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LETTER

Emergent effects of multiple predators on prey survival: the importance of depletion and the functional response

Michael W. McCoy,[1,2]* Adrian C. Stier[2] and Craig W. Osenberg[2]

[1]*Department of Biology, East Carolina University Greenville, NC, 27858, USA*

[2]*Department of Biology, University of Florida Gainesville, FL, 32611-8525, USA*

**Correspondence: E-mail: mccoym@ecu.edu*

Abstract

The combined effects of multiple predators often cannot be predicted from their independent effects. Emergent multiple predator effects (MPEs) include risk enhancement, where combined predators kill more prey than predicted by their individual effects, and risk reduction, where fewer prey are killed than predicted. Current methods for detecting MPEs are biased because they assume linear functional responses and/or no prey depletion. As a result, past studies overestimated the occurrence of risk enhancement for additive designs, and tended to overestimate the occurrence of risk reduction for substitutive designs. Characterising the predators' functional responses and accounting for prey depletion reduces biases in detection, estimation, interpretation and generalisation of the emergent effects of predator diversity on prey survival. These findings have implications beyond MPE's and should be considered in all studies aimed at understanding how multiple factors combine when demographic rates are density dependent.

Keywords

Additivity, density dependence, handling time, MPE, predation, Roger's random predator model.

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INTRODUCTION

Traditionally, ecological systems have been studied as subsets of pairwise interactions, with the hope that these can then be assembled to predict the spatial and temporal patterns of intact communities (e.g. Oksanen *et al.* 1981; Wootton 1994). If pairwise interactions are independent of other members of the community, then community dynamics can be assembled piecemeal. However, if interaction strengths are not independent of other players in the system, then observed community dynamics will depart from simple predictions based on pairwise interactions (Wootton 1994; Bolker *et al.* 2003; Werner & Peacor 2003). Such departures can reveal the action of complex and potentially novel forms of species interactions, which have been referred to as higher order interactions, trait mediated indirect interactions or non-consumptive predator effects (Wootton 1994; Bolker *et al.* 2003; Werner & Peacor 2003). As predator–prey interactions comprise the foundation of food web dynamics, many studies have focused on examining possible interactions among multiple predator species and a shared prey (Polis & Holt 1992; Sih *et al.* 1998; Ives *et al.* 2005; Vance-Chalcraft & Soluk 2005; Griffen 2006; Schmitz 2009). In general, these studies show that the effects of multiple predators are often not independent, suggesting that a better understanding of these higher order interactions is needed to fully appreciate food web dynamics.

Interactions between predators (e.g. cooperation, competition or intraguild predation), as well as anti-predator responses by prey, can lead to emergent 'multiple predator effects' (MPEs), where prey consumption rates by multiple predators foraging in concert cannot be predicted by knowing the independent effects of each predator on prey survival (e.g. Polis & Holt 1992; Sih *et al.* 1998; Ives *et al.* 2005; Vance-Chalcraft & Soluk 2005; Griffen 2006; Schmitz 2009). Multiple predator species can combine: (1) independently, (2) synergistically (leading to prey risk enhancement: Soluk & Collins 1988; Losey & Denno 1998; Sih *et al.* 1998) or (3) antagonistically (e.g. leading to prey risk reduction: Sih *et al.* 1998; Vance-Chalcraft & Soluk 2005). Studies of MPEs typically compare observed patterns of prey survival in the presence of combinations of predators to expected survival predicted from the independent effects of each predator species in monoculture (Soluk & Collins 1988; Billick & Case 1994; Sih *et al.* 1998; Griffen 2006). Early studies of MPEs misinterpreted results by using incorrect models to calculate expected effects (see critiques by Billick & Case 1994; Wootton 1994), or by ignoring prey losses in the absence of predators (see clarifications by Sih *et al.* 1998; Vonesh & Osenberg 2003). Indeed, considerable debate in the literature focused on understanding how statistical models used to make predictions about the expected effects of multiple predators are linked to the underlying biological models assumed to drive prey survival (Soluk & Collins 1988; Billick & Case 1994; Sih *et al.* 1998; Vonesh & Osenberg 2003; Griffen 2006). A consensus approach emerged (Vonesh & Osenberg 2003; Vance-Chalcraft & Soluk 2005; Griffen 2006). Specifically, if two predators have independent effects, then expected proportion of prey surviving (hereafter prey survival) in the presence of both predators, $\hat{S}_{1,2}$ can be calculated based on prey survival in the presence of each predator species in monoculture, S_1 and S_2, corrected for background mortality (i.e. survival in no predator control treatment S_{ctl}) (also see Table 1):

$$\hat{S}_{1,2} = \frac{S_1 \cdot S_2}{S_{ctl}} \quad (1)$$

Equation 1 is a corrected version of the 'Multiplicative Risk Model' of Soluk & Collins (1988) based on an additive design (i.e. where the combined effects of predator species 1 and 2, at densities C_1 and C_2, are predicted based on their isolated effects, also evaluated at C_1 and C_2) (Vonesh & Osenberg 2003). An analogous null model for a substitutive design (where total predator density is the same in both the monoculture and mixed species treatments) yields:

$$\hat{S}_{\frac{1,2}{2}} = \frac{S_1^{0.5} S_2^{0.5}}{S_{ctl}} \quad (2)$$

Despite general consensus among ecologists on these calculations of expected survival in combined predator treatments, the derivation (Table 1) and underlying assumptions of eqns 1 and 2

Table 1 Application of the Multiplicative Risk Model for additive and substitutive designs for predicting effects of two predators (1 and 2) from their effects quantified in isolation. These expressions assume both predators have a Type I functional response (so that mortality rates are constant and independent of prey number or density, N). P is proportion of prey surviving from specific mortality sources, μ is the per capita prey mortality rate from those sources, S is the proportion of prey surviving in a specific treatment and ($\hat{S}$) is predicted survival from the Multiplicative Risk Model for additive and substitutive experimental designs. Subscripts 1, 2 indicate predator identity (1 or 2) *ctl* indicates the control treatment (i.e. no predator)

		Absolute number surviving	Proportion survival
Control	N_{ctl}	$N_{ctl} = N_0 C^{-\mu_{ctl}} = N_0 P_{ctl}$	$S_{ctl} = P_{ctl}$
Predator (1)	N_1	$N_1 = N_0 C^{-\mu_{ctl}-\mu_1} = N_0 P_{ctl} P_1$	$S_1 = P_{ctl} P_1$
Predator (2)	N_2	$N_2 = N_0 C^{-\mu_{ctl}-\mu_2} = N_0 P_{ctl} P_2$	$S_2 = P_{ctl} P_2$
Additive (1 + 2)	$N_{1,2}$	$N_{1,2} = N_0 C^{-\mu_{ctl}-\mu_1-\mu_2} = N_0 P_{ctl} P_1 P_2$	$\hat{S}_{1,2} = P_{ctl} P_1 P_2 = \frac{S_1 S_2}{S_{ctl}}$
Substitutive [(1 + 2)/2]	$N_{1,2}$	$N_{1,2} = N_0 C^{-\mu_{ctl}-\frac{\mu_2-\mu_1}{2}} = N_0 P_{ctl} P_1^{0.5} P_2^{0.5}$	$\hat{S}_{\frac{1,2}{2}} = P_{ctl} P_1^{0.5} P_2^{0.5} = \frac{S_1^{0.5} S_2^{0.5}}{S_{ctl}}$

(hereafter referred to as the Multiplicative Risk Model) are still not well appreciated. Most notably, the Multiplicative Risk Model is often assumed to account for prey depletion (Sih *et al.* 1998; Griffen 2006), ostensibly because it adjusts survival probabilities so that they are conditional (i.e. prey cannot be eaten twice). However, the Multiplicative Risk Model assumes that prey per capita mortality rates imposed by predators are constant over the course of the experiment, which is only valid when predators have linear (i.e. Type I) functional responses, when prey are continuously replenished, or experimental durations are sufficiently short that depletion is inconsequential (Collins *et al.* 1981; Juliano & Williams 1985; Juliano *et al.* 2001). These assumptions are violated in most empirical studies, because most predators have nonlinear functional responses (Hassell *et al.* 1976, 1977; Jescheke *et al.* 2004) and because most predation studies run sufficiently long that prey density declines appreciably from the start to end of trials. For example, we examined 100 multiple predator studies reviewed by Vance-Chalcraft *et al.* (2007) and found that on average prey were depleted by 70% over the course of these experiments. Therefore, the assumption of constant instantaneous per capita feeding rates (i.e. temporally invariant mortality rates of prey) is probably severely violated in studies of MPEs, and this could affect how we interpret and generalise the results of multiple predator studies.

To our knowledge, no studies have examined explicitly how prey depletion in combination with a nonlinear functional response influences the expected results of multiple predator studies. In this study, we show that depletion of prey by predators in combination with a nonlinear functional response leads to conclusions of risk enhancement or risk reduction even when predators have independent effects. Moreover, we show that the direction and magnitude of bias is in part a consequence of the experimental design. As a result, past studies (including meta-analyses and syntheses of the literature: e.g. Sih *et al.* 1998; Schmitz 2007) likely misestimated the importance of MPEs. Specifically, studies that used additive experimental designs with prey depletion were more likely to conclude risk enhancement, and studies that used substitutive designs were usually more likely to conclude risk reduction, even when predators affected prey independently (Fig. 1). We also suggest some approaches for dealing with depletion in studies of MPEs that will reduce biases in detection, estimation and interpretation of emergent MPEs.

METHODS

Although a variety of nonlinear functional responses are possible, here, we assumed that predators exhibited a Type II functional response:

$$F = \frac{aNC}{1 + ahN} \tag{3}$$

where F is the instantaneous feeding rate of one predator, C is predator density, N is prey density, a is attack rate and h is handling time (Holling 1959). Type II functional responses are thought to characterise the feeding rate of many types of predators (Jescheke *et al.* 2004). We also assumed that mortality of prey in the absence of predators was negligible: that is, survival in the control treatment was 100% ($S_{ctl} = 1$). As a result, prey survival, S, in the presence of a predator assemblage with n predator species (with predator species i at density C_i), can be found by integrating the aggregate feeding rates over the duration of the study (from time 0 to T), as prey density (N_t) declines:

$$S = 1 - \frac{\int_0^T \sum_{i=1}^{n} \frac{a_i N_t C_i}{1 + a_i h_i N_t} dt}{N_0} \tag{4}$$

We simulated foraging trials by solving eqn 4 numerically. First, we did this for two predator species in monoculture, which yielded S_1 and S_2. Second, we generated the survival of prey in the presence of both predator species under the assumption of independence (i.e. the true value of $S_{1,2}$) using eqn 4 and the same parameters used to generate the monoculture results. Third, we applied eqns 1 or 2 (depending on the experimental design being examined) to generate $\hat{S}_{1,2}$ (i.e. the standard null expectation under the Multiplicative Risk Model). We then compared the true value of $S_{1,2}$ under the assumption of independence with that expected from the Multiplicative Risk Model, $\hat{S}_{1,2}$ (eqns 1 and 2). The deviation between $\hat{S}_{1,2}$ and $S_{1,2}$ (expressed as both a difference, $\hat{S}_{1,2}$-$S_{1,2}$, and a ratio, $\hat{S}_{1,2}/S_{1,2}$) was used as a measure of bias in the Multiplicative Risk Model formulation. We then determined how bias changed as a function of study duration, T, handling time, h and attack rate, a, all of which influence depletion and/or the nonlinearity of the functional response (Rogers 1972; Thompson 1978; Juliano & Williams 1985; Juliano *et al.* 2001), for both additive designs and substitutive designs.

After providing a simple illustration of bias with a limited set of parameters, we systematically explored how variation in attack rates, handling times and study duration affected bias in applications of the Multiplicative Risk Model.

(1) To start, we assumed that the two predator species were identical ($a_1 = a_2 = a$ and $h_1 = h_2 = h$), and we simultaneously varied a (from 0.1 to 0.5), h (from 0 to 5) and T (from 5 to 20).

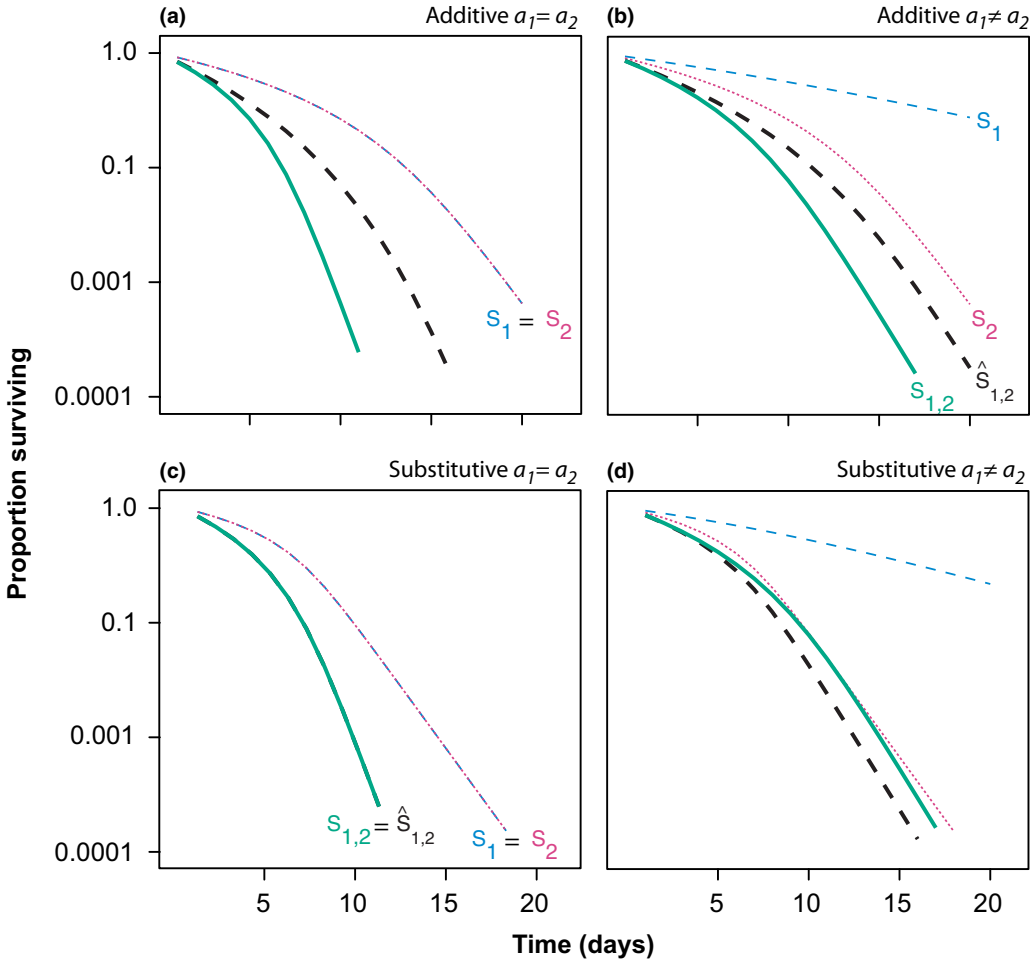

Figure 1 Examples illustrating bias in the Multiplicative Risk model. Each panel shows the simulated prey survival (eqn 4) with two predators in isolation (S_1, blue dashed line; S_2, red dotted line) and in combination ($S_{1,2}$, green line), as well as the predicted survival ($\hat{S}_{1,2}$, bold dashed) based on the Multiplicative Risk Model (eqns 2 or 3). Panels a and c depict an additive experimental design; panels b and d depict a substitutive experimental design. Panels a and b have identical attack rates and handling times (for these simulations: $a_1 = a_2 = 0.5$); panels c and d depict predators with unequal attack rates ($a_1 = 0.1$; $a_2 = 0.5$). All other parameters were identical in the four scenarios ($h_1 = h_2 = 1$; $N_0 = 10$, $T = 20$).

(2) We then relaxed the assumption of predator equivalency for the remaining scenarios. First, we assumed predators had identical handling times ($h_1 = h_2 = h$), fixed the attack rate of one predator ($a_2 = 0.5$), and varied the attack rate of the other predator (from 0.1 to 0.5). We also varied handling time (from 0 to 5) and experimental duration ($T = 5, 10, 20$) to assess how these factors interacted.
(3) We more fully explored how disparity in the functional responses affected bias, by fixing handling time ($h_1 = h_2 = 2.5$) and experimental duration ($T = 20$), but varying the two attack rates (from 0.01 to 1.0).
(4) We then fixed the attack rates ($a_1 = a_2 = 0.25$) and experimental duration ($T = 20$) and let handling times vary (from 0 to 5).

In all scenarios, we set $N_0 = 10$, and for the monoculture treatments, we set predator densities equal to one another ($C_1 = C_2 = 1$ for additive experimental designs; $C_1 = C_2 = 2$ for substitutive designs).

RESULTS AND DISCUSSION

Bias was affected by the amount of depletion, the nonlinearity of the functional response and the study design. Absolute and relative bias measures yielded qualitatively similar results, as would be expected (i.e. if $\hat{S}_{1,2}$-$S_{1,2} > 0$, then $\hat{S}_{1,2}/S_{1,2} > 1$). Specifically, if predators had Type II functional responses and prey were allowed to deplete over the course of the study, then predicted prey survival based on the Multiplicative Risk Models (eqns 1 and 2) was always greater than expected for additive experimental designs (i.e. $\hat{S}_{1,2} > S_{1,2}$) and typically (but not always) lower than expected for substitutive experimental designs (i.e. $\hat{S}_{1,2} < S_{1,2}$; Fig. 1 and Supplement 1). These biases occurred because the per capita mortality rates of the prey (i.e. proportional survival) increased as prey density declined (Fig. 1); however, the Multiplicative Risk Models assumes per capita mortality rates remain constant as prey are depleted.

Despite these qualitative similarities, absolute and relative biases diverged in their quantitative patterns. For example, as depletion became extreme, *absolute* bias decreased and approached zero not because the two models were similar but because both $\hat{S}_{1,2}$ and $S_{1,2}$ approached zero (Supplement 1) so that their difference also approached zero. Thus, this apparent reduction in bias was a poor reflection of the errors inherent to the application of the Multiplicative Risk Model. In contrast, *relative* bias often continued to increase under severe depletion: that is, even as $\hat{S}_{1,2}$ and $S_{1,2}$ approached

zero (e.g. Figs 1 and 2a). Thus, here, we focus on the relative bias results, but provide results for absolute bias in Supplement 1. Relative bias also is a relevant focus of our analyses because relative changes are often used to quantify effect sizes (Osenberg *et al.* 1997; Hedges *et al.* 1999).

For additive experimental designs, the Multiplicative Risk Model, combined with nonlinear functional responses and prey depletion, always led to a conclusion of risk enhancement when, in fact, predators had independent effects (e.g. Fig. 1 a and c). With identical predators (i.e. a situation equivalent to doubling the density of a single predator species), relative bias increased as the duration of the experiment increased and as attack rates increased (Fig. 2 a–c). In fact, with high levels of depletion the Multiplicative Risk Model generated predicted survival probabilities that were only 1/400th of the actual survival expected if predators had independent effects (eqn 4; Fig. 2c). In contrast to the case with additive designs, application of the Multiplicative Risk Model for substitutive designs (eqn 2) was unbiased when predators had identical functional responses (Fig. 1b). The lack of bias for a substitutive design with identical predators arose because this design simply replaced individuals of one species with the same number of individuals of the other (equivalent) species: that is, there was no functional change in the predator assemblage.

In contrast, with non-identical predators, bias existed for *both* additive and substitutive designs (Fig. 3). For the additive design, bias increased as attack rates increased (Figs 3a–c and 4a). This bias existed for additive designs because per capita prey risk increased (due to the Type II functional response) as prey density declined (i.e. prey were depleted). Thus, because total predator abundance was greater in mixed predator treatments, depletion was also greater, and this created a bias in favour of risk enhancement.

Depletion alone, however, cannot explain these results for additive designs. For example, bias was not monotonically related to handling time (Figs 2a–c and 4b). As handling time initially increased, depletion decreased, yet bias increased (e.g. see the right hand edge of Fig. 2c for small values of *h*); however, as handling time increased further (and thus depletion decreased), bias eventually decreased (as expected due to depletion).

This disparity between the results for changing attack rates vs. handling times exists because the parameters (attack rate and handling time) have two types of effects: they affect depletion but they also affect the shape of the functional response (and how depletion affects prey mortality). For attack rates, these effects operate in the same direction: increasing attack rates, increases depletion (which increases bias) and makes the functional response more nonlinear (which also increases bias) (Fig. 4a). In contrast, the effects of increased handling time on depletion and the shape of the functional response act in opposition to one another, creating a humped pattern of bias: that is, as handling time increases, depletion is reduced (which decreases bias), but the functional response becomes more nonlinear (which increases bias) (Fig. 4b).

For substitutive designs with non-identical predators, use of the Multiplicative Risk Model with substitutive designs also led to bias; however, the direction of the bias depended on how the predators differed. If predators differed only in attack rates, the bias was typically in the *opposite* direction from that observed with additive designs (but see Fig. 4d). Use of the Multiplicative Risk Model (eqn 2) led to erroneous conclusions of risk reduction (Figs 3d–f and 4c) even when predators acted independently. This bias arose because prey depletion was greater with a monoculture of the more efficient predator than with the same total density of predators in a mixed predator scenario (because some of the predators were less efficient). The single predator treatments thus gave an expectation that prey would be more depleted than they actually were in the mixed predator treatment.

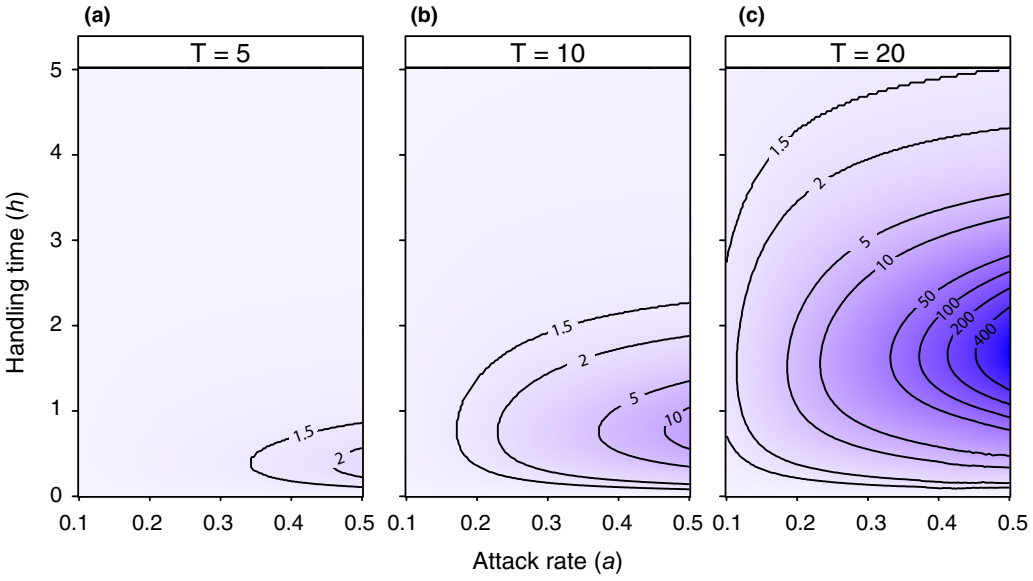

Figure 2 Contour plots showing bias as the fold change (i.e. $\hat{S}_{1,2}/S_{1,2}$) between predicted survival based on the Multiple Risk Model (eqn 1) and true survival, when predators have independent effects (eqn 4). Here, predators have the same attack rates and handling times. The degree of bias is affected by handling time (*y*-axis) and attack rate (*x*-axis) and is shown at three experimental durations: 5 days (panel a), 10 days (panel b) and 20 days (panel c). Darker blue indicates higher bias (i.e. predicted survival is too low based on the Multiplicative Risk Model).

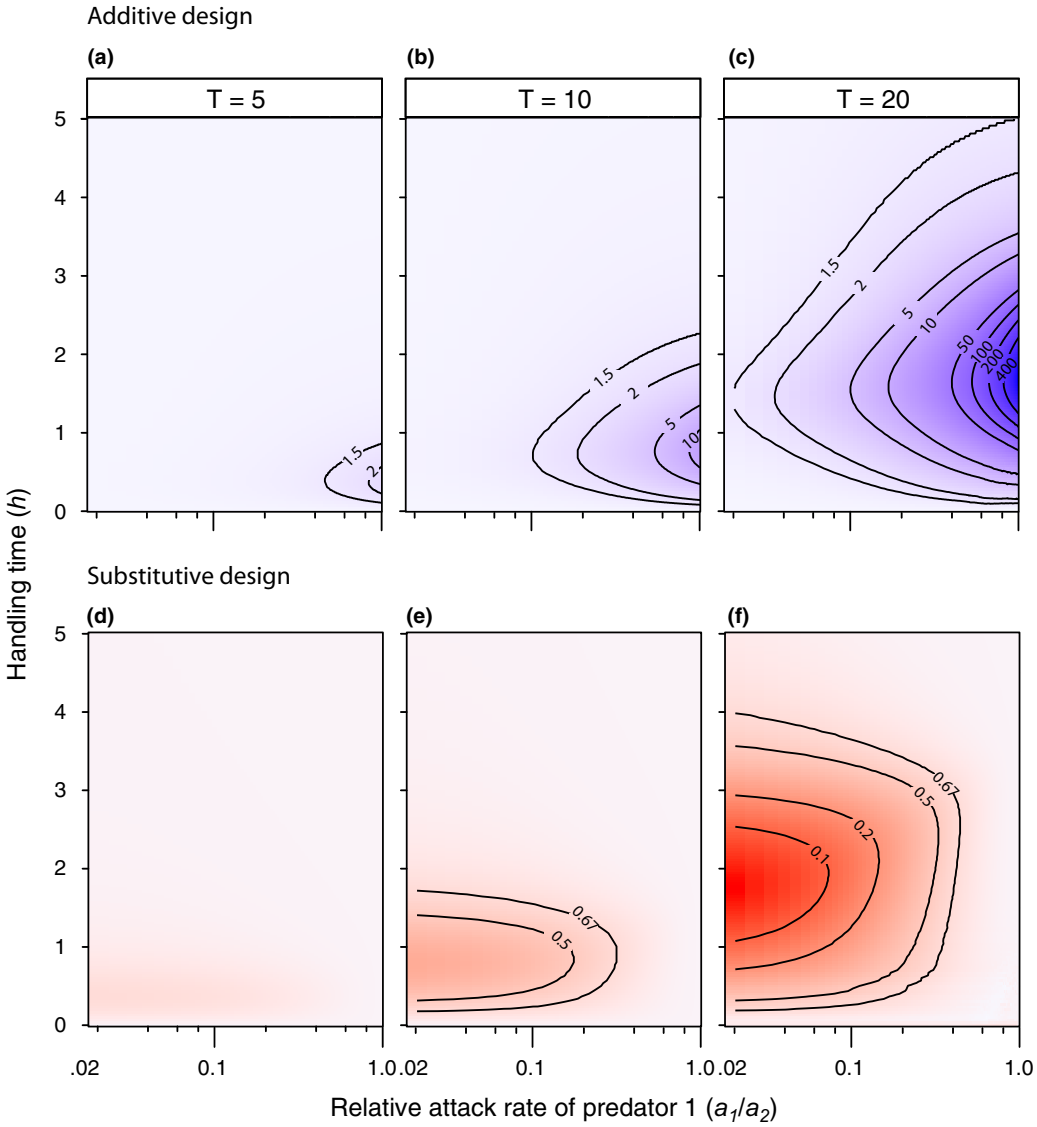

Figure 3 Contour plots showing bias (i.e. $\hat{S}_{1,2}/S_{1,2}$) as the fold change between known survival when predators have independent effects $S_{1,2}$, eqn 4) and that predicted based on the Multiple Risk Model ($\hat{S}_{1,2}$, eqn 1) for experiments using additive (Panels a–c) or substitutive designs (Panels d–f). Handling times (y-axis) were the same for both predators ($b_1 = b_2 = 1$ to 5), but attack rates (x-axis) and experimental durations varied. For additive designs (panels a, b, c), the Multiplicative Risk Model predicts survival probabilities that are too low (magnitude of bias towards risk enhancement increases with darker blues). In contrast, for substitutive designs (panels d, e, f) the Multiplicative Risk Model predicts survival probabilities that are too high (magnitude of the bias towards risk reduction increases with darker reds).

Variation in handling times between the predators in substitutive designs had the most complex effects. As substitutive designs control for density effects, bias was absent when attack rates and handling times were similar for the two predators: note the absence of bias along the 1 : 1 line in Fig. 4d (see also Fig. 4c). As predators diverged in handling time, the direction of bias depended on specific parameter values: for smaller handling times, bias led to erroneous conclusions of risk enhancement, whereas for larger handling times, bias was towards risk reduction (Fig. 4d). When predators have equal attack rates (as in Fig. 4d), and one predator has handling time near zero (i.e. see the dark blue regions of Fig. 4d), that predator's functional response is approximately linear and thus imposes the maximum per capita mortality rate over the duration of the study. A monoculture of the other predator (with a more nonlinear functional response) will not deplete prey as much as in the mixture. As a result, in the mixture, this predator will impose a greater mortality rate as prey deplete (through the actions of the more efficient predator). Thus, a researcher applying the Multiplicative Risk Model would be more likely to conclude risk enhancement even when predators are acting independently.

The bias reverses when handling times are large. Again, consider an extreme case, where predators are feeding at their maximum rates (i.e. at the asymptote of the functional response) because, for example, handling times are large (or initial prey densities are high). In this case, each predator has a feeding rate of 1/h (it is handling time limited). If depletion is insufficient to reduce this feeding rate, then relative bias can be calculated directly:

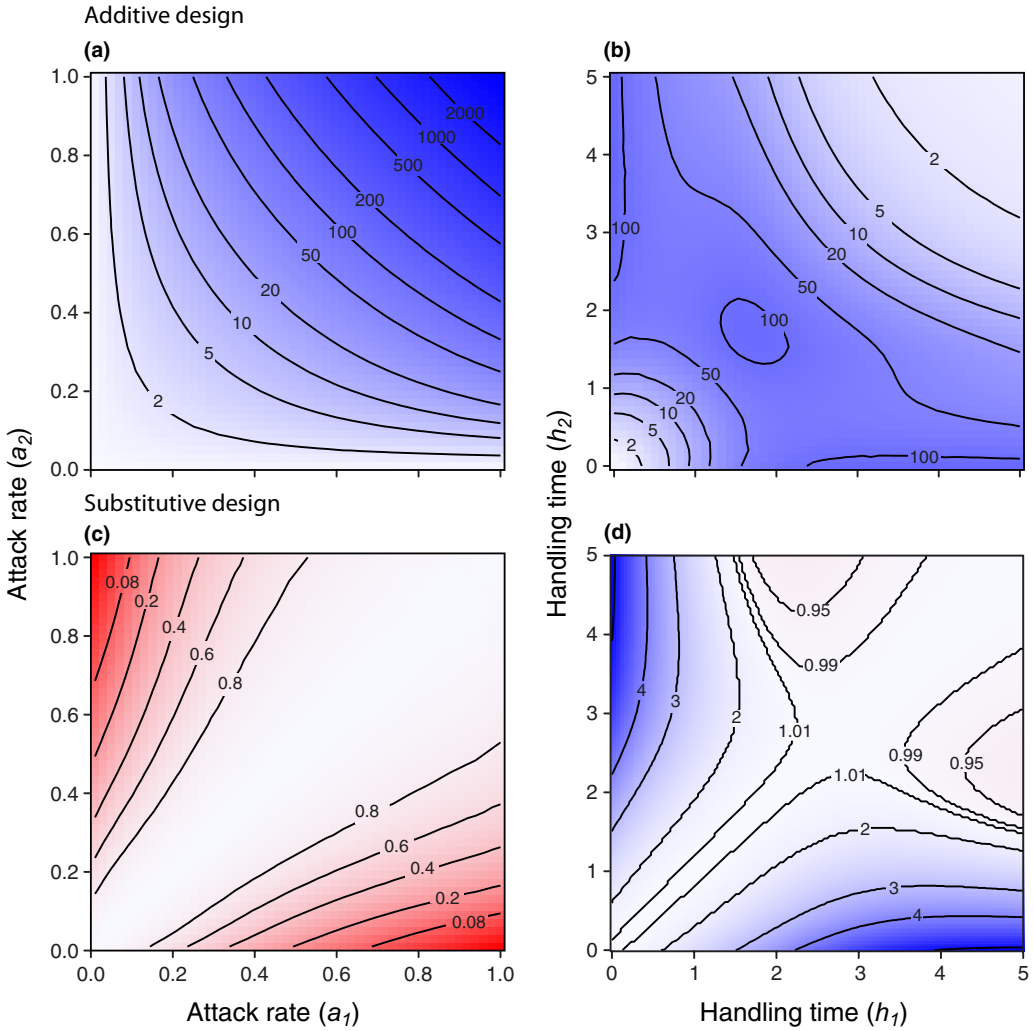

Figure 4 Contour plots showing relative bias (i.e. $\hat{S}_{1,2}/S_{1,2}$) of the Multiple Risk Model ($\hat{S}_{1,2}$, eqn 1) for experiments using additive (Panels a–b) or substitutive designs (Panels c, d). Panels a and c depict relative bias as a function of the attack rates of the two predators (other parameters were fixed: $h_1 = h_2 = 2.5$, $T = 20$, $N_0 = 10$. Panels b and d depic relatives bias as a function of the handling time of the two predators (other parameters were fixed: $a_1 = a_2 = 0.25$, $T = 20$, $N_0 = 10$). The degree of bias is indicated by more intense colours: blue indicates a bias towards risk enhancement, whereas red indicates a bias towards risk reduction. Note that in substitutive designs, there is no bias when attack rates and handling times are equivalent for the two predators (i.e. along the 1 : 1 lines in panels c and d).

$$Bias = \frac{\hat{S}_{1,2}}{S_{1,2}} = \frac{S_1^{1/2} S_2^{1/2}}{S_{1,2}} = \frac{(C_1 T/h_1 N_0)^{1/2} (C_2 T/h_2 N_0)^{1/2}}{(C_2 T/2h_2 + C_2 T/2h_2)/N_0} \quad (5)$$

When $C_1 = C_2$, this simplifies to:

$$Bias = \frac{(h_1 h_2)^{1/2}}{(h_1 + h_2)/2} \quad (6)$$

Thus, bias is the ratio of the geometric to arithmetic means of the handling times. As the geometric mean is always less than the arithmetic mean, relative bias is always < 1, indicating that the Multiplicative Risk Model predicts risk reduction even when predators are independent of one another. Indeed, in accord with eqn 6, the area with bias < 1 expands under larger values of handling time (or as initial prey density increases).

In all cases examined, the magnitude of bias was determined by the values of the parameters of the functional responses (attack rate and handling time) and the duration of the experiment (Figs 2–3). Bias is also affected by initial prey densities, but we did not explicitly explore that factor because it affects where the system starts on the functional response and that effect can be incorporated by the other factors. For example, if experimental durations were sufficiently short that depletion was slight and thus prey mortality rates imposed by predators did not dramatically change over the course of the study, then bias was negligible. However, as prey were depleted and prey mortality rates increased, bias increased as did the potential for incorrect inferences. The effect of depletion depended on the degree of nonlinearity of the functional response (e.g. as reflected in handling time). Although we have derived these predictions for Type II functional responses, these general results likely hold for any decelerating functional response. Results will be complex for more complex responses, such as Type III responses in which negative and positive density dependence occurs.

Our literature review suggests that MPE studies often allow high levels of depletion (on the order of 70%) and most predators exhi-

bit saturating functional responses; the application of eqns 1 and 2 has likely led to a biased impression of MPEs in food webs. For studies using additive designs, this bias has been towards concluding risk enhancement even in cases where predators have independent effects (e.g. Buskirk 1988; Soluk & Collins 1988; Morin 1995; Losey & Denno 1998; Son & Thiel 2006). Despite this bias, meta-analyses suggest that it is more common to find 'risk reduction' (Sih *et al.* 1998) in studies using additive experimental designs. As a result, the application of eqn 1 has likely resulted in underestimates of the magnitudes of risk reduction. In other words, risk reduction is likely *even stronger* than past analyses have suggested. For studies using a substitutive design, the bias inherent in eqn 2 likely also led to bias, although the direction of that bias is more ambiguous.

The magnitude of the bias in past studies (and associated meta-analyses) is impossible to infer without reliable estimates of depletion in combination with handling times and attack rates for predators used in those studies. Thus, instead of reanalysing those data (which is not possible), we require new approaches. One option, which does not require assessment of the functional response, is to do very short-term experiments or to replace prey as they are consumed to prevent depletion in foraging trials. This is a robust approach that can be effective no matter the shape of the functional response (or nature of density dependence). However, preventing depletion is often not feasible, so other approaches are required.

If the predators exhibit Type II functional responses, then we recommend the application of Roger's random predation model to estimate parameters of the functional response while accounting for prey depletion (e.g. Juliano *et al.* 2001; Bolker 2008; McCoy & Bolker 2008; Stier *et al.* 2012). Note, however, that functional response parameters are likely to be affected by the predator's level of satiation and physiological status as well as the experimental venue. Thus, quantification of the functional responses and evaluation of the MPEs are best conducted in parallel using similar conditions. Of course, these parameter-based approaches assume that feeding rates are suitably described by a specific form of functional response (we have emphasised a Type II response in our discussion). For many organisms, we still lack the information needed to assess the general form of this function, let alone how it can be estimated in the presence of prey depletion (e.g. as in the case of Roger's model for a Type II functional response).

Our insights about studies of MPEs likely apply to a range of other ecological phenomena (e.g. effects of toxicological mixtures on survival assays, and effects of multiple parasites on their common host) in which depletion and density dependence likely occur. For example, over the past two decades a wealth of studies have aimed to understand if diversity within trophic levels affects ecosystem function (Cardinale 2002; Ives *et al.* 2005; Balvanera *et al.* 2006; Cardinale *et al.* 2006; Duffy & Cardinale 2007; Schmitz 2007). Often these studies compare the function of ecosystems with mixtures of consumers to the function expected based on results from systems with a single species of consumers (Ruesink & Srivastava 2001; Cardinale 2002; Balvanera *et al.* 2006). Greater ecosystem function with diverse consumer assemblages is often interpreted as an emergent property of consumers that occurs from niche partitioning, or facilitative interactions (e.g. cooperative hunting) among consumers. Reduced ecosystem function is often attributed to intraguild interactions and interference among consumers (Cardinale *et al.* 2006; Duffy & Cardinale 2007). However, as we have shown here, some of the differences in consumption rates between monocultures and mixtures may be the result of nonlinearities in consumer functional responses (also discussed in Ives *et al.* 2005), combined with changes in the densities of resources via depletion. A better appreciation of the shapes of consumer functional responses and the effects of prey density on expected prey survival (and predator feeding rates) in diverse mixtures of consumer species will enhance our ability to predict how species loss in consumer trophic levels will affect ecosystem function and stability. Moreover, understanding when emergent effects of multiple predators are expected to arise is fundamental for understanding the trophic interactions that characterise all food webs.

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AUTHOR CONTRIBUTIONS

MWM, ACS and CWO developed the idea and conceptual framework of the study, MWM performed modelling work and analysed output data, and ACS performed the literature review on prey depletion. MWM wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

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Electronic Supplement 1

EMERGENT EFFECTS OF MULTIPLE PREDATORS ON PREY SURVIVAL: THE IMPORTANCE OF DEPLETION AND THE FUNCTIONAL RESPONSE

by

M. W., McCoy, A. C. Stier, And C. W. Osenberg

This supplement provides additional results from simulated multiple predator effects experiments as described in McCoy et al. For each simulation we compared the true survival (i.e. value of $S_{1,2}$, Eq. 4) under the assumption of independence with that expected from the Multiplicative Risk Model (i.e. value of $\hat{S}_{1,2}$, Eq. 1 and 2), expressed as both a difference, $\hat{S}_{1,2} - S_{1,2}$, and a ratio, $\hat{S}_{1,2}/S_{1,2}$. Absolute bias is presented here and relative bias is presented in the main document.

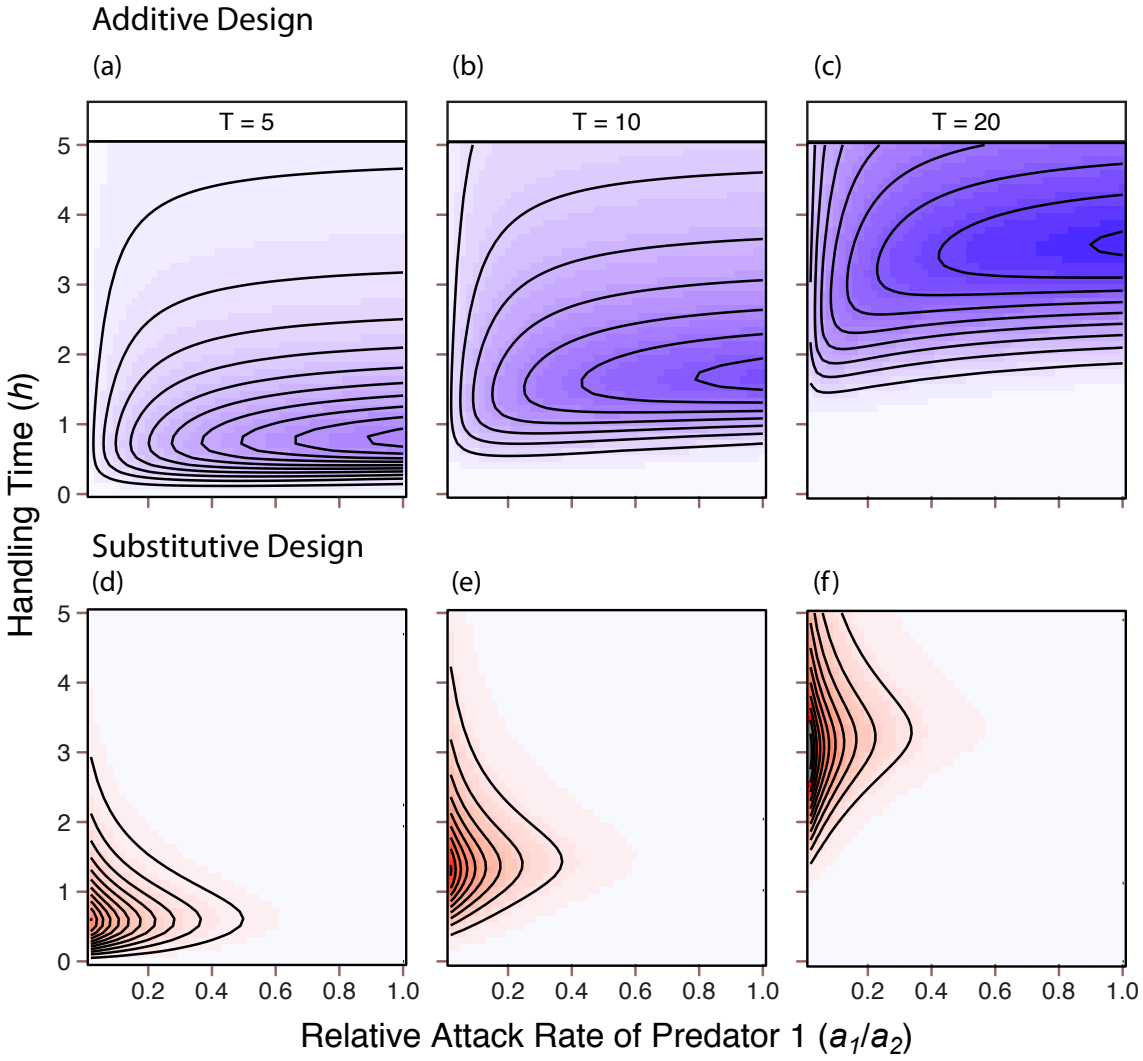

Figure S1. Contour plots showing absolute bias (i.e., $\hat{S}_{1,2} - S_{1,2}$) for the Multiple Risk Model ($\hat{S}_{1,2}$, Eq. 1) based on experiments using additive (Panels **a-c**) or substitutive designs (Panels **d-f**). Handling times (y-axis) were the same for both predators (h_1=h_2=1 to 5), and the attack rate for predator 2 was fixed (a_2=1), but the attack rate for predator 1 (x-axis) and experimental duration was varied. For additive designs (panels **a**, **b**, **c**), the Multiplicative Risk Model predicted survival probabilities that were too low. In contrast, for substitutive designs (panels **d**, **e**, **f)** the Multiplicative Risk Model predicted survival probabilities that were too high. The direction of bias is indicated by color, while the magnitude of bias is indicated by intensity. Compare this figure to that for relative bias provided in the text (Fig. 2)

Electronic Supplement 2

EMERGENT EFFECTS OF MULTIPLE PREDATORS ON PREY SURVIVAL: THE IMPORTANCE OF DEPLETION AND THE FUNCTIONAL RESPONSE

by

M. W., McCoy, A. C. Stier, And C. W. Osenberg

This supplement provides sample computer code for the R statistical programming environment (R Development Core Team, 2011) that can be used to help understand depletion and to reduce the potential for bias when making inferences about multiple predator effects. The code provided in this supplement is divided into four sections. Here, we deal only with the case in which predator functional responses can be described with a Holling's Type II model (Holling, 1959). In the first section, code is provided that helps to illustrate how depletion affects the shape of the predator's functional response.

The second and third sections provide a guide for attaining accurate estimates of functional response parameters when depletion has occurred. Conducting experiments in which depletion is not an important factor is often not feasible. One alternative is therefore, to conduct functional response experiments and then apply Roger's random predation model (which accounts for prey depletion) to estimate functional response parameters (B M Bolker, 2008; Juliano, 2001; M W McCoy & Bolker, 2008; Rogers, 1972; James R Vonesh & Bolker, 2005). Section 2 provides code for a data simulator that generates code that one might expect to get from a functional response experiment. In

this case the experiment is run for 2 days and depletion occurs. Code is then provided that employs a maximum likelihood approach to analyze the simulated data. This analysis assumes a binomial error model and uses the lambertW function to fit Roger's random predator equation to the data (Bolker, 2008; McCoy & Bolker, 2008). Section 3 outlines how one might go about estimating one unknown parameter for a Type II functional response. This approach may be most useful for well-studied systems where one parameter, such as handling time, is known. In the example provided, attack rate is estimated for the case where prey survival and handling time is known.

Section 4 provides code to implement a numerical integration (alternate approaches can be used) to generate correct predictions of expected prey survival for two predator species foraging independently.

Section 1--Illustration of depletion effect

Taken from the help page for the lambertW function in the R package "emdbook". The original code can be accessed in **R** via the command-- `?emdbook::lambertW`

```
##Load libraries

require(deSolve)
require(bbmle)
require(ggplot2)
require(emdbook)

## Rogers random predator equation:
rogers.pred <- function(N0,a,h,T) {
  N0 - lambertW(a*h*N0*exp(-a*(T-h*N0)))/(a*h)
}
##Holling's Type II Functional Response
holling2.pred <- function(N0,a,h) {
  a*N0/(1+a*h*N0)
}

curve(rogers.pred(x,a=1,h=0.2,T=1),from=0,to=60,
```

```
      ylab="Number eaten/unit time",xlab="Initial
number",ylim=c(0,5),
      main="Predation: a=1, h=0.2")
curve(rogers.pred(x,a=1,h=0.2,T=5)/5,add=TRUE,lty=2,from=0)
curve(rogers.pred(x,a=1,h=0.2,T=0.2)*5,add=TRUE,lty=3,from=0)
curve(rogers.pred(x,a=1,h=0.2,T=10)/10,add=TRUE,lty=4,from=0)
curve(holling2.pred(x,a=1,h=0.2),add=TRUE,lty=1,lwd=2,from=0)
abline(h=5)
legend(30,3,
       c(paste("Rogers, T=",c(0.2,1,5,10),sep=""),
         "Holling type II"),lwd=c(rep(1,4),2),lty=c(3,1,2,4,1))
```

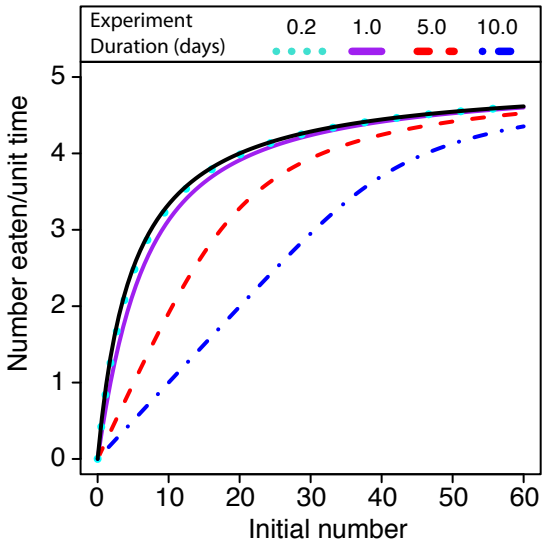

Figure 1: Illustration of depletion effects on the shape of the predators function response. As duration of the experiment, T, increases the apparent shape of the predators functional response becomes increasingly linear and the deviation from the Holling Type II functional response model increases. Fitting the Holling Type II model to data for T>0, fitting without accounting for depletion will generate parameters estimates substantially different from the true predator functional response depicted by the bold solid line (the Holling type II curve).

Section 2--Estimating attack rate and handling time parameters from data

For this example we have modified the supplemental code file provided in (McCoy, Bolker, Warkentin, & Vonesh, 2011). First we generate simulated data that mimics a functional response experiment. The simulation gives consumption by predators across a prey gradient ranging from 10 to 100 individuals with each trial replicated 6 times. The simulated experiment runs for two days allowing depletion to occur.

In this simulation, variation among replicates is introduced by allowing attack rate, *a*, to vary randomly across replicates around a median value of 0.75 and with a proportional variation of 10%.

We then apply Rogers random predator model to obtain estimates of the parameters of the functional response.

```
## Generate data
```

```
set.seed(1001) ## set random-number seed for reproducibility

simdata <- function(nrep){
  test.vals <- expand.grid(N0=seq(10,100,by=10),
                           replicate=1:nrep)

  a <- rlnorm(nrep,meanlog=log(0.75),sdlog=.10)
  p <- with(test.vals,rogers.pred(N0=N0,
                                  a=a[replicate],
                                  h=0.02,T=2)/N0)
  z <- rbinom(nrow(test.vals),prob=p,size=test.vals$N0)
  data.frame(test.vals,killed=z)
}
x <- simdata(6)

## Plot results ...
g=ggplot(x,aes(N0,killed))+geom_point(aes(colour=replicate,shape=
 factor(replicate))) + labs(x = "Initial Density",y="# Prey
 Eaten") + stat_function(fun = function(x,a,h,T) { x -
 lambertW(a*h*x*exp(-a*(T-h*x)))/(a*h)},
 args=list(a=.75,h=.02,T=2),colour = "red") +
 opts(panel.grid.minor=theme_blank(),
 panel.grid.major=theme_blank())+
 (axis.title.x = theme_text(face="bold", size=16),
 axis.text.x  = theme_text( size=12),
 axis.title.y = theme_text(face="bold", angle=90,size=16),
 axis.text.y  = theme_text( size=12))
g # print graph
```

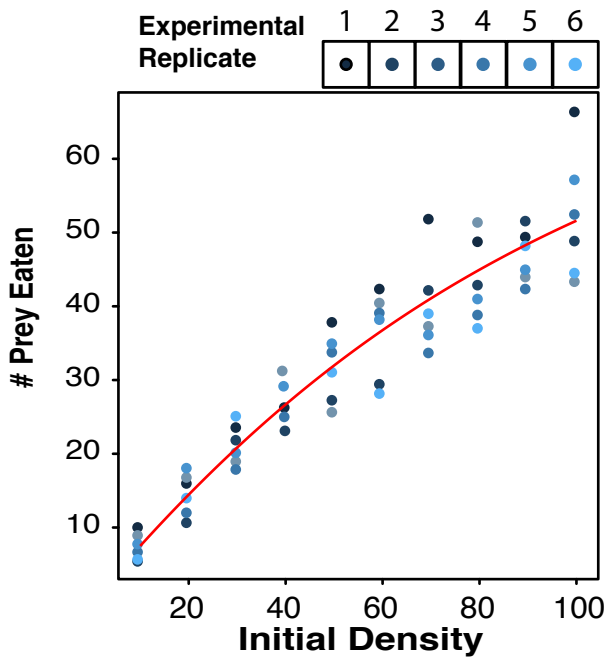

Figure 2: Plot of simulated functional response data. Colors depict among replicate variability.

```
##Now estimate parameters by fitting Rogers random predator
equation using a maximum likelihood approach and assuming a
binomial error structure.

m1= mle2(killed~dbinom(size=N0,prob=rogers.pred(N0,a,h,T=2)/N0),
start=list(a=.75,h=0.02), method="L-BFGS-B",lower=1e-5,,data=x)

coef(m1) ##Gets the estimates of the parameters
## The should be very close to the true parameters that were used
to generate the simulated data.
```

Section 3—Estimating attack rate post hoc.

For some MPE studies, there may be existing estimates of handling time. In these cases, one need not perform detailed functional response studies. Instead, the data from the MPE study can be used along with the existing handling time estimates to estimate attack rates.

To illustrate how to estimate an attack rate when handling time and proportional survival is known, we use the same data depicted in supplemental figure 2 that was generated above. Specifically, we know that h=0.02 and that when the initial density of prey was 100, ~52 prey were eaten (N_{eaten}) on average. So, we can specify the known values of *N0*, $N_{eaten,}$ and *h* to solve for attack rate, *a* by finding the value of

a whereby the expected number eaten based on the functional response model is equal to the observed number eaten.

```
Neaten=52
u1=uniroot(function(a){rogers.pred(N0=100, a=a, h=.02, T=2)-Neaten
      },lower=0.00001,upper=20)

u1$root ## ~0.75.
```

The attack rate and handling time parameters (estimated with a model that accounts for depletion) can now be used in section 4 to derive the expected survival of prey in the mixed predator trials.

Section 4—Predicting independent effects of two predators assuming both have Type II functional responses

Armed with the correct parameters for handing time and attack rate for each predator species, for example by using Roger's random predation model as above, one can generate the predicted combined effects of the two predators if they act independently. Below we provide an example that uses numerical integration

```
#First define a gradient function for the numerical integration

frgrad <- function(t,y,parms) {
  with(c(as.list(parms),y),
      list(-N0*(a1/(1+a1*h1*N0)+a2/(1+a2*h2*N0)),
            NULL))
}
```

Second, define a function to compute number eaten by time T. Here, we represent the starting prey densities as N10, and N20 for predator 1 and 2 respectively. Similarly, we use *a1,* and *a2* for the attack rates and *h1* and *h2* for the handling times of the two predators.

```
rogers.lsoda <- function(N0,a1,a2,h1,h2,T) {
  L1 <- lsoda(y=c(N0=N0),times=seq(0,T,length=2),
              parms=c(a1=a1,a2=a2,h1=h1,h2=h2),
              func=frgrad)
 (N0-L1[2,-1])/N0

}
```

Then we can run the numerical integration by specifying the values of each of these parameters.

```
rogers.lsoda(N0=100,a1=0.75,a2=0.5,h1=0.02,h2=0.02,T=2)
```