

The consequences of consumer diversity loss: different answers from different experimental designs

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Abstract. Predators are often the most vulnerable group to extinction, yet the consequences of changing predator diversity are poorly understood. One source of confusion has been different experimental designs. The multiple-predator effects literature typically employs an additive design, while the biodiversity ecosystem function literature typically uses a replacement design. Separately, these designs each detect only a subset of the changes in food web interactions caused by predator loss. Here, we measure the impact of consumer diversity on sessile marine invertebrates using a combination additive–replacement design. We couple this with a meta-analysis of previous combination experiments. We use these two approaches to explore how each design can detect different types of interactions among predators. We find that, while high diversity does lead to more negative interspecific interactions, the strength of these interactions is often weaker than negative intraspecific interactions caused by increasing the density of a single species alone. We conclude that a hybrid design is the optimal method to explore the mechanisms behind the effects of changing predator diversity. If researchers merely want to know the consequences of changing predator diversity, at a bare minimum, the experimental design must mimic the actual changes in both predator density and diversity in their system of interest. However, only a hybrid design can distinguish the consequences of shifting the balance of interspecific and intraspecific interactions within a community, an issue of great importance when considering both natural diversity loss and pest biocontrol.

Key words: additive design; biodiversity ecosystem function; Bodega Bay, California, USA; fouling community; intraguild predation; multiple-predator effects; replacement design; species interactions.

INTRODUCTION

In seeking to understand the consequences of global declines in consumer species diversity (Pauly et al. 1998, Petchey et al. 1999, Myers and Worm 2003, Baum and Myers 2004, Byrnes et al. 2007, Bruno and Cardinale 2008), researchers have unified the conceptual frameworks of the multiple-predator effects literature and the biodiversity and ecosystem function literature (Ives et al. 2005). Experimentally changing species number alters ecosystem structure and function under a wide variety of scenarios (for review, see Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006, Worm et al. 2006, Stachowicz et al. 2007) with the effects only increasing with increasing duration (Tilman et al. 2001, Cardinale et al. 2007). Consumer and predator extinctions can have large effects on the structure and function of natural communities (Estes and Palmisano 1974, Duffy et al. 2003, 2005, Dyer and Letourneau 2003, Borrvall and Ebenman 2006). Biocontrol of pests can similarly be altered dramatically as additional predator and parasit-

oid species are added (Rosenheim et al. 1993, 1995, Cardinale et al. 2003, Snyder et al. 2006). Generalities regarding the impact of altering consumer diversity have been slow to emerge, however (Bruno and Cardinale 2008).

One reason for the lack of generality may have to do with the broad range of potential interactions between predators themselves (Sih et al. 1998, Nilsson et al. 2006). Combining predators can lead to increases in top down control due to predator synergisms (Hixon and Carr 1997, Losey and Denno 1998, Cardinale et al. 2003), complementary prey usage (Stachowicz and Whitlatch 2005), or complementary behavioral responses by prey (Byrnes et al. 2006, Van Son and Thiel 2006). Alternatively, high predator diversity can increase the likelihood of antagonistic interactions that decrease top-down control by multiple predators (Finke and Denno 2004, 2005) and omnivory can short-circuit the potential for increases in top-down effects (Bruno and O'Connor 2005).

A second potential factor slowing the emergence of generalities may be the type of experimental design used in these experiments (Schmitz 2007). The multiple-predator effect literature has tended to utilize additive designs, in which prey consumption in treatments composed of a single individual of particular species

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TABLE 1. A comparison of different multiple-predator effect designs including what effects are detected, as well as the problems and drawbacks of each design.

What does it clearly detect?	Additive	Replacement	Combination
Synergistic interactions	x		x
Complementarity		x	x
Interspecific predation	x	x	x
Intraspecific predation		x	x
Effects of interspecific interactions	x		x
Effects of intraspecific interactions			x
Problems:	conflates density and diversity; partitioning of space, time, or resource base makes consumers appear substitutable	conflates changes in intraspecific and interspecific interactions; high-density monocultures may be logistically infeasible; can be biologically artificial	may be logistically infeasible

are compared to treatments with single individuals of two (or more) species. Less commonly used is the replacement, or substitutive, design in which predator density is held constant and only predator diversity is manipulated. Replacement designs, however, are common in the biodiversity and ecosystem function literature (Hooper et al. 2005, Spehn et al. 2005).

The central problem in comparing the results from these two types of designs lies in the types of interactions they reveal (Table 1). Additive experiments compare the effects of a species at low density to the effects of low densities of two or more species as modified by interspecific interactions. Replacement designs compare the effects of two or more units of low density of a single species as modified by intraspecific interactions to the effects of single units of low densities of two or more species as modified by interspecific interactions.

This is not a new problem, as animal competition studies have long used combination additive–replacement designs (Underwood 1978). However, the issue of whether to use either an additive or replacement design for multispecies experiments has been somewhat more contentious in the plant competition literature (Connolly 1988, 1997, Snaydon 1991, 1994, Sackville Hamilton 1994, Inouye 2001). Recent multiple-predator experiments using combinations of both types of designs (Vance-Chalcraft et al. 2004, Vance-Chalcraft and Soluk 2005, Griffen 2006, Griffen and Byers 2006, Douglass et al. 2008, Griffen et al. 2008) have again pointed out the potential problems in comparing results between the two. Additive experiments conflate density with diversity, while replacement experiments conflate reductions in intraspecific interactions with increases in interspecific interactions (Table 1). One solution is to run experiments over multiple generations until some equilibrium is reached, so that starting densities do not influence the final outcome. This may not be possible for long-lived species or species dependent on open recruitment. A short-term solution for pairwise competition experiments is to employ a response surface varying the density of two species in a factorial design (Underwood 1978, Inouye 2001). This gives a more accurate estimate of both

interspecific and intraspecific competitive effects. Furthermore, any design with both single- and multiple-individual treatments will be able to explicitly detect the relative strength of interspecific vs. intraspecific interactions. This piece of knowledge is critical to understanding the effects of altering predator diversity on top-down control of species, both with respect to diversity loss (i.e., extinction scenarios) or gain (e.g., pest biocontrol or species invasions), particularly when other predators change their density in response to changes in diversity.

Here we adopt this hybrid approach to understanding the effects of predator–predator interactions on top-down control of prey abundance by using a combination additive–replacement design, with both low- and high-density monocultures compared with polycultures of a constant density but at multiple levels of diversity. Using this design, we examine the role of consumer density and diversity in regulating the abundance of sessile marine invertebrates. We compare observed predation effects in polycultures with predicted values from both additive and replacement designs in order to tease apart the role of intraspecific and interspecific effects in modifying the strength of top-down control. We also perform a meta-analysis on other combination additive–replacement multiple-predator studies performed to date. We find that both our experiment and the meta-analysis point to a need to understand trade-offs between interspecific effects and intraspecific effects when consumer diversity is lost.

METHODS

Experimental study system

The docks and piers of Spud Point Marina in Bodega Bay, California, USA (38°19'41.60" N, 123°3'23.11" W) are covered by a diverse mixture of native and nonnative ascidians, bryozoans, sponges, polychaetes, and algae collectively referred to as a “fouling community” (for description, see Stachowicz and Byrnes 2006, Nydam and Stachowicz 2007). Sessile invertebrate biomass and composition on docks and nearby substratum can be under strong consumer control (Osman and Whitlatch 2004, Stachowicz and Whitlatch 2005, Nydam and

Stachowicz 2007). Four of the most common conspicuous mobile consumer taxonomic groups in Spud Point are crabs, limpets, urchins, and chitons (J. Byrnes, *personal observation*). Although these groups are comprised of multiple species, for our experiment, we used the most common single species from each of these taxonomic groups, the lined shore crab *Pachygrapsus crassipes*, the limpet *Lottia limatula*, the purple urchin *Strongylocentrotus purpuratus*, and mossy chiton *Mopalia muscosa* (J. Byrnes, *personal observation*). Hereafter we shall refer to them by taxonomic group.

Field experiment

Our experiment was designed to assess how different experimental designs altered conclusions regarding the effects of changing consumer diversity on top-down control of an early successional invertebrate community. We used a strict combination additive–replacement design, rather than similar designs that also seek to assess the role of density and diversity (O'Connor and Crowe 2005, Benedetti-Cecchi 2006), both for ease of interpretation and the ability to mechanistically tease out effects of intra- and interspecific interactions in modifying the impact of individual consumers. Treatment densities consisted of zero, one, or four individual consumers (Table 2). Treatments with four individuals were comprised of either one species, one of the possible two species pairs, or all four species (Table 2). During the second week of June 2007, consumers were placed on 17×20 cm roughened gray PVC panels that had been deployed two weeks earlier to allow for initial prey settlement. While panels had some initial settlers, cover of sessile species was typically $<1\%$. Panels were suspended within plastic cages with 1 cm diameter holes that allowed water flow and larval settlement, but prevented predators from migrating in or out of the cages. Panels faced downward at ~ 1 m depth. We checked cages after one month and replaced any missing consumers. At the end of August 2007, we sampled each box and recorded the percentage of bare space, the percent cover of each sessile species, and the number of missing consumers of each species on the embedded panel. Three cages failed, and data from these were excluded from the analysis (two controls and one crab–urchin treatment).

To assess the relationship between diversity and bare space (our measure of predator control of prey abundance) in cages that contained four individuals (i.e., excluding treatments with a single individual), we used a generalized linear model with a logit link and a binomial error (McCullagh and Nelder 1989) using R (R Development Core Team 2007). We used this function as values were bounded at 0 and 100 and the error changed with the predicted mean. To evaluate an intuitive measure of fit (Cameron and Windmeijer 1996), we provide the R^2 of the observed vs. predicted values (R^2_{COR}). In order to determine whether each individual species was actually exerting stronger top-

TABLE 2. Treatments from our dock predator caging experiment conducted at Spud Point Marina in Bodega Bay, California, USA.

Treatment	Sample size
Control (no predators)	6
1 urchin	6
1 chiton	6
1 limpet	6
1 crab	6
4 urchins	6
4 chitons	6
4 limpets	6
4 crabs	6
2 urchins, 2 chitons	4
2 urchins, 2 limpets	4
2 urchins, 2 crabs	4
2 chitons, 2 limpets	4
2 chitons, 2 crabs	4
2 limpets, 2 crabs	4
1 urchin, 1 chiton, 1 limpet, 1 crab	8

down control in high densities as compared to low densities, we compared the amount of bare space in low- and high-density monocultures for each consumer with a one-tailed t test. To correct for multiple comparisons, we used adjusted P values (P_{adj}) using the sharpened false discovery rate (FDR) correction (Verhoeven et al. 2005). We used the FDR because it imposes a linear adjustment on P values, rather than the more restrictive exponential adjustment of the Bonferroni correction.

To examine whether replacement or additive designs led to different conclusions about the effect of diversity, we performed a two-way factorial ANOVA with the difference between observed vs. predicted values for each high-density multispecies treatment as the response variable and treatment and prediction type as the independent variables. Due to the unbalanced nature of our experiment, we used type II sums of squares to assess significance (Langsrud 2003). We then used t tests to assess whether differences between observed and predicted values differed from 0. As there were 14 separate comparisons, we used adjusted P values (P_{adj}) using the sharpened FDR correction (Verhoeven et al. 2005). We performed the same analysis on monoculture observed vs. predicted values using only additive predictions.

To calculate the predicted additive value for a polyculture we used the following formula:

$$S_m = \min \left[\bar{S}_c + \sum_{g=1}^R N_g (\bar{S}_g - \bar{S}_c), 100 \right] \quad (1)$$

where S_m is the predicted amount of bare space in the mixture, constrained to be no more than 100%; S_g is the amount of bare space for species g in each low-density monoculture of species 1 through R that comprise a given mixture; N_g is the number of individuals of species g in the polyculture; S_c is the amount of bare space in the control. For S_g and S_c , we use mean values. As the experiment was sampled early in the successional

sequence of these docks (Nydam and Stachowicz 2007), they contained bare space that had not yet been colonized or overgrown. We corrected for predictions over 100% bare space by using 100 as a maximum possible value for S_m . We used a ceiling for our prediction rather than applying the commonly used multiplicative risk model (Sih et al. 1998) as the latter is based on encounter probability with prey decreasing as predators remove prey individuals (Soluk and Collins 1988). Typically, this is due to a fixed number of prey being stocked at the beginning of an experiment or new prey coming from reproduction within experimental enclosures. In contrast, (1) our plates were open to a constant rain of new settlers from the surrounding docks on the order of a hundred new individuals per 100 cm² fortnightly (J. J. Stachowicz, *unpublished data*) and (2) predators were able to freely wander around the entire cage and have access to all available prey. Individuals of each species were even observed moving from one side of plates to the other during deployment and subsequent sampling.

We calculated replacement design predicted values for a given polyculture by averaging the amount of bare space in the high-density monocultures that composed the polyculture of interest. To look for any nonadditivity in the density response of a single species, we used the additive model to calculate the predicted amount of bare space for each high-density monoculture.

We also examined the data for evidence of mechanisms that would lead to deviations from additivity: intraguild predation and the presence of grazing-resistant species. To detect intraguild predation, we compared the number of missing consumers among high-density treatments using ANOVA. We specifically tested the hypothesis that there is no difference in the number of consumers lost in treatments containing crabs vs. those not containing crabs using an a priori contrast. We also wanted to examine whether some consumers were unable to clear an area due to resistant or unpreferred species. Previous work (Nydam and Stachowicz 2007) and preliminary observations indicated that many encrusting bryozoans are not as readily removed by some consumers. We therefore compared bryozoan cover among high-density treatments using ANOVA and compared all treatments to the control using a Dunnett's test. For both ANOVAs, all assumptions regarding normality and homoscedasticity were met.

Meta-analysis of combination additive-replacement experiments

While full additive-replacement design experiments are uncommon, we performed a meta-analysis on results from the literature along with our own to determine whether there are any consistent trends. We searched the ISI Web of Knowledge (Thomson Scientific 2008) with the terms "additive AND (replace* or substitut*) AND (predat* or consum*)", "intraspecific AND interspecific AND predat*." We then evaluated results for whether

(1) studies had some measure of prey consumption or top-down control, (2) experiments actually used a simultaneous additive-replacement design, and (3) studies contained monocultures with single individuals (i.e., no intraspecific interactions). We found six papers that matched these criteria (Appendix). These studies yielded 13 comparisons between single- and mixed-species treatment and 18 comparisons between low- and high-density monocultures.

We extracted data that measured top-down effects (percentage of survivorship of prey, number of prey eaten, etc.) from papers using ImageJ (Rasband 1997). When possible, we also extracted additive or replacement predicted values. When predicted values were not directly provided, we calculated predictions for high-density monocultures and polycultures using the formula presented in a given study (i.e., we assumed the authors had selected the correct model for their particular study). Where no formula was provided for additive predictions, we either used a straight additive model or a multiplicative risk model, depending on whether prey encounter probabilities were likely to be reduced as predation continued in the study based on the authors' discussion of the predators' behavior. We calculated replacement design predictions by averaging high-density monocultures.

To compare predicted and observed values across studies we created a log response ratio (Hedges et al. 1999) of $\ln(\text{predicted}/\text{observed})$. We used this measure of deviation from predicted values with a weighted mixed-model categorical meta-analysis (Gurevitch and Hedges 2001). We compared deviations from predicted values from replacement and additive portions of the design in multispecies treatments. We then compared deviations from additivity for both multispecies treatments and equivalent density single-species treatments. Lastly, we repeated this analysis including our own data (seven multispecies comparisons and four single-species comparisons) to determine whether they would change the results.

RESULTS

Field experiment

Examining the high-density treatments and the no-consumer control, consumer diversity was positively correlated with the amount of bare space (diversity coefficient of link function = 0.937 ± 0.43 [mean \pm SE], $z = 2.175$, $P = 0.031$, $R^2_{\text{COR}} = 0.319$, $df = 57$; Fig. 1A). The relationship had plateaued, however, by four species because nearly all replicates achieved close to the maximum 100% bare space. Indeed, any treatment with a chiton present (regardless of diversity) had nearly 100% bare space. The relative importance of chitons becomes more apparent when looking at the high- vs. low-density monocultures (Fig. 1B). One chiton was equally capable of clearing an entire plate as four chitons ($t_{10} = -1.141$, $P_{\text{adj}} = 0.070$). For all other species, the high-density treatment had more bare space than

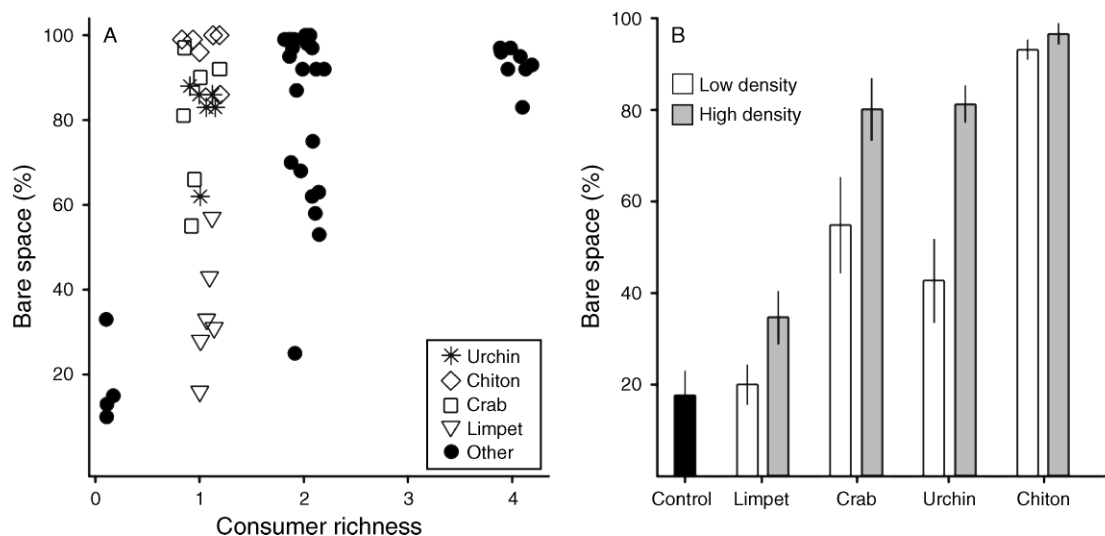


FIG. 1. (A) The percentage of bare space generated by different levels of consumer diversity. Diversity is positively correlated with bare space, although this relationship saturates fairly quickly due to treatment with chitons having nearly 100% bare space. Data points are offset for clarity. (B) Bare space generated by high-density (four individuals) vs. low-density (one individual) monocultures (mean \pm SE); one chiton is sufficient to nearly clear a plate. The experiment was conducted at Spud Point Marina in Bodega Bay, California, USA.

low-density treatments (crabs, $t_{10} = -2.045$, $P_{\text{adj}} = 0.034$; limpets, $t_{10} = -2.048$, $P_{\text{adj}} = 0.023$; urchins, $t_{10} = -3.890$, $P_{\text{adj}} = 0.004$), and no other monoculture was capable of creating 100% bare space on its own, even at high density.

Predicted amounts of free space differed from observed values in more than half of all species combinations (Fig. 2A). However, the frequency and sign of these deviations differed among additive and replacement models, resulting in an interaction between prediction

type and treatment ($F_{6,48} = 3.305$, $P = 0.008$). This can be seen on a biplot of the deviation from additivity vs. the deviation from replacement predictions, with most points clustering in the upper left quadrant, where deviation from replacement values are generally positive and deviation from additivity values are generally negative or neutral. In four of the five cases in which replacement predictions differed from 0, the difference was positive (crab–limpet, $t_{48} = 6.57$, $P_{\text{adj}} < 0.001$; limpet–chiton, $t_{48} = 7.133$, $P_{\text{adj}} < 0.001$; polyculture, $t_{48} =$

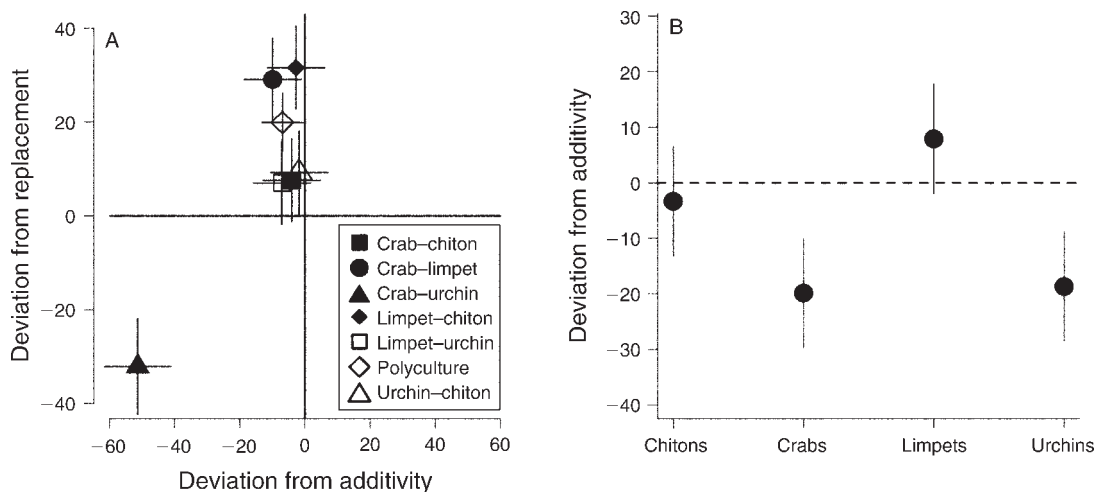


FIG. 2. The difference between observed and predicted levels of bare space using either additive or replacement design predictions and 95% confidence intervals. (A) Mixed-species treatments are placed on a biplot for clarity. They show a general pattern of observed values being greater than replacement predictions, leading to positive values, but equal to or less than additive predictions. The crab urchin treatment is an exception, with underyielding for both the additive and replacement treatments. (B) High-density (four individuals) monocultures in which observed values for both crabs and chitons are less than predicted under an additive model.

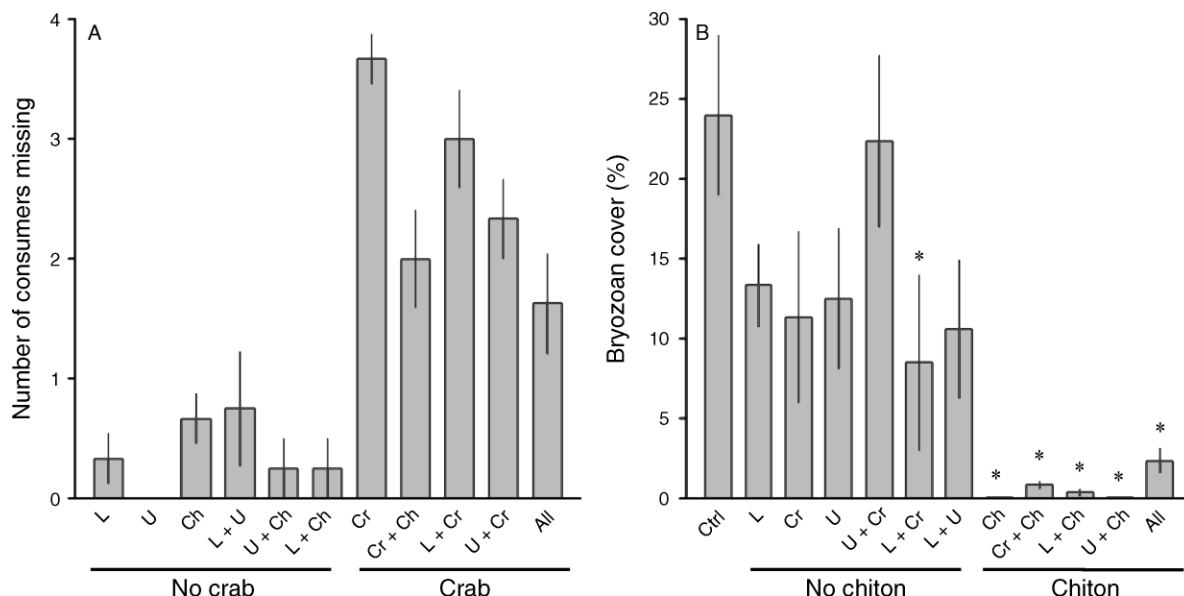


FIG. 3. Potential mechanisms explaining deviations from predicted values. (A) The number of missing consumers by treatment. Treatments that include crabs have lost more individuals than treatments without crabs ($P < 0.0001$). (B) Bryozoan cover by treatment. Bars indicate mean \pm SE. Bryozoans proved resistant to some, but not all, species. Treatment abbreviations are: L, limpet; U, urchin; Ch, chiton; Cr, Crab; All, all four species. Asterisks represent those treatments significantly different (at $P \leq 0.05$) from the control (Ctrl) using a Dunnett's test. With the exception of the limpet and crab treatment, the only treatments significantly different from the control included chitons.

6.361, $P_{\text{adj}} < 0.001$; urchin–chiton, $t_{48} = 2.089$, $P_{\text{adj}} = 0.026$), indicating that observed values of bare space were greater than predicted (i.e., overyielding). Notably, the only treatment in which the observed value was less than the replacement prediction (i.e., underyielding) was the crab–urchin treatment ($t_{48} = -6.275$, $P_{\text{adj}} < 0.001$). The observed amount of bare space for the crab–urchin treatment was also less than the additive prediction ($t_{48} = -10.040$, $P_{\text{adj}} < 0.001$). This was also true for the crab–limpet treatment and the polyculture (crab–limpet, $t_{48} = -2.240$, $P_{\text{adj}} = 0.025$; polyculture, $t_{48} = -2.197$, $P_{\text{adj}} = 0.024$). Observed values were never greater than their additive predictions.

Two of the four high-density monocultures differed from their additive predicted values, resulting in a significant ANOVA ($F_{4,20} = 8.303$, $P < 0.001$; Fig. 2B). Both high-density crab and urchin treatments had less bare space than predicted (crab, $t_{20} = -4.002$, $P_{\text{adj}} = 0.002$; urchin, $t_{20} = -3.767$, $P_{\text{adj}} = 0.002$). This indicates that something other than lack of consumptive ability was limiting these species from clearing off all panels at high densities.

We found some evidence that intraguild predation by crabs and the presence of resistant prey caused these differences between observed and predicted values. Treatments with crabs (including crab monocultures) lost more consumers than treatments without crabs ($P < 0.0001$; Fig. 3A). Some treatments had lower bryozoan cover than others ($F_{11,47} = 5.1871$, $P < 0.0001$; Fig. 3B). All treatments containing a chiton had less bryozoan cover than the control ($P < 0.05$). Chitons appeared to

be the only species capable of reducing bryozoan cover on their own, as all treatments with at least one chiton had negligible cover of bryozoans. Interestingly, the crab–limpet treatment also had less bryozoan cover than the control ($P = 0.048$). No other treatments differed from the control with respect to cover of bryozoans ($P > 0.05$).

Meta-analysis of combination additive–replacement experiments

Similar to some of our experimental results, deviations between additive and replacement predicted values for multispecies treatments differed ($Q_b = 26.00$, $df = 1$, $P < 0.001$), with observed values being higher than replacement predictions but less than additive predictions. This did not change when we incorporated our data ($Q_b = 38.93$, $df = 1$, $P < 0.001$; Fig. 4). This suggests that reducing intraspecific interactions while increasing interspecific interactions can lead to greater top-down control, whereas adding interspecific interactions when no other interactions are present generally decreases the strength of top-down control per individual predator. Observed values in both mixed- and single-species treatments were less than additive predictions, but data from experiments published to date showed no difference between the magnitude of single-species and multispecies deviations from predictions ($Q_b = 0.915$, $df = 1$, $P = 0.76$). However, once we added our data to the set, this changed; single-species treatments deviated more from predicted values than mixed-species treatments ($Q_b = 4.519$, $df = 1$, $P = 0.034$; Fig. 4). This agrees

with the analysis of additive vs. replacement predictions, as it suggests that intraspecific interactions are stronger than interspecific interactions between consumers.

DISCUSSION

Many designs are possible to examine the emergent effects of multiple consumers, each with their own advantages and drawbacks. While the strict additive and replacement series designs may be among the most easily interpretable, each is still limited in the mechanisms they can detect (Table 1). Failure to recognize these limitations can lead to erroneous conclusions. However, strong ecological interactions should transcend choices of design. For example, both the replacement and additive portions of our experiment showed that (1) chitons had a strong impact on top-down control at a small spatial scale and (2) combinations of crabs and urchins exert less top-down control than one would predict from looking at either species in isolation, regardless of density. Additional conclusions about differences between monocultures and polycultures in both our experiment and the broader literature (Fig. 4) differ based on choice of design. For example, in our experiment, results from a replacement design would have suggested that increasing consumer diversity increased the strength of top-down control, reducing cover of sessile species. Although we would have observed intraguild predation and resistant prey with this design, we would have concluded that their role in mitigating top-down control of sessile species over the summer was negated by the combination of a sampling effect (including chitons, a strong consumer) and weak complementarity between crabs and limpets. By contrast, if we had only used an additive design, we would not have observed that increasing the density of crabs and urchins does not increase top-down control as expected. This could have caused us to erroneously suggest that any monoculture, in high-enough density, would compensate for lost species.

Results from our field experiment agree with our meta-analysis of published studies that used combination additive–replacement designs with respect to differences between the two designs. Additive predictions, when they deviated from observed values, predicted a greater impact of multiple consumers on prey than observed. Replacement values almost always predicted less top-down control than was observed. Our results differ from the average results of previous work included in the meta-analysis, however, in that we show that the effect of within-species interactions are stronger than between-species interactions. One possible explanation for this difference is a publication bias of multiple-predator experiments using combination additive–replacement designs. In almost all cases, investigators chose consumers specifically to investigate interference effects among predator species and explore their consequences for prey populations (e.g., Griffen and Byers 2006). Our study, in contrast, chose the most common consumers found in the

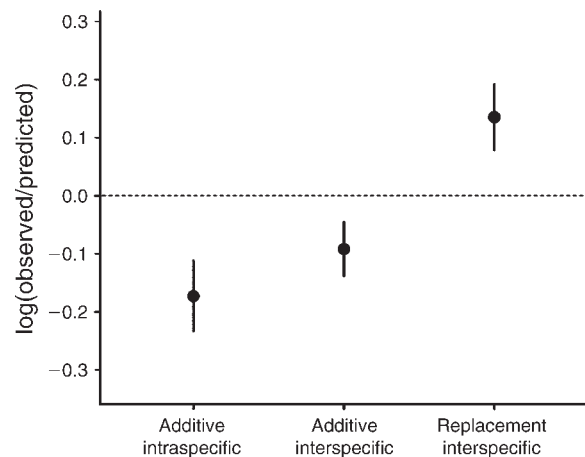


FIG. 4. The log ratio of deviations from predicted values in combination additive–replacement design experiments including data from our study. Bars represent 95% confidence intervals calculated using the weighted variance of each group of studies and the pooled variance of all three groups (Gurevitch and Hedges 2001). Observed values in high-density single-species treatments and mixed-species treatments are less than additive predictions. The deviation for single-species treatments is greater. Observed values in mixed-species treatments tend to be greater than replacement predictions.

field, with no preexisting bias toward the types of interactions in which they engage. Indeed, as several of the species we chose consume sessile species exclusively, negative interactions were more likely to occur from competition rather than intraguild predation. Non-agonistic interactions are likely underrepresented in previous combination experiments in comparison to their frequency in nature. Indeed, in some systems, both predator synergisms and inhibition are present (Nilsson et al. 2006). Similarly, most previous studies used only a single prey type. Multiple prey types allow for greater niche complementarity (Byrnes et al. 2006, Snyder et al. 2006, Griffen et al. 2008) and hence may reduce interspecific interference.

In many ways, replacement series designs represent a best-case scenario for community recovery following extinction. They assume that the community is able to compensate for the loss, and total number of consumers remains unchanged. Declining predation due to losses in diversity, however, cannot necessarily be made up for by increases in density of remaining species, particularly when intraspecific interactions are stronger than interspecific interactions. In such a case, as diversity declines and intraspecific density increases, intraspecific interactions that decrease the per capita strength of top-down control will come to replace the interspecific interactions formerly present in the community. The replacement portion of both our experiment and other additive–replacement experiments show that, when intraspecific interactions are replaced by interspecific interactions, mixed-species assemblages often perform as well or better than would be expected. As strong intraspecific

agonistic interactions may facilitate coexistence in nature (Rudolf 2007), this scenario may be common. Replacing interspecific interactions with intraspecific interactions therefore may actually limit top-down control as consumer diversity further declines, a point relevant to both natural diversity loss and considerations for pest biocontrol.

Additive, replacement, and combination designs are all useful, but the choice of design must be guided by the underlying research question and interactions of interests by a given investigator (Table 1). All three designs should be able to determine whether species within a guild actually prey upon one another, although the relative influence of this factor on predicted consumption of prey will vary among designs. If a researcher wishes to examine how interspecific interactions among predators affect top-down control, an additive design is more appropriate, as its predictions will not be confounded by the release from intraspecific interactions. If, on the other hand, the issue of interest is comparing the functioning of a system dominated by intraspecific vs. interspecific interactions, a replacement design should be used. Furthermore, a replacement design is better suited to detecting complementarity. If predators partition prey species, spatial locations, or time of foraging, an additive design is likely to erroneously conclude that prey are functionally substitutable (Schmitz 2007) as their impact in polyculture may combine linearly despite predators performing very different and nonoverlapping roles in a food web. One reason for the popularity of the replacement design in biodiversity and ecosystem function research is precisely for its ability to avoid this problem. However, a replacement design may overemphasize the strength of complementarity and synergistic interactions relative to agonistic interactions, compromising the estimate of the net diversity effect. If a researcher is solely interested in particular mechanisms behind multiple-predator effects, an additive or replacement design may be all that is necessary. However, if the goal is to estimate the overall impact of consumer diversity gain or losses, researchers may want to consider either a combination or full response surface design.

Different inferences can be drawn from combination designs depending on the abundance of individuals in low-density single-species treatments. In our experiment, our low-density single-species treatments contained a single individual. This allowed us to fully calculate the effects of within- and between-species interactions. By definition, however, our design could not have included a multispecies treatment with the same density. Thus, we were unable to statistically evaluate a richness \times density interaction in a full factorial model. Other experiments that have used the number of species as the low-density abundance and then included a high-density abundance at twice the number of species or more can use such a model (Douglass et al. 2008, Griffin et al. 2008). However, they cannot accurately calculate the effect of

species interactions on per capita feeding rates due to the lack of a single-individual treatment.

While a combination additive-replacement design may be the best design to evaluate the consequences of changing consumer diversity, under some scenarios it may be unnecessary or logistically not possible. For example, if an experiment is run over sufficient predator generations that equilibrium is reached, the two designs should produce qualitatively similar results. As densities of species in each treatment should move closer to this level of equilibrium, one might expect the initial signature of the additive or replacement design to disappear (see Tilman et al. [2001] for terrestrial grasslands, Duffy et al. [2003] for herbivores, Stachowicz et al. [2008] for seaweeds), although this may not always be the case. The strengthening of diversity effects over time (Cardinale et al. 2007) may be a signature of communities moving beyond the initial constraints of their design. Natural patterns of density and diversity may also suggest one design or the other. For example, if density is constant regardless of diversity (e.g., for many sessile invertebrates and plants), then a replacement design may be all that is needed. However, this will still yield an incomplete answer with respect to the effects of changing the types of species interactions. If a combination design is not logistically tractable, we recommend separate investigations into intraspecific vs. interspecific effects (Guidetti 2007), although results might not be directly quantitatively comparable, making net diversity effects difficult to estimate. Similarly, it is possible that a response surface design, manipulating density and diversity without regard to strict monocultures, may be useful in situations in which a researcher wishes to look at a large number of species and get an estimate of the functional form of the effects of density and diversity.

As we are confronted by the continued loss of biological diversity worldwide and as some species become established in new regions as a result of human-mediated invasions, it is crucial to understand the consequences of these changes in diversity for food web structure and ecosystem function. Given that extinction can be biased toward higher trophic levels (Pauly et al. 1998, Petchey et al. 1999, Myers and Worm 2003, Baum and Myers 2004, Byrnes et al. 2007), we need to understand the consequences of changes to food web architecture (Bruno and Cardinale 2008). It is imperative that, in asking these questions, researchers employ approaches that will lead to clear, concise answers rooted in how nature works, rather than their particular experimental design. This may require a design such as that presented here or something simpler. In the end, it must be the question and mechanisms of interest that drive the design and analysis, lest important interactions and roles of species be overlooked.

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APPENDIX

References used for the meta-analysis (*Ecological Archives* E090-204-A1).

SUPPLEMENT

Table of all data used in the meta-analysis (*Ecological Archives* E090-204-S1).