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Multiple predator effects result in risk reduction for prey across multiple prey densities

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Abstract Investigating how prey density influences a prey's combined predation risk from multiple predator species is critical for understanding the widespread importance of multiple predator effects. We conducted experiments that crossed six treatments consisting of zero, one, or two predator species (hellgrammites, greenside darters, and creek chubs) with three treatments in which we varied the density of mayfly prey. None of the multiple predator effects in our system were independent, and instead, the presence of multiple predator species resulted in risk reduction for the prey across both multiple predator combinations and all three levels of prey density. Risk reduction is likely to have population-level consequences for the prey, resulting in larger prey populations than would be predicted if the effects of multiple predator species were independent. For one of the two multiple predator combinations, the magnitude of risk reduction marginally increased with prey density. As a result, models predicting the combined risk from multiple predator species in this system will sometimes need to account for prey density as a factor influencing per-capita prey death rates.

Keywords Prey density · Multiple predator species · Risk reduction · Functional response · Artificial stream tanks

Introduction

Food web and biological control models investigate the effects of predation on populations and communities. These models typically assume that the effects of multiple predator species on their prey are independent (Sih et al. 1998). Although a number of studies have reported that the effects of multiple predators are independent (Van Buskirk 1988; Wilbur and Fauth 1990; Sokol-Hessner and Schmitz 2002), others report effects that are not independent (Rahel and Stein 1988; Spiller and Schoener 1994; Wootton 1994; Sih et al. 1998; Eklov and Van Kooten 2001; Vonesh and Osenberg 2003; Vance-Chalcraft et al. 2004). If multiple predator effects are strong and not independent, models that assume independence of predator effects will either underestimate or overestimate the prey's predation risk depending upon whether risk enhancement or risk reduction, respectively, occurs for the prey. Both risk reduction (Soluk and Collins 1988a; Huang and Sih 1990; Wissinger and McGrady 1993) and risk enhancement for prey (Soluk and Collins 1988a; Martin et al. 1989; Losey and Denno 1998; Sih et al. 1998) have been documented and neither appears to be ubiquitous across systems. For us to understand these multiple predator effects further, it will be important for us to predict the conditions under which independent predator effects, risk reduction, and risk enhancement occur. Currently risk reduction is thought to occur when there are negative interactions between predators (e.g., interference or intraguild predation) or when changes in prey microhabitats or behaviors in response to one or both of the predators reduce the prey's risk of predation. Risk enhancement occurs when there is facilitation between predators or when changes in prey microhabitats or behaviors in

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response to the predator(s) increase the prey's risk of predation. However, other factors such as prey density also may contribute to these outcomes.

We were interested in determining if the occurrence or magnitude of risk reduction or enhancement is a function of prey density. Addressing this question is important for three reasons. First, it could provide insight into why some multiple predator studies find evidence for independent predator effects while others find evidence for risk reduction or enhancement. If the prey's risk of predation is a function of prey density, prey could experience both risk reduction and risk enhancement over a natural range of prey densities. Consequently, the different studies may represent the testing of multiple predator effects at different locations along this response surface. Second, changes in the extent of risk reduction or enhancement with prey density would suggest that the accurate prediction of a prey's mortality risk requires the development of more sophisticated models than are typically used (e.g., multiplicative risk model). Third, addressing this question provides information on the ability of predators to be a potential regulating factor for prey populations. In order for predators to be potential regulators for a prey population, the prey's per-capita risk of predation must increase with prey density. In areas with multiple predator species, changes in the extent of risk reduction or enhancement with prey density would influence both the ability of predators to act as a regulating factor and the abundance about which the prey population could be regulated (Fig. 1). Even if the predators are not a potential regulating factor, they may be a limiting factor for a prey population if prey abundance would increase in the absence of predators.

Prey density is widely understood to influence mortality rates caused by single predator species (Hirvonen and Ranta 1996; Kratz 1996; Yasuda and Ishikawa 1999; Connell 2000; Elliott 2003) and there is evidence suggesting that the predation risk from multiple predator species also may be influenced by prey density. For example, Soluk (1993) reported that the predictability of the prey's risk in the presence of multiple predator species varied with prey density and that the influence of prey density varied for different prey species. Using stoneflies and sculpins as predators and *Baetis* mayfly larvae as prey, he found that the degree of risk reduction for the prey increased with prey density. In contrast, with *Ephemerella* mayfly larvae as prey, risk enhancement occurred for the prey at low prey densities, but the predator effects were independent at higher prey densities (Soluk 1993). In addition, Losey and Denno (1998) reported that the strength of risk enhancement for aphids in the presence of one foliar-foraging and one ground-foraging predator increased with prey density.

The independence of multiple predator species effects may be affected by prey density if the intensity of interspecific interactions between predators changes with prey density, which is known to happen. For example, intraguild predation and other interspecific interactions have been found between ladybird beetles of different

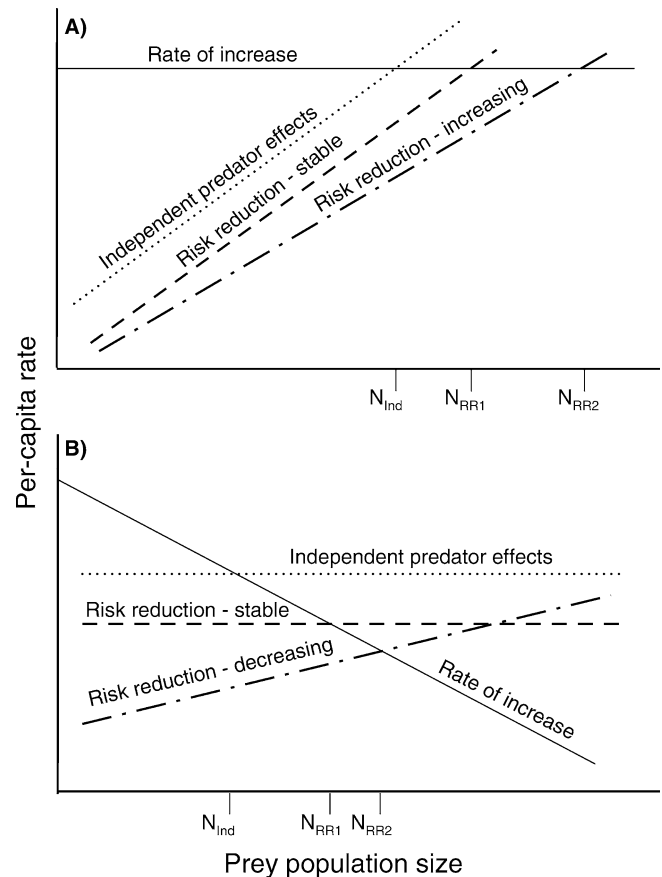


Fig. 1 Conceptual figure demonstrating how the presence of risk reduction (RR) and changes in the magnitude of RR with prey population size influence (i) the equilibrium abundance of the prey population and (ii) the ability of the predators to potentially regulate the prey population. **A)** The equilibrium abundance of the prey population is identified as the point of intersection between the prey population's per capita rate of increase due to immigration and births (solid line) and the prey's risk of mortality due to multiple predator species. When the amount of RR does not change with prey population size (Risk reduction—stable) (---), RR causes the equilibrium population size (N_{RR1}) to be greater than what would be expected (N_{Ind}) if the effects of multiple predators are independent of each other (....). If the magnitude of RR increases with prey population size (—), the equilibrium population size of prey (N_{RR2}) would be even higher. The ability of predators to regulate the size of a prey population around a particular equilibrium abundance requires that their combined effect on a prey's risk of mortality change with prey population size. Consequently, these predators may regulate the prey population in all cases. These predictions would still be true if the prey's per capita rate of increase declines with prey density. **B)** Symbols are the same as in A. Multiple predator species cannot regulate a prey population around an equilibrium size if the independent effects of predators do not increase with population size or the magnitude of RR does not change with prey population size. Predators may regulate the prey population, however, if the amount of RR decreases with increasing prey density (thus increasing the prey's risk of predation). This figure could be expanded to determine the outcome when risk enhancement is present and when the lines are non-linear

species at low aphid (prey) density but not at high prey density (Obrycki et al. 1998; Kajita et al. 2000). Furthermore, Peckarsky (1991) reported interference

between predatory stoneflies that only affected their feeding rates at intermediate prey densities and not at either high or low prey densities. Aggressive responses between stonefly species also increased at intermediate prey densities and decreased at the highest prey densities (Peckarsky and Penton 1985). One reason why the intensity of interspecific interactions between predators could change with prey density is that the likelihood of the predators' becoming satiated will often be a function of prey density. If one of the predators becomes satiated, it may decrease its direct aggression toward other predators which could increase the prey's risk of predation from these other predators.

Prey density may affect the independence of multiple predator species effects also if the degree to which the prey's antipredator defenses against one predator affects their risk of being killed by another predator changes with prey density. For example, Soluk (1993) suggested that sculpins only benefited from the presence of predatory stoneflies at low to moderate *Ephemerella* densities where the behavior that this prey used as a defense against the stoneflies made them more detectable by sculpins. At higher prey densities, enough of the prey were already in exposed places that small increases in prey accessibility or visibility did not further increase sculpin capture rates.

The evidence outlined above suggests that the effects of multiple predators on their prey should change with prey density. Here we report the results of an experimental analysis of the effects of prey density on interactions between pairs of two common fish and one invertebrate predator from Midwestern streams. We exposed single and multispecies pairs of hellgrammites, greenside darters, and creek chubs to a range of densities of a common mayfly prey (*Stenonema* sp.) and evaluated whether the effects of the predators were independent at all prey densities.

Methods

Study species

Hellgrammites [Corydalidae: *Corydalis cornutus*], greenside darters [Percidae: *Etheostoma blennioides*], and creek chubs [Cyprinidae: *Semotilus atromaculatus*] all co-occur in many streams in the eastern United States where they prey upon mayfly larvae and other benthic invertebrates. Hellgrammites forage on rocks and wood (Roell and Orth 1991; Phillips 1996; Kirk and Smock 2000) while greenside darters and creek chubs consume prey from the upper surfaces of rocks and from the drift (Smith 1979; Magnan and FitzGerald 1984; Dahl and Greenberg 1996; Welsh and Perry 1998). Large hellgrammite larvae (mean head width = 8.0 ± 0.3 mm) and small fish (mean mass = 3.1 ± 0.2 g for darters and 6.0 ± 1.1 g for creek chubs; mean TL = 70.0 ± 1.1 mm for darters and 78.3 ± 4.2 mm for creek chubs) were used to

avoid intraguild predation. Preliminary experiments (H.D. Vance-Chalcraft, unpublished data) showed that individuals of these sizes are unlikely to consume each other. *Stenonema* sp. mayfly larvae (Heptageniidae) in the size range between 5.4 mm and 11.4 mm total length were used as prey in all treatments. *Stenonema* are grazers that use stones and wood as substrate and crawl away or rely on cryptic coloration to avoid predators (Peckarsky 1980; Jacobi and Benke 1991; Peckarsky and Cowan 1995; Dudgeon 1996).

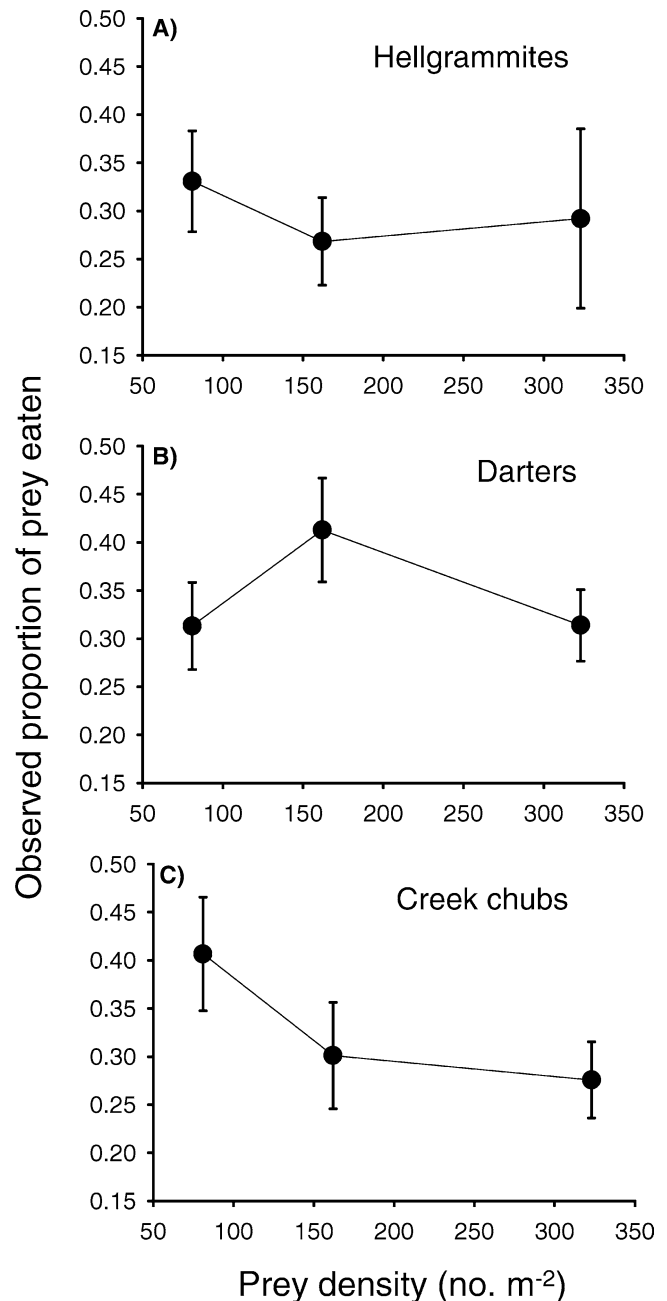


Fig. 2 Observed proportion of mayfly prey eaten (mean \pm 1 SE) at each prey density (no./m²) by **A)** hellgrammites alone, **B)** darters alone, and **C)** creek chubs alone

All experimental organisms were collected from the Vermilion River drainage system (Vermilion County, IL, USA) or Shivering Sands Creek (Door County, WI, USA). Before being used in an experiment, invertebrates were held in a cold room for at least 24 h and fish for at least 1 week. All the predators were fed live mayflies, oligochaetes, or zooplankton. Fish diets were supplemented with frozen mosquito larvae. All predators were starved for 24 h before being used in an experiment. Prey were held in a container with algae-covered rocks as food.

Experimental protocols

Our experiment was conducted in replicate recirculating stream tanks (Soluk and Collins 1988a, Fig. 2), (30.48×30.48×60.96 cm, average current velocity 0.12 m/s) housed in a cold room set to mimic natural summer temperatures (20°C) and diel conditions (14 h light:10 h dark cycle). This experiment measured the risk of mortality to prey in six different predator treatments (no predators, 1 hellgrammite, 1 darter, 1 creek chub, 1 hellgrammite + 1 darter, and 1 darter + 1 creek chub) at three different prey densities (81, 162, and 323 mayflies/m²) that reflected a range of natural field densities (H.D. Vance-Chalcraft, unpublished data), for a total of 18 treatments. These densities correspond to adding 15, 30, and 60 mayflies per tank for the low, intermediate, and high prey densities, respectively. We did not include the 1 hellgrammite + 1 creek chub combination at the three prey densities because of space constraints in the cold room and logistical considerations related to collecting experimental animals. Each of the low (81/m²) and high (323/m²) prey density treatments was represented once in each of six temporal blocks, for a total of six replicates. The data for the intermediate (162/m²) prey density treatments came from a prior experiment (Vance-Chalcraft 2003; H.D. Vance-Chalcraft and Soluk, in review) using the same experimental protocols and conducted immediately before this experiment in the summer of 2002. The intermediate prey density treatments were replicated nine times, once in each of nine temporal blocks. Within each temporal block, each treatment was randomly assigned to an independent stream tank and barriers between aquaria prevented visual cues between organisms in different tanks.

We added water (approximately 56 l), an air diffuser, and nine large rocks to each stream tank prior to the initiation of each replicate. The rocks were haphazardly chosen from a pool of approximately 300 natural stream rocks held submerged for at least 2 weeks in a tank under a plant growth lamp to allow for algae and periphyton growth. We added the appropriate number of mayflies to each tank 24 h after the tanks were filled, and added predators to the appropriate tanks and placed lids on each tank 2 h later. After 4 days, the predators were removed and measured (total length and wet mass for all the predators and also head width for

the hellgrammites) and all surviving mayflies were counted by carefully searching each tank. All experimental animals were only used once.

Statistical analyses

To determine whether the predator treatments caused significant prey mortality, we used ANOVA on the proportion of prey missing at the end of the experiment, followed by multiple comparisons with Bonferroni adjustments to determine which treatments differed significantly from the control for that same prey density. We used a two-way ANOVA to determine whether the observed proportion of prey eaten in any single predator species treatment was dependent on the identity of the predator present and/or prey density. To account for the loss of individuals not due to predation, the actual proportion of prey eaten within each block in the x th treatment (p_x) was calculated as:

$$p_x = \frac{n_c - n_f}{n_c} \quad (1)$$

where n_c is the final number of prey found in the control tank (without predators) for that prey density in that block, and n_f is the final number of prey recovered in the x th experimental tank.

To test for independence between predator effects, we compared the observed predation rate to the predicted values generated by the multiplicative risk model (Soluk and Collins 1988a; Soluk 1993; Sih et al. 1998). This model predicts the combined risk to the prey when two predator species are present but their effects are independent. Specifically, the multiplicative risk model predicts that the expected proportion of prey killed by predator species A and B together (p_{AB}) is:

$$p_{AB} = p_A + p_B - p_A p_B \quad (2)$$

where p_A is the probability of being consumed by predator species A in isolation, and p_B is the probability of being consumed by predator species B in isolation. The $p_A p_B$ term in the model accounts for prey removal as a prey individual eaten by one predator is no longer available to other predators. This model assumes that (1) the effect of each predator species on a prey species is not influenced by the presence of other predator species, (2) there is no reproduction during this time period, and (3) the instantaneous rate of prey consumption (i.e., \ln [proportion of prey consumed]/unit time) by a predator species is relatively constant for the length of the experiment. No reproduction occurred during our experiment and Vance-Chalcraft (2003) has shown that the assumption of constant consumption rates through time is valid for the predators considered here. As a result, a significant difference between observed and predicted predation risk is the result of a prey's risk of predation by one predator species changing in the presence of another predator species. We calculated an expected proportion of prey eaten by each of the two

predator combinations at each prey density by substituting the observed proportion of prey eaten in tanks with one predator individual into this model (Eq. 2). The expected proportion of prey eaten in a particular tank was always based on observed proportions of prey eaten that were recorded in tanks in the same block. Thus, we had an observed and expected proportion of prey eaten for each tank with two predator individuals in each block.

We used a three-way ANOVA to determine simultaneously: (1) if predator effects were independent when two predators were together, (2) whether the magnitude of risk reduction or risk enhancement was similar for both multiple predator combinations, (3) whether the magnitude of risk reduction or risk enhancement was similar for all prey densities, and (4) whether the multiple predator combinations differed in how the magnitude of risk reduction or risk enhancement varied with prey density. The two multiple predator combinations represent one factor, the three levels of prey density represent the second factor, and the categories “observed” and “expected” represent different levels of the third factor. The response variable for each tank was the proportion of prey eaten (both observed and expected values). The observed and expected proportion eaten for each enclosure are independent of each other because the expected values were derived from different independent experimental units. If the observed and expected proportions eaten are equivalent, the effects of the predators are independent (addresses question 1). A larger observed risk than expected risk would indicate risk enhancement for the prey, while a smaller observed risk than expected would indicate risk reduction for the prey. Although interpretation of the “predator combination” and the “prey density” effects is meaningless (as they pool both observed and predicted values within each of these main effects), a non-significant interaction between the factors “predator combination” and “observed versus expected” would indicate that the magnitude of risk reduction or enhancement is similar for both multiple predator combinations (addresses question 2). Likewise, a non-significant interaction between the factors “prey density” and “observed versus expected” would indicate that the magnitude of risk reduction or enhancement was similar for all three levels of prey density (addresses question 3). A non-significant three-way interaction would indicate the multiple predator combinations did not differ in how the magnitude of risk reduction or risk enhancement varied with prey density (addresses question 4). We used this large-scale ANOVA approach to avoid using multiple tests and risk artificially inflating our error rate.

We repeated each of these analyses with only the low and high prey density treatments (which were conducted during the same experiment, unlike the intermediate prey density treatments) and included temporal block effects to determine if combining the results from two experiments influenced the results.

Results

All predators inflicted significant mortality on the prey at each prey density compared to the predator-free control for that density (overall: $F_{17,108} = 10.278$; $P < 0.001$; all comparisons to controls: $P < 0.003$). The proportion of prey consumed did not differ among single predator treatments (i.e., predator identity; $F_{2,54} = 0.581$; $P = 0.563$) or among the three levels of prey density ($F_{2,54} = 0.666$; $P = 0.518$). The interaction between identity of the single predator species and prey density also was not significant ($F_{4,54} = 1.093$; $P = 0.369$; Fig. 2).

The observed and expected proportions of prey eaten significantly differed ($F_{1,74} = 8.627$; $P = 0.004$), indicating that the predator effects were not independent. Specifically, the prey experienced risk reduction (Fig. 3). There was no significant interaction between the “predator combination” and “observed versus expected” factors ($F_{1,74} = 0.107$; $P = 0.744$), indicating that the strength of risk reduction did not differ between the predator combinations with two predators. There was also no significant interaction between the “prey density” and

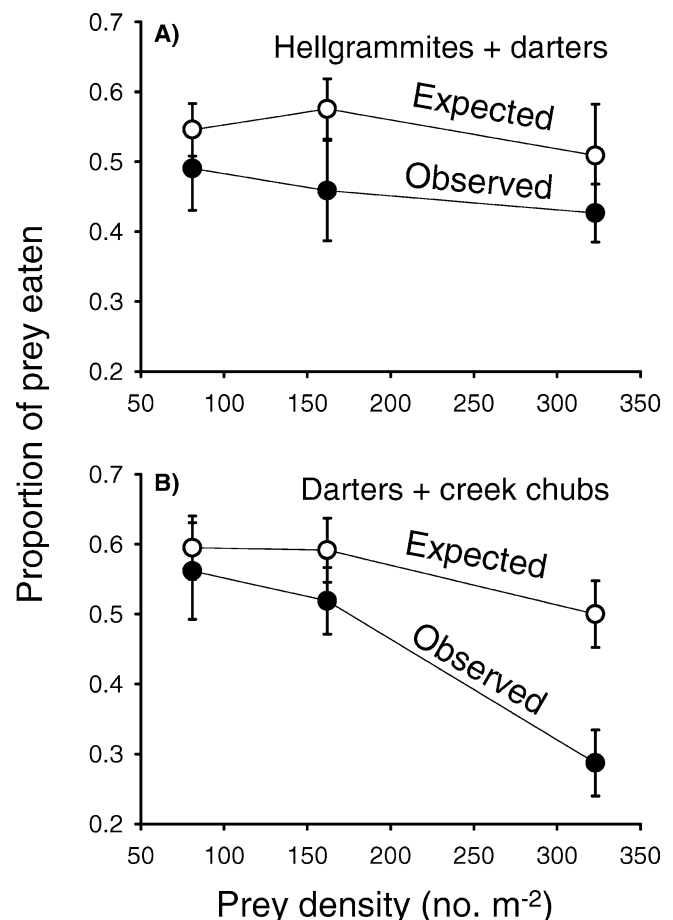


Fig. 3 Observed (filled circle) and expected (open circle) proportion of mayfly prey eaten (mean \pm 1 SE) at each prey density (no./m²) by **A)** hellgrammites and darters together and **B)** darters and creek chubs together

“observed versus expected” factors ($F_{2,74}=0.743$; $P=0.479$), so the strength of risk reduction did not differ among the three levels of prey density (Fig. 3). Although there appears to be a greater magnitude of risk reduction at the highest prey density with the darter + creek chub combination than the hellgrammite + darter combination (Fig. 3), this difference was not statistically significant (three-way interaction term $F_{2,74}=0.699$; $P=0.500$). Thus, the magnitude of risk reduction did not vary with prey density for either multiple predator combination.

To further examine the trend of greater risk reduction at the highest prey density in the darter + creek chub combination, we used a posthoc ANOVA to determine if the magnitude of the risk reduction (observed–expected proportion of prey eaten) was significantly greater at the high prey density than the low prey density for this predator combination. We found that the difference in the magnitude of risk reduction between these two prey densities was marginally significant ($F_{1,10}=4.504$; $P=0.060$), with a higher magnitude of risk reduction in the high prey density than the low prey density treatments.

None of the results changed qualitatively if only the low and high density treatments (which were conducted at the same time) are considered and a temporal block effect is included.

Discussion

Risk reduction

We found risk reduction at all three levels of prey density with both multiple predator combinations. Our experimental design purposely prevented intraguild predation and predator numerical responses; thus, the risk reduction in our system was caused by trait-mediated interactions. Trait-mediated interactions occur when interactions among individuals depend on phenotypic responses (in this case behavioral responses) of the predators or prey to each other (Abrams 1995; Bolker et al. 2003). Specifically, the risk reduction may have been caused by interference between predators or the presence of prey defenses that made one or both predators less effective in the presence of the other predator. Aquatic predators are known to interfere with each other (Peckarsky and Penton 1985; Soluk and Collins 1988a, b; Peckarsky 1991; Soluk 1993) primarily because of food resource limitation and the risk of intraguild predation (Peckarsky 1991; Soluk 1993). Although intraguild predation did not occur in the experiment, the fear of intraguild predation may still have influenced the predators. Based on preliminary work using predators of varying sizes, creek chubs appeared much more likely to be intraguild predators on darters than darters were on hellgrammites. It is also possible that the prey used defenses that made one or

both predator species less effective in the presence of the other predator. This mechanism could explain the risk reduction if, for example, the prey reduced their activity and remained on the bottoms of rocks increasingly when both the darter and creek chub were present than when either one alone was present. This mechanism is not as likely for the hellgrammite + darter treatment, though, since hellgrammites can crawl along the bottoms of stream rocks. Direct (e.g., aggression) or indirect (e.g., fear of intraguild predation) interference between predators is the more likely explanation.

Regardless of the mechanism responsible, risk reduction may allow the prey population to have lower mortality rates from predation and sustain higher population growth rates than would be predicted by independent predator effects. Risk reduction may also influence the distribution of predators because costs (i.e., lower consumption rates) would be associated with being spaced too closely with other predators. Alternatively, the predators may switch the type of prey they consume when other predators are present. Finally, the presence of risk reduction indicates that food web and biological control models must take these trait-mediated interactions into account in order to avoid overestimating a prey's predation risk in the presence of multiple predators.

Effects of prey density

Prey density had little impact on the prey's risk of predation in the single predator treatments as a similar proportion of prey was eaten in all prey density treatments. Thus, there was no demonstrable benefit or risk for the prey from being near increasing levels of conspecifics. In addition, these single predators do not appear to be potential regulating factors for the prey. However, the per-capita consumption of the predators was influenced by prey density because a greater number of prey were consumed in the high density treatments. Thus, the predators benefited from the presence of increasing numbers of prey. This result for the predators is consistent with previous studies which found that per-capita consumption by predators increased with prey density (Yasuda and Ishikawa 1999; Elliott 2003).

We also found a similar amount of risk reduction at all three levels of prey density with both multiple predator combinations, and this result has many implications. First, it does not provide support for the idea that variation in prey density explains why some multiple predator studies find evidence for independent predator effects while others find evidence for risk reduction or enhancement. We found no evidence for prey experiencing both risk reduction and risk enhancement over a natural range of prey densities. Second, this result suggests that complex models that account for changes in the magnitude of risk reduction with prey density are not necessary at some prey densities. The marginal increase in risk reduction with prey

density in the darter + creek chub combination, however, suggests that it may be necessary to develop more complex models at other prey densities. These more complex models could introduce a term that accounts for risk reduction or enhancement changing with prey density. Third, this result indicates that these multiple predator combinations could not regulate the prey's population size because the prey's per-capita risk of predation did not vary with prey density. Instead, the presence of risk reduction allows the predators to increase prey population size above that which would be expected on the basis of independent predator effects (e.g., Fig. 1b). Hence, the predators have the ability to limit, but not regulate, prey abundance.

Although prey density did not have a strong effect on the strength of risk reduction with either multiple predator combination, there was a marginally significant effect of prey density in the darter + creek chub treatment. These results are not consistent with previous studies on this topic, which found a substantial influence of prey density on risk reduction or enhancement in the presence of multiple predator species. In those studies, either the intensity of predator-predator interactions (Soluk 1993) or the degree to which the prey's antipredator defenses against one predator changed their risk of being killed by another predator (Soluk 1993; Losey and Denno 1998) changed with prey density. In our system, these phenomena must not have been influenced by prey density in the hellgrammite + darter treatments or were not influenced strongly by prey density in the darter + creek chub treatments.

Why the magnitude of risk reduction did not change strongly with prey density

Without detailed behavioral study, we cannot be sure why risk reduction did not change strongly with prey density in this system. However, we have enough information to advance some testable hypotheses. We will first examine why we think the intensity of predator-predator interactions did not change with prey density for the hellgrammite + darter treatment. We will then explain why we think the intensity of these interactions may have changed with prey density for the darter + creek chub treatment. Finally, we discuss the possibility that in either of the multiple predator treatments the degree to which the prey's antipredator defenses against one predator affected their risk of being killed by another predator changed with prey density.

The fact that the intensity of predator-predator interactions did not change with prey density is not surprising for the hellgrammite + darter treatment. These predators forage on *Stenonema* in very different ways with hellgrammites largely acting as ambush predators on the bottoms of rocks and darters pursuing prey in a much more active manner (H.D. Vance-Chalcraft and Soluk, unpublished manuscript). Furthermore, once hellgrammites reach the size of

individuals in this experiment these two predators are unlikely to view each other as a potential intraguild predator since both species consume prey much smaller than the size of these predators. As a result, we would not expect these two predators to engage in many predator-predator interactions (e.g., interference or intraguild predation) under any prey density.

On the other hand, the slightly greater magnitude of risk reduction in the high than the low prey density treatments when a darter and a creek chub were present together may have been due to the intensity of predator-predator interactions changing with prey density. This could happen if the darters perceived a tradeoff between risk of intraguild predation and ability to forage (i.e., the rule of minimizing the ratio of mortality to growth rates described by Werner and Gilliam 1984). Creek chubs of the size used in this experiment were unlikely to consume darters, but slightly larger creek chubs did consume darters in preliminary trials (H.D. Vance-Chalcraft, personal observation). If the darters did perceive the creek chubs to be a potential intraguild predator, they would likely decrease their foraging activity and increase refuge use in the presence of creek chubs. However, these behaviors have a cost associated with decreased foraging and these costs will be influenced by *Stenonema* density. Thus, it seems reasonable to hypothesize that the darters would decrease their foraging activity less in the high prey density treatments than in the low prey density treatments. As a result, the darters and creek chubs may be more likely to directly interfere with each other in the high prey density treatments. The risk reduction in the presence of these two predators then would be due to the darters' perceived risk of intraguild predation in the low prey density treatments and due to direct predator interference in the highest prey density treatments. This direct interference would lead to a greater magnitude of risk reduction for the prey than would the darters' perceived risk of intraguild predation, resulting in a greater magnitude of risk reduction in the high prey density treatments than in the low prey density treatments. Alternatively, it is possible that darters have a simple rule of hiding as much as possible when creek chubs are around. The costs of hiding in terms of lost foraging opportunities would increase with prey abundance, and could lead to the observed result.

Although we cannot rule it out, we have no reason to believe that the degree to which the prey's antipredator defenses against one predator affected their risk of being killed by another predator changed with prey density. The only way in which this would have happened in a system with risk reduction is if the predators were more likely to focus on patches with larger numbers of prey (e.g., Connell 2000) or if detection of the predator depended on prey density (e.g., Krause et al. 1998). The spatial scale in our system was such that the predators could not sample many different areas and focus on an area with high prey density more than one with low prey density. In addition, most predators in aquatic systems are detected via chemical cues (Peckarsky and Dodson

1980; Stauffer and Semlitsch 1993; Eklov 2000), which would not be influenced by prey density unless the chemicals detected are from killed conspecifics instead of the predators themselves. While detection of cues from killed conspecifics is common in some systems, we have no evidence for it happening here in the presence of any of the predators alone (i.e., the prey's per-capita risk did not change with prey density in the single predator treatments). Thus, it seems unlikely it would occur when multiple predators are together.

Summary

In conclusion, the ability of studies conducted at a single prey density to accurately predict the combined risk from multiple predator species over a wide range of prey densities depends on the identity of the predator species in this system. Although prey density may marginally influence the magnitude of risk reduction in this system, it does not influence the presence of risk reduction. The findings from this study and others (Vance-Chalcraft 2003, 2004; H.D. Vance-Chalcraft and Soluk, in review) indicate that risk reduction in this system is widespread under both lab and field conditions, a variety of intra and interspecific predator combinations, and a range of prey densities. This risk reduction may have population-level consequences for the prey, allowing the prey population to grow to a greater extent than would be predicted if the effects of multiple predator species were independent. Since prey density does influence the likelihood of finding independent predator effects with some species (also see Soluk 1993; Losey and Denno 1998), empirically derived functional response curves should be developed for other multiple predator species systems.

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