exclusion ($R^2 = 0.050$, $P = 0.033$), and over half of the variability in plot composition could be attributed to removal treatment either in combination with predation as an interaction or as a main effect (Table S1). Finally, following recovery, all treated plots converged back toward the untreated plots in composition (Figures 5, 6, S6, S7). The interaction of removal
and predation treatments subsided as demonstrated by lower $R^2$ values, as did the main effect of removal, although the latter was still significant (interaction $R^2 = 0.07$, $P=0.06$; removal $R^2 = 0.06$, $P=0.01$). The persistence of the removal treatment effect was mostly due to the incomplete recovery of open plots with barnacle or mussel removals, as mussel cover did not increase in these plots the way it did in the remaining treatments (Figure S7). Support for both removal and predation disappeared entirely when these two treatment combinations were excluded, although the interaction between predation and removal remained just as in the pre-treatment timepoint.

From nMDS it is apparent that there was greater variance in composition across plots during the last census, meaning that there was some variability in terms of which species came to dominate cover in any given plot. Nevertheless, PERMANOVA indicates community composition converged across treatments in aggregate.

**Discussion**

Coexistence is a question of whether each species in an assemblage can increase from rarity, i.e., show positive population growth when it is at low abundance, while the remaining species are at their stable states (Chesson 2000; Barabás et al. 2018). I approximated an empirical test of the invasibility criterion in the rocky intertidal zone by removing one species each from a set of plots and tracking recovery. I further specifically tested the effect of predation on reinvasion by experimentally manipulating predator access to plots via caged exclusion and cage-controls with two open sides.

This study showed very clearly that barnacles, mussels, and fucoid algae all increased from rarity following removals. An overwhelming majority of plots with species removals showed an
increase in cover by the removed species during the recovery period (Figure 2a), and the increase in cover was usually quite large (Figure 2b). Moreover, each species increased its cover the most in the plots where it was the target of removal (Figure 4). In contrast, only mussel cover increased in plots with no removals, whereas barnacles and fucoids declined in the no-removal plots (Figure S2). The general pattern of recovery to a pre-disturbance state is consistent with other studies of disturbance and patch dynamics in the intertidal (Paine & Levin 1981; Berlow 1997).

Species dynamics translated into recovery at the community composition level, too. nMDS ordination, individual species coverages, and PERMANOVA showed modest differences in plot composition across assigned treatments at the outset. This indicates that some plots had meaningfully different cover from the rest at the start of the study, even though I assigned treatments randomly. The interaction persisted at approximately the same magnitude for the duration of the study (Table S1). Upon experimental removal, plot composition clearly diverged by removal treatment, as expected (Table S1, Figures 5, 6, and S4-S7). Differences by removal and predation treatment combination remained modest at this point, which was unsurprising since the treatment census occurred within 30 days or fewer of the Initial (pre-treatment) census, and within less than two weeks on average, leaving little time for predation effects to develop.

After that, all focal species were significantly more likely than not to increase their cover in plots where they were removed, with 85-100% of plots exhibiting positive growth of removed species (Table 1, Figure 2). Whereas regrowth proportions were consistent across and independent of predator exclusion treatments (Table 2), there was a marginal effect of predation on plot composition (dissimilarity), conditional upon removal treatment (Table S1). In particular, final coverage of mussels was lowest in open plots where mussels or barnacles had been removed.
(Figure S7). Prior work has shown that full recovery of a patch can take several years (Paine & Levin 1981), so it is possible that recovery was still in progress. Repeating the PERMANOVA analysis after jointly excluding barnacle and mussel counts from the data eliminated the marginal main effect of predation, whereas excluding Fucus from the data strengthened support for predation. This suggests that barnacles and mussels were in fact the species that responded most strongly to predator exclusion treatments.

The weak effect of predation on reinvasion per sé is surprising, however, and difficult to attribute without further study. Various cage effects have been documented in rocky intertidal settings (Schmidt & Warner 1984; Hayworth & Quinn 1990; Tomanek & Sanford 2003; Miller & Gaylord 2007), but the similarity of outcomes across all predator exclusion treatments suggests cage effects were negligible in this study. Possible explanations are that predation was not strong enough to influence reinvasion at the scale of my study, or the negative effects of consumption outweighed the positive effects of frequency-dependence (Socolar & Washburne, 2015; Hutson Chapter 2). The widespread presence of mussels and barnacles, especially at sites L2 and L3, might indicate high wave exposure that limited predation (Menge 1976; Menge & Sutherland 1976).

Based both on reinvasion success and convergence of composition between disturbed and undisturbed plots, all species appeared to satisfy the invasibility criterion. I did not repeat removals while waiting for the remaining species to equilibrate in the plots following the initial disturbance, so this experiment was most directly a test of recovery following disturbance, showing clearly that all removed species can return following a disturbance. The “ideal” experiment of invasibility might be difficult to implement for two key reasons. First, the community might continue to collapse following removal of one species, with a chain of
secondary extinctions (Barabás et al. 2016). At that point, it would be a different community that
the original species was attempting to invade, muddying inference about coexistence. For
example, removing barnacles often resulted in incidental removal of fucoids that had been
growing on top of the barnacles. Second, in a variable environment, the whole concept of an
equilibrium state is questionable. Along the shores of New England, the standing members of the
rocky intertidal community experience predation and competition for space, desiccation, wave
shock, and scouring by cobbles or ice (Menge 1976; Lubchenco & Menge 1978; Lubchenco
1980). The intertidal is famously in a constant state of flux, with patches prone to appear at any
time (Dayton 1971; Menge 1976; Paine & Levin 1981). A summertime storm destroyed one of
my cages (L2-2) between July and August, with a hard object gouging a trail through the middle
of the plot – incidentally, comparable in size and shape to some of the mussel removal scars I
had created (Figure S7). Thus, even with removals, the patches I created with targeted removals
were comparable to real patches, but carefully controlled and replicated.

There are two cautionary points to make regarding experiment effects with Fucus. The first is
that incidental removal of Fucus from barnacle removal plots could have influenced plot
conditions. Mean incidental removal of Fucus from barnacle removal plots was 12% of total
cover, or 33% of fucoid cover (before/after paired t-test t-statistic = -4.91, df = 26, p = 4.3e-5).
Algal removal could reduce the whiplash effect, whereby wave-tossed fronds impede larval
Conversely, the negative effect of algal canopy on barnacle recruitment can be moderated by the
presence of adult barnacles (Jenkins et al. 1999) and further counteracted by the positive effect
of canopy on post-recruitment survival (Jenkins et al. 1999; Beermann et al. 2013). I found that
barnacles increased in cover in a lower proportion of plots where Fucus was the target of
removal (0 of 27) compared to plots where no species was removed at all (3 of 27). But algal regrowth could have masked barnacle regrowth in the first group, since *Fucus* forms a canopy, obscuring barnacles below, so I cannot dismiss the possibility outright. In any case, the only way to reduce incidental loss of *Fucus* would have been to remove fewer barnacles, which would impede interpretation of barnacle recovery from the invader state.

Fucoid canopy might also increase sampling bias and error. The canopy biases lower the coverage in photographs of all understory organisms, including barnacles, mussels, and encrusting algae, and can increase sampling error by obscuring shifts in their relative proportions. Nonetheless, the overwhelming regrowth of barnacles following removal – including in plots where I measured greater algal cover following barnacle removal than at the initial census – suggests that barnacle recovery was not merely an artifact of experimental or sampling bias. Likewise, the reinvasion of mussels also appears robust to sampling bias. *Fucus* cover did not show a strong trend from Treatment to Recovery time in plots without species removals (mean difference -6.5 % cover, paired t-test t-statistic = -1.06, df = 26, p = 0.30), although there was a marginally significant decrease in cover in the subset of plots without any cages at all (mean difference -18 % cover, paired t-test statistic = -2.20, df = 8, p = 0.059). Therefore changes in *Fucus* cover are likely insufficient to explain why mussel cover increased in 26 out of 27 plots following targeted removal of mussels.

Also of note, winter storms in particular are an important driver of patch creation (Paine & Levin 1981; Sousa 1984), and the early timing of barnacle recruitment, prior to the start of mussel recruitment, appears suitable for rapid colonization of patches. Barnacles are inferior space competitors against mussels, yet mussels are limited in their ability to recruit to bare substrate (Dayton 1971; Menge 1976; Menge *et al.* 2011). Indeed, in the variable environment of the
rocky intertidal, patchy extinction-colonization dynamics are likely to play an important role in regional diversity (Caswell 1978; Paine & Levin 1981; Leibold et al. 2004; Gouhier et al. 2011). But similar to other studies in the intertidal (Menge 1991; Caro et al. 2010; Rilov & Schiel 2011), regional dynamics alone do not sufficiently explain patch-level dynamics like invasibility, unless perhaps the timing of my experiment happened to align (Paine & Levin 1981) such that all species could invade. For a single round of removals, initiating the experiment in late winter was a reasonable choice given that patches of open space are likely to form in winter, when large storm-driven waves are likely to batter the shore (NOAA National Data Buoy Center 2018). However, it would be instructive to continue or repeat removals staggered throughout the year, e.g. after barnacle recruitment has concluded but while the other species are still recruiting, to determine whether invasibility is consistent or dependent on removal timing. Predators might be another important driver of patch creation (Dayton 1971; Sousa 1984), and their effects need not be constrained to winter. Therefore the exclusion cages could have a different effect on reinvasion for an experiment initiated in spring or summertime.

For competitive assemblages to persist over long time spans, their members must minimize average fitness differences or experience stabilization (Chesson 2000; Adler et al. 2007). This study contributes evidence that local coexistence is possible for a widely recurring set of species, conditional on recurring disturbance across the landscape. Predation did not affect the likelihood of reinvasion in my study, but predators might still potentially act as a spatially variable agent of patch creation, thus enabling coexistence via a storage effect or competition-colonization tradeoff (Chesson 2000). In nature, multiple mechanisms might be operating in tandem or in opposition (Chesson & Kuang 2010; Kuang & Chesson 2010; Stump & Chesson 2017), and teasing these mechanisms apart will require identifying first whether species are coexisting, and
second which mechanisms are responsible. A growing body of theory and evidence (Wildová et al. 2012; Zhao et al. 2016; Stump 2017; Stump & Chesson 2017) indicates ecologists should also not overlook the role of equalizing forces that reduce average fitness differences and make lengthy co-occurrence more likely. Where species are very similar, dynamics can even appear neutral (Siepielski et al. 2010). There might even be evolutionary pressure for similar competitors to evolve yet greater similarity (Abrams 1987; Fox & Vasseur 2008; terHorst et al. 2010). Ultimately, the communities we observe in nature are likely mixtures of differentiated coexisting species and similar co-occurring ones. Future efforts should continue to untangle the mechanisms that could allow diverse species to persist, so that we are not caught off guard if those mechanisms fail.

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Ecol.*, 324, 31–43.


with Evidence for Differences in the Time of Maximal Settling along the Connecticut


Table 1. Proportions of plots where each species (columns) had positive growth following treatment application, defined as greater coverage after recovery period than immediately following removal. Results of a test for equality of proportions shown in two bottom rows. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1.

<table>
<thead>
<tr>
<th></th>
<th>Fucus</th>
<th>Barnacle</th>
<th>Mussel</th>
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<tr>
<td>Proportion of plots</td>
<td>0.85</td>
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<td>0.96</td>
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<td>with regrowth of focal species (n = 27)</td>
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<tr>
<td>χ² statistic,</td>
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<td>H₀: proportion ≤ 0.5</td>
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<tr>
<td>Holm-Bonferroni adjusted p-value (df=1)</td>
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<td>&lt; 0.001 ***</td>
<td>&lt; 0.001 ***</td>
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Table 2. Proportions of plots with regrowth of focal species, by predator treatment. N = 9 plots per removal:predator treatment combination. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1.

<table>
<thead>
<tr>
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<tr>
<td>focal species regrowth by predator treatment (open / roof / cage)</td>
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<td>1.0 / 1.0 / 1.0</td>
<td>1.0 / 0.89 / 1.0</td>
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<td>$\chi^2$ statistic for equality of proportions</td>
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<td>p-value (df=2), no correction</td>
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<td>1.0</td>
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Figure 1. Study location. I established site L1 at 41°27’18”N, 71°24’3”W; site L2 at 41°27’6”N, 71°24’2”W; and site L3 at 41°27’3”N, 71°23’50”W. Sites L2 and L3 are both exposed to open ocean, whereas site L1, set approximately 0.75 km back into the westernmost channel into Narragansett Bay, is partially sheltered from the heaviest surf. Note different map orientation in...
closest view. Maps generated with Google Earth using images from TerraMetrics, and data from Landsat/Copernicus, SIO, NOAA, US Navy, NGA, and GEBCO.
Figure 2. A. Proportion of plots where cover of focal species increased following experimental removal of that species, i.e., cover was greater at the Recovery timestep than at the Treatment timestep. B. Amount of increase in cover following removal. Bars show 95% confidence intervals.
Figure 3. Amount of bare space over time, by treatment. Points show means, vertical bars show standard error.
Figure 4. Change in cover of each focal species over recovery period, by resident vs. invader status. All species are residents in control plots. Points show means, vertical bars show standard error.
Figure 5. Non-metric multidimensional scaling plots of community dissimilarity with 95% confidence ellipses, grouped by removal treatment. Individual points represent experimental plots. Location of labels within figures, relative to the origin, indicates approximately how species abundances compare to axes, e.g., an increase along MDS1 axis corresponds to a decrease in fucoid cover in the present configuration. Ordination is for all plot measurements across all times, to allow comparison across panels, but each panel represents plot measurements from only a single time step. Time steps are pre-treatment (top panel), post-treatment (middle panel), and post-recovery (bottom panel). Note, ordinations are in k=3 space, but only the first two MDS axes are shown. Remaining MDS axes are shown in supplemental figures; images are qualitatively similar. nMDS ordination stress is 0.14.
Figure 6. Cover over time of focal species (in panels) by removal treatment. Points show means, vertical bars show standard error. Interacting effects of removal and predator exclusion treatment are shown in Supplemental Information Figure S6.
**Supplementary Information**

Table S1. Results of PERMANOVAs evaluating effect of predation and removal on community dissimilarity, with 999 permutations stratified by site, for the three timesteps (Initial, Treatment, and Recovery). Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1.

**Initial (Pre-Treatment)**

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<th>R²</th>
<th>Pr (&gt;F)</th>
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**Treatment**

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**Recovery**

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Figure S1. Amount of bare space over time, by removal and predator exclusion treatment. Points show means, vertical bars show standard error.
Figure S2. Change in cover of focal species over recovery period, by removal treatment (here denoted “Invader” to aid interpretation). All species are residents in control plots. Points show means, vertical bars show standard error. Change in cover corresponds to the difference between percent cover at Treatment and at Recovery time steps shown in figure S6.
Figure S3. Non-metric multidimensional scaling plots of community dissimilarity with 95% confidence ellipses, grouped by removal treatment (A = Fucoid algae, B = barnacle, M = mussel). Ordination results split into pre-treatment (top panels), post-treatment (middle panels), and post-recovery (bottom panels). Note, ordinations are in k=3 space, but only the first two MDS axes are shown. Leftmost panels in each row show MDS axes 1 vs 2, central panels show MDS axes 1 vs 3, and rightmost panels show MDS2 vs MDS3. nMDS ordination stress is 0.14.
Figure S4. nMDS ordination of Bray-Curtis community dissimilarity with 3 dimensions (stress = 0.14). Time shown is initial (pre-treatment). Centroids and 95% confidence ellipses shown for treatment combinations of species removal and predator exclusion (A = fucoid algae; B = barnacles; M = mussels). Note, only two MDS axes are shown.
Figure S5. nMDS ordination of Bray-Curtis community dissimilarity, with 3 MDS dimensions (stress = 0.14). Time shown is following species removals. Centroids and 95% confidence ellipses shown for treatment combinations of species removal and predator exclusion (A = fucoid algae; B = barnacles; M = mussels).
Figure S6. nMDS ordination of Bray-Curtis community dissimilarity, with 3 MDS dimensions (stress = 0.14). Time shown is post-recovery. Centroids and 95% confidence ellipses shown for treatment combinations of species removal and predator exclusion (A = fucoid algae; B = barnacles; M = mussels).
Figure S7. Cover over time of focal species (panels) by removal and predator exclusion treatment. Points show means, vertical bars show standard error.
Figure S8. Plot L2-2 on June 21 (left panel) and on August 21, 2017 (right panel). The cage was likely destroyed during the two weeks preceding the second photograph, when two storm systems brought heavy surf to the New England coast. Note the diagonal path gauged into the plot from the top left corner of the right panel.
Sensitivity of ordinations to estimates of missing plot coverages

To evaluate sensitivity of ordination results to my estimates of initial coverage for plots L2-19, L2-23, L2-24, and L2-26, I excluded all four from the dataset and repeated all ordination analyses for the Initial timestep. Ordinations look similar (Figure S9) and PERMANOVA results are unchanged (Table S2).

Table S2. PERMANOVA tables for Initial (Pre-Treatment) time step, with and without estimated plots included. Predation and removal treatments are predictors, community dissimilarity is response, with 999 permutations stratified by site. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1.

Estimated plots included

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Estimated plots removed

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Figure S9. nMDS ordination of Bray-Curtis community dissimilarity, with 3 dimensions (stress = 0.14). Left panel shows ordination with all plots included, right panel shows ordination with estimated plots excluded. Time shown is initial (pre-treatment). Centroids and 95% confidence ellipses shown for treatment combinations of species removal and predator exclusion (A = fucoid algae; B = barnacles; M = mussels). Note, only two of three MDS axes are displayed.