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Detecting emergent effects of multiple predator species

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Abstract When foraging together, multiple predator species that share a single prey often cause prey mortality that cannot be predicted based on knowledge of predation by each species separately. Modeling and managing the effects of multiple predator species depend on accurately assessing these combined effects. Two methods are currently used to experimentally examine combined predation by multiple predator species: the additive and substitutive experimental designs. I simultaneously employed both experimental designs to examine predation by two crab species on shared mussel prey. I show that the two methods yield results that disagree both quantitatively and qualitatively, leading to very different conclusions about the way that predator species combine to affect prey mortality. This discrepancy occurred because the two methods examine complementary, but not interchangeable questions. I advocate using an experimental design that incorporates both additive and substitutive designs to achieve a more complete understanding of the combined effects of multiple predator species.

Keywords Additive · Predator interference · Replacement series · Risk enhancement · Risk reduction

Introduction

Much of the early empirical and theoretical work on predator–prey interactions involved only a single species of predator. However, in reality most prey are subject to consumption by multiple predator species. This awareness has led to considerable attention over the last

decade on the combined effects of multiple predator species on shared prey. Predation by multiple species often does not combine additively, rather predation is often greater (risk enhancement) or less (risk reduction) than additive. Both of these impacts are broadly termed emergent multiple predators effects (Sih et al. 1998), because they cannot be predicted based on knowledge of predation by each species separately. However, nonadditive predation can also occur when multiple predators of the same species forage together (Beddington 1975; Hassel 1978). Thus effects of multiple predator species are only truly emergent if the degree of nonadditivity is different than that resulting from interactions between conspecific predators (Sih et al. 1998; Vance-Chalcraft et al. 2004).

Emergent effects of multiple predator species can have important ecological and economic implications. For example, the effects of predator species may combine synergistically to limit the expansion of nonindigenous prey (Harvey et al. 2004) or to suppress pest species in agroecosystems (Losey and Denno 1998) with subsequent positive effects on economically important crops (Cardinale et al. 2003). Alternatively, predator species may interfere with and/or consume each other, thus decreasing their combined effectiveness for controlling prey populations (Sih et al. 1985; Rosenheim 1998). The practical importance of emergent multiple predator effects to wider community dynamics illustrates the need to accurately detect their presence.

Two different experimental designs are commonly employed to detect risk reduction or risk enhancement: additive and substitutive (also referred to as replacement series) experimental designs. Both designs compare predation by each species separately to predation when the species are combined. However, the two methods differ in the way that predator density and predator identity are manipulated. The more common additive experimental design establishes a set density for each predator species and maintains that density in both single-species and multiple-species treatments. By combining predators in this way, additive designs confound

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changes in the number of predator species present with changes in total predator density. These two factors can therefore not be disentangled to determine which has caused any resultant risk reduction or risk enhancement.

While less frequently used, the substitutive design is becoming more common (Schmitz and Sokol-Hessner 2002; Sokol-Hessner and Schmitz 2002; DeWitt and Langerhans 2003; Siddon and Witman 2004; Vance-Chalcraft et al. 2004; Griffen and Byers 2006, in review). Substitutive experiments hold total predator density constant while manipulating predator species richness. Total predator density and predator species richness are therefore not confounded in substitutive experiments. However, while total predator density remains constant, substitutive experiments have different relative densities of each species in single-species and multiple-species treatments. Thus substitutive experiments only examine the effects of interactions between species relative to effects of interactions between conspecifics.

These experimental designs are not only used in multiple predator studies, but have commonly been used to examine interspecific competition. Historically, substitutive designs have been used most extensively (Cousens 1991; Gibson et al. 1999). Yet the validity of this approach for assessing competition has been challenged, and the use of additive designs has been encouraged (reviewed in Snaydon 1991). Because these designs address complimentary, but not identical hypotheses, the appropriate design depends on the question of interest. While the additive design asks whether nonadditive effects due to interspecific interactions occur at all, the substitutive design asks whether nonadditive effects due to interspecific interactions are as strong as those due to intraspecific interactions (Jolliffe 2000). Thus, the most comprehensive assessment of competition may be gained from a combination of the two designs, where the density of each competing species is varied independently (Snaydon 1991).

Similarly, it has been recommended that additive and substitutive designs be used in tandem for multiple predator studies to detect nonadditive effects and then to determine whether these effects arise from adding a second predator species or from increasing total predator density (Sih et al. 1998). Two studies published to date have followed this recommendation and have shown that nonadditive effects in multiple predator systems were duplicated with single-species predator pairs (Vance-Chalcraft et al. 2004; Vance-Chalcraft and Soluk 2005a), indicating that these effects were a consequence of changes in predator density rather than predator species richness. However, the majority of studies have frequently employed only the additive (e.g., Losey and Denno 1998; Eklöv and Werner 2000; Eklöv and VanKooten 2001; Finke and Denno 2002; Lang 2003; Warfe and Barmuta 2004) or only the substitutive (e.g., Schmitz and Sokol-Hessner 2002; Sokol-Hessner and Schmitz 2002; DeWitt and Langerhans 2003; Siddon and Witman 2004) design when testing for risk reduction and risk enhancement.

Under some conditions, the two experimental methods may yield the same results. However, because the two methods manipulate predator densities differently, they may yield different results whenever predator density is important in eliciting emergent effects. For example, multiple predator species often interfere with each other, reducing predation rates and resulting in risk reduction for shared prey (Peckarsky 1991; Soluk 1993; Eklöv 2000; Eklöv and Werner 2000; Warfe and Barmuta 2004; Griffen and Byers 2005). Similarly, conspecific predators also frequently interfere, thus reducing predation rates (Abrams and Ginzburg 2000). Because the substitutive design incorporates conspecific interference, while the additive design does not, results obtained using the two methods may disagree when conspecific interference occurs.

While the above discussion highlights the importance of predator density, studies using additive designs have also demonstrated that prey density can influence the strength of multiple predator effects (Soluk 1993; Losey and Denno 1998; Vance-Chalcraft and Soluk 2005b). However, the strength of interference among conspecific predators can also vary with prey density, similarly altering per capita predation (Arditi and Ginzburg 1989; Abrams and Ginzburg 2000). Because of the use of additive experiments, it is therefore uncertain whether reported changes in the magnitude of nonadditivity with changes in prey density are a unique outcome of the multiple predator system, or are similar to dynamics expected with a single predator species.

Here I compare multiple predator effects in a field experiment by simultaneously employing both an additive and substitutive design at two different prey densities. I demonstrate that results obtained from the two designs differed when two species of intertidal crab foraged together on shared mussel prey. I show that differences in the relative strength of interference between conspecific and heterospecific predators lead to qualitatively opposite results using the additive and substitutive experimental designs, and that the difference in results obtained using the two designs increased with prey density.

Materials and methods

Natural history

To examine the impacts of multiple predators on shared prey, I used two established invasive predators on the east coast of North America, the European green crab *Carcinus maenas*, and the Asian shore crab *Hemigrapsus sanguineus*. Both predators are common on the New England coast and presently are the dominant shore crabs in the southern Gulf of Maine. The two crab species are often found together (McDermott 1998) and may compete for food resources (Tyrrell and Harris 1999; Jensen et al. 2002). The blue mussel, *Mytilus edulis*, an important prey resource for both of these

species (Tyrrell and Harris 1999; Lohrer and Whitlatch 2002), was used as shared prey in this experiment. Previous experiments with these two species foraging on shared amphipod prey demonstrated that both species decrease foraging effort in the presence of the other species (Griffen and Byers 2005). Thus I expected that their predation on mussel prey may not be additive. Additionally, both species decrease foraging effort in the presence of conspecifics (Griffen and Byers 2006, in review).

The experiment was conducted on a semi-exposed beach at South Odiorne Point, New Hampshire, that is dominated by various sized boulders and cobble overlying a substrate of sand and shell. Both species of crab are patchily abundant at this site [*C. maenas* density, $7.28 \pm 7.71 \text{ m}^{-2}$ (mean ± 1 SD); *H. sanguineus* density, $3.6 \pm 5.97 \text{ m}^{-2}$; range = 0–48 and 0–38 individuals m^{-2} for each species, respectively]. Additionally, individuals of the two species are found in close association, frequently inhabiting space under the same rock. Mussels at Odiorne Point are mainly found attached sporadically underneath or around boulders and cobble. Mussel densities in lower intertidal regions of Odiorne Point where this experiment was conducted are highly variable: $198 \pm 107 \text{ m}^{-2}$ (Tyrrell and Harris 1999).

Experimental design

I examined predation by *C. maenas* and *H. sanguineus* on mussels in field enclosures (0.6×0.5×0.3 m, length×width×height) that were constructed of lobster wire and lined by 0.5-cm plastic mesh, and were deployed at approximately mean low water. Small boulders were cleared from the substrate to allow for enclosure placement and were subsequently placed inside enclosures to mimic ambient habitat. Naturally occurring flora and fauna were not removed from these boulders and could have been used as an alternative prey source by either predator. Other potential prey items within experimental enclosures included urchins, limpets, snails, amphipods, polychaetes, small sea stars (<2 cm diameter), brittle stars, small fish, juvenile crabs, and macroalgae. Evidence of predation was at times apparent on some of these alternative prey. While this may dilute effects of predation on the focal prey (mussels), it was desirable to further approximate the natural habitat where the predators have multiple prey choices.

I used the full complement of predator treatments necessary to compare the additive and substitutive experimental designs: one *C. maenas*, two *C. maenas*, one *H. sanguineus*, two *H. sanguineus*, one *C. maenas* plus one *H. sanguineus*, and a no predator control. Adult males of both species were used, as female *H. sanguineus* in preliminary experiments did not consume the size of mussels used in this experiment (10–20 mm shell length). Adult *C. maenas* in the Gulf of Maine are typically much larger than adult *H. sanguineus*, and this was reflected in

the carapace widths of crabs used in these experiments [*C. maenas*, $53 \pm 7 \text{ mm}$ (mean \pm SD); *H. sanguineus*, $24 \pm 3 \text{ mm}$].

To provide multiple opportunities for comparison between the additive and substitutive designs, I conducted experiments at two mussel densities that were both within the middle range of mussel densities at Odiorne Point. Henceforth, I refer to these as high (80 mussels per enclosure = 267 m^{-2}) and low (40 mussels per enclosure = 133 m^{-2}) densities. Each of the six predator treatments listed above were conducted at each of these prey densities, thus allowing for two separate comparisons between the additive and substitutive designs.

Mussels were introduced into experiments by haphazardly scattering them within enclosures and allowing them to form byssal thread attachments between or under boulders. Crabs were introduced to the enclosures 24 h later. Crabs were allowed to forage for 6 days, following which, the contents of each enclosure was removed and the number of surviving mussels was counted. Each treatment was replicated 5 times, once during each of five separate, consecutive trials conducted during July and August 2004. Individual animals were only used in a single trial.

Data analysis

I determined whether an emergent multiple predator effect occurred by comparing observed prey survival when both predator species foraged together to expected values in the absence of risk reduction or risk enhancement. Expected survival assuming the predators have independent effects ($E_{1,2}$) can be calculated for the additive experimental design using the number of prey surviving when single predators of each species foraged alone as follows (Billick and Case 1994; Vonesh and Osenberg 2003; Vance-Chalcraft and Soluk 2005a):

$$E_{1,2} = \frac{N_1 \times N_2}{N_c}, \quad (1)$$

where N_1 , N_2 , and N_c are the number of prey that survive each trial with *C. maenas*, *H. sanguineus*, and the no predator control, respectively. When prey survival is 100% in the no-predator control, Eq. 1 yields the same expected consumption as the multiplicative risk model proposed by Soluk (1993) and commonly employed by researchers using an additive experimental design with prey depletion (Sih et al. 1998). Because Eq. 1 explicitly includes survival in the absence of predators, it may be preferred in the presence of predator-free “background” prey mortality. Data can be statistically compared to this expected null model by conducting a two-way ANOVA on log transformed prey survivorship data, with the presence/absence of each predator species treated as a separate factor (Billick and Case 1994; Sih

et al. 1998). A significant interaction term between the two factors indicates the presence of an emergent effect of combining the two predator species. Because I examined the additivity of *C. maenas* and *H. sanguineus* at two prey densities, I conducted a three-way ANOVA ($\alpha=0.05$), analogous to the two-way ANOVA just described, but with high and low prey density as two levels of a third factor. A significant two-way interaction (*C. maenas*×*H. sanguineus*) would thus still indicate the presence of nonadditivity, and a significant three-way interaction (*C. maenas*×*H. sanguineus*×prey density) would indicate that emergent effects of the two predators change with prey density.

I next assessed the effects of combining both predator species together while holding total predator density constant using the substitutive experimental design. I again compared observed prey survival to expected survival if each predator species has independent effects. I used a null model of expected survival derived from the same independent effects model from which Eq. 1 was derived (Billick and Case 1994). The original model [Eqs. 11–14 in Billick and Case (1994)] was modified for use with the substitutive design by dividing prey mortality rates when predators were combined by two because the substitutive design uses half the number of predators in the combined predator treatment as in the single predator treatments. This yielded:

$$E_{1,2} = (N_{1,1} \times N_{2,2})^{0.5}, \quad (2)$$

where $N_{1,1}$ and $N_{2,2}$ are the number of prey surviving when foraged on by pairs of *C. maenas* and *H. sanguineus*, respectively. Expected prey survival ($E_{1,2}$) was calculated separately for each replicate trial. The presence of an emergent multiple predator effect was then detected using ANOVA with the number of prey surviving to the end of each trial as the response variable, observed and expected as two levels of one factor, and high and low prey density as two levels of a second factor (Vance-Chalcraft et al. 2004; Vance-Chalcraft and Soluk 2005b). This was followed by planned linear contrasts to separately compare observed and expected prey survival at high and low prey densities. Previous studies have used the average of the single species treatments as the null model when using a substitutive experimental design (e.g., Siddon and Witman 2004). For comparison I also analyzed the data using this null model. However, as this did not alter the patterns seen or the conclusions drawn from the study, only results using Eq. 2 are given here.

I conducted an additional statistical test to more fully understand the influence of predator density on predation by *C. maenas* and *H. sanguineus* when the two species foraged alone. I conducted a two-way ANOVA ($\alpha=0.05$) on log transformed prey mortality (to correct for heteroscedasticity in the data) with predator identity (*C. maenas* or *H. sanguineus*) as one factor, and predator density (one or two individuals) as a second factor.

Trial was initially included as a blocking factor in each of these analyses described above, but was later removed as it was not responsible for a significant amount of the variation in any of the analyses. Removal of the blocking factor did not alter the conclusions of the analyses. Statistical analyses were performed using JMP IN (SAS Institute 2003) version 5.1.

Results

No predator mortality occurred during any of the trials. Approximately 50% of the mussel prey were consumed by *C. maenas* (significant main effect of *C. maenas*, $P<0.0002$; Table 1, Fig. 1). While 25–30% of the prey were consumed by *H. sanguineus*, the decrease in prey survival was not statistically significant (main effect of *H. sanguineus*, $P=0.95$; Table 1, Fig. 1). Single predators of both species more than doubled their prey consumption with increasing prey density (significant main effect of prey density, $P=0.0001$; and there were no significant interactions between prey density and either species, $P=0.59$ for *C. maenas* and $P=0.85$ for *H. sanguineus*; Table 1, Fig. 1). Similarly, when two conspecifics foraged together, increasing prey density caused *C. maenas* and *H. sanguineus* to increase predation by 50 and 200%, respectively (significant effect of prey density, $P<0.0001$; Table 1, Fig. 1).

Prey survival when the two predator species foraged together was greater than expected based on the additive experimental design at both prey densities examined (significant *C. maenas*×*H. sanguineus* interaction, $P=0.04$; and nonsignificant three-way interaction, $P=0.62$; Table 1, Figs. 1, 2). However, qualitatively opposite results were obtained using the substitutive experimental design, and results depended on prey density. Prey survival when the two predator species foraged together at the high prey density was less than expected based on the substitutive experimental design (significant difference between observed and expected in linear contrast, $P=0.02$; Table 1, Figs. 1, 2), and was similar to expected at low prey density (nonsignificant difference between observed and expected in linear contrast, $P=0.78$; Table 1, Figs. 1, 2).

Finally, each predator species consumed the same number of mussels in single-species treatments regardless of whether one or two predators were present (nonsignificant main effect of predator density, $P=0.69$; and nonsignificant interaction of predator density×predator species, $P=0.19$; Table 1, Fig. 1).

Discussion

Results of this study are consistent with previous studies that have examined predation by these and other crab species. I found that *C. maenas* consumed approximately 50% more mussels than did *H. sanguineus* (Fig. 1).

Table 1 Results of ANOVAs used to compare observed predation by *Carcinus maenas* and *Hemigrapsus sanguineus* to expected predation based on an additive and a substitutive experimental design, and to examine the influence of predator density on prey consumption

Source	<i>df</i>	SS	<i>F</i>	<i>P</i>
Test of additive design (three-way ANOVA)				
<i>C. maenas</i>	1	2.7936	17.521	0.0002
<i>H. sanguineus</i>	1	0.0008	0.005	0.946
Prey density	1	3.0858	19.354	0.0001
<i>C. maenas</i> × <i>H. sanguineus</i>	1	0.7086	4.445	0.043
<i>C. maenas</i> ×prey density	1	0.0463	0.291	0.594
<i>H. sanguineus</i> ×prey density	1	0.0058	0.036	0.850
<i>C. maenas</i> × <i>H. sanguineus</i> ×prey density	1	0.0412	0.259	0.615
Error	32	5.1022		
Source	<i>df</i>	SS	<i>F</i>	<i>P</i>
Test of substitutive design (two-way ANOVA)				
Observed/expected	1	150.4717	2.584	0.322
Prey density	1	3,242.1151	55.669	< 0.0001
Observed/expected×prey density	1	235.5829	4.045	0.062
Error	16	931.8226		
Linear contrast				
Observed versus expected at high prey density	1, 16	381.3051	6.547	0.021
Observed versus expected at low prey density	1, 16	4.7494	0.082	0.779
Source	<i>df</i>	SS	<i>F</i>	<i>P</i>
Effect of predator density (two-way ANOVA)				
Predator species	1	18.9498	24.826	< 0.0001
Predator density	1	0.1234	0.162	0.690
Predator species×predator density	1	1.3340	1.748	0.195
Error	36	27.4793		

^a*df* are given as numerator, denominator

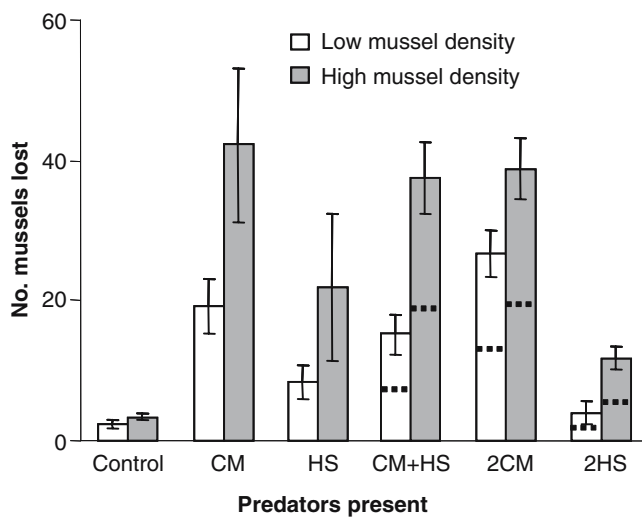


Fig. 1 Number of mussel prey lost (mean \pm 1 SE, $n=5$) at low (133 m^{-2}) and high (267 m^{-2}) mussel densities by *Carcinus maenas* (CM) and *Hemigrapsus sanguineus* (HS) foraging as single individuals and in heterospecific and conspecific pairs. The average per capita number of mussels consumed at low and high mussel densities is also shown as dotted lines for treatments with two crabs foraging together. No predators were present in the control

Previous studies with smaller mussels ($< 10 \text{ mm}$ shell length) have reported higher predation rates for both crab species than those reported here (Brousseau et al.

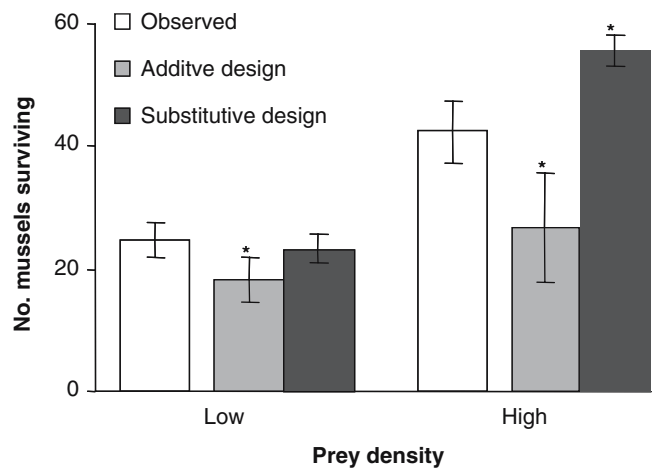


Fig. 2 Observed and expected mussel prey survival based on the additive and substitutive experimental designs at low and high mussel densities. Expected values based on the additive and substitutive experimental designs were determined using Eqs. 1 and 2, respectively. * $P \leq 0.05$ (expected survival significantly different than observed survival). Bars are mean \pm 1 SE ($n=5$)

2001; Lohrer and Whitlatch 2002) and reported approximately 30% higher consumption rates for adult *C. maenas* than for adult *H. sanguineus* (Lohrer and Whitlatch 2002). The lower consumption rates and

greater disparity between the consumption rates of the two crabs observed here may result first from the use of larger mussel prey than in previous studies, as *H. sanguineus* preferentially consumes mussels < 10 mm shell length (Bourdeau and O'Connor 2003), and second from the inclusion of alternative prey items in the experimental enclosures, as *H. sanguineus* is more omnivorous than *C. maenas* (Tyrrell and Harris 1999). Finally, the findings here that predation increases with prey density and decreases with predator density are consistent with previous studies of crab predation on bivalve prey (Mansour and Lipcius 1991; Clark et al. 1999; Taylor and Eggleston 2000; Sietz et al. 2001; Mistri 2003).

Results of this study indicate that the strength of multiple predator effects (both risk reduction and risk enhancement) can vary with prey density. Previous studies using additive experiments have also reported increases in both risk reduction (Soluk 1993) and risk enhancement (Losey and Denno 1998) with increasing prey density, while others have reported similar levels of risk reduction across prey densities (Vance-Chalcraft and Soluk 2005b). Limited behavioral options for mussel prey imply that nonadditive effects in this study were likely due to changes in predator foraging in the presence of competitors. Increased departure from expected results at high prey densities may be attributed to the increased foraging effort of predators in all treatments at high prey densities (Fig. 1). Higher foraging effort may, in turn, result in more frequent interactions between crabs, thus increasing the magnitude of the effects of interference.

While these results were consistent with established foraging ecology of crab predators and previous studies demonstrating the impacts of prey density on multiple predator effects, they highlight a discrepancy between conclusions that may be drawn from additive and substitutive experimental designs, and demonstrate that the two cannot be used interchangeably. Emergent effect of the two predators in my experiment depended on prey density and which experimental design was used (Fig. 2). Prey survival was greater than expected based on the additive experimental design at both prey densities. However, using the substitutive experimental design, prey survival was not different than expected at low prey density, and was lower than expected at high prey density. Taken independently these results would be interpreted as risk reduction at both prey densities using the additive design, with no emergent effect at low prey density and risk enhancement at high prey density using the substitutive design.

The discrepancy between the results of the two approaches can be attributed to how the two experimental methods "deal with" intraspecific interference and its strength relative to interspecific interference. The detection of risk enhancement with the substitutive design does not imply that predator facilitation occurred. Rather, interference occurred between the two species, decreasing predation rates below those observed when individuals of each species foraged independently

(Fig. 1). Using additive experiments, many researchers with similar results have concluded that risk reduction occurs in their study systems (Sih et al. 1998; Finke and Denno 2002; Crumrine and Crowley 2003; Harvey et al. 2004; Warfe and Barmuta 2004; Griffen and Byers 2005). However, per capita predation by both species was higher when single individuals foraged than when two conspecifics foraged together, indicating that intraspecific interference also decreased predation rates (Fig. 1). Further, intraspecific interference in my experiment was stronger than interspecific interference (i.e., per capita predation decreased more when conspecifics were combined than when heterospecifics were combined; Fig. 1). This is consistent with a laboratory behavioral study in which conspecific pairs of these predators foraging on mussels spent 68% more time in aggressive behaviors than heterospecific predator pairs (B. D. Griffen, unpublished data). The substitutive design, in effect, compares the impacts of interspecific interference relative to intraspecific interference. Therefore, while the two predator species did not facilitate one another's prey consumption, risk of predation was higher for prey when heterospecific predators foraged together than when conspecific predators foraged together (i.e., risk enhancement).

Four previously published studies employed the same treatments used here to examine systems with multiple predators (Evans 1991; Peckarsky 1991; Vance-Chalcraft et al. 2004; Vance-Chalcraft and Soluk 2005a). Of these, two examined prey mortality/survival, and therefore lend themselves to comparison of the additive and substitutive experimental design for detecting emergent effects of multiple predators (Vance-Chalcraft et al. 2004; Vance-Chalcraft and Soluk 2005a). I obtained survivorship data from the original authors of these studies and then used Eqs. 1 and 2 to determine expected prey survival when predator species foraged together (Table 2). Only average survival across all replicates of a given treatment was available, and therefore no estimate of error is presented. Consistent with this study, a discrepancy exists in the direction of emergent effects in six of the seven multiple predator comparisons, with the additive design detecting risk reduction, and the substitutive design detecting risk enhancement (Table 2). A single comparison detected risk reduction with both designs, but the magnitude of risk reduction based on the additive design was nearly an order of magnitude greater than that based on the substitutive design.

The inconsistencies between results obtained with the additive and substitutive designs in this and the previous studies indicate a consistent trend that interference with conspecific predators often reduces predation as much as, or more than, interference between heterospecific predators. This finding highlights the importance of including intraspecific interactions when assessing the impacts of multiple predator species to ensure that observed multiple predator effects are an emergent result of interactions between predator species, and not simply an outcome of altering predator density (Sih et al. 1998).

Table 2 Comparison of additive and substitutive experimental designs from previously published studies. Predicted survival was calculated (using Eqs. 1, 2) from average survivorship across all replicates. Observed survival that is greater than or less than expected indicates risk reduction or risk enhancement, respectively. Mayflies were used as prey in each of these studies

Predators	Predicted		Observed	Publication
	Additive	Substitutive		
Dragonfly and hellgrammite	0.325	0.571	0.600	Vance-Chalcraft et al. (2004)
Dragonfly and hellgrammite	0.582	0.694	0.674	Vance-Chalcraft and Soluk (2005b)
Dragonfly and darter	0.465	0.666	0.604	Vance-Chalcraft and Soluk (2005b)
Dragonfly and creek chub	0.555	0.710	0.659	Vance-Chalcraft and Soluk (2005b)
Hellgrammite and darter	0.428	0.618	0.537	Vance-Chalcraft and Soluk (2005b)
Hellgrammite and creek chub	0.511	0.659	0.637	Vance-Chalcraft and Soluk (2005b)
Creek chub and darter	0.409	0.632	0.478	Vance-Chalcraft and Soluk (2005b)

It should also be noted that mechanisms besides predator interference could also lead to conflicting results from the two experimental methods. For example, changes in prey behavior are at times the underlying mechanism that causes risk reduction (Eklöv 2000) or risk enhancement (Swisher et al. 1998). Prey often alter behavior proportionally to the threat of predation (Lima 1998), which can in turn be influenced by predator density. If prey alter behavior in response to total predator density, then additive experiments that confound changes in total predator density and predator species richness may lead to erroneous conclusions. Similarly, if prey respond to each predator species individually (rather than to total predator density) (e.g., DeWitt and Langerhans 2003), then substitutive experiments that confound changes in individual predator species density and predator species richness could lead to erroneous conclusions.

Thus in this study system, as well as others where predator density is an important component of interactions between predators and prey, the most complete understanding of the effects of multiple predators may be obtained using an experimental design that combines additive and substitutive components, as has previously been encouraged (Sih et al. 1998). Using only the additive design would have led to the conclusion that the two species interfere, but the roles of total predator density and unique interspecific effects would have been unclear. Predation that is greater than expected was detected with the substitutive design; thus using only this design would have led to the false conclusion that these two predators facilitate each other's predation. Only by using both methods together is it possible to obtain the full picture that these species interfere with each other, but that this interference does not decrease per capita predation rates to the same extent as conspecific interference.

The need to develop predictive patterns in the effects of multiple predators on shared prey highlights perhaps the greatest shortcoming of using the different experimental designs interchangeably in examining predation

by multiple species. When different experimental designs are used, interference between predator species in one study, for example, is not equivalent (or comparable) to that in another. Therefore, it becomes more difficult to detect patterns across studies in the strengths of emergent multiple predator effects that can eventually facilitate predictions. Thus, using a combined additive and substitutive experimental design not only provides more information on the system under study, but should also advance our understanding of the effects of multiple predators across systems at an accelerated pace.

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