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LETTER

Multi-predator effects across life-history stages: non-additivity of egg- and larval-stage predation in an African treefrog

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Abstract

The effects of multiple predators on their prey are frequently non-additive because of interactions among predators. When prey shift habitats through ontogeny, many of their predators cannot interact directly. However, predators that occur in different habitats or feed on different prey stages may still interact through indirect effects mediated by prey traits and density. We conducted an experiment to evaluate the combined effects of arboreal egg-stage and aquatic larval-stage predators of the African treefrog, *Hyperolius spinigularis*. Egg and larval predator effects were non-additive – more *Hyperolius* survived both predators than predicted from their independent effects. Egg-stage predator effects on aquatic larval density and size and age at hatching reduced the effectiveness of larval-stage predators by 70%. Our results indicate that density- and trait-mediated indirect interactions can act across life-stages and habitats, resulting in non-additive multi-predator effects.

Keywords

Complex life history, density-mediated indirect interaction (DMII), higher order interaction (HOI), Hyperoliidae, multiple predators, phenotypic plasticity, predator-induced hatching, trait-mediated indirect interaction (TMII).

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INTRODUCTION

A growing number of studies demonstrate that pair-wise predator-prey interactions can be altered by the presence of additional predators, such that aggregate predator effects cannot be predicted from their independent effects (reviewed in Sih *et al.* 1998; Bolker *et al.* 2003; Werner & Peacor 2003). Given that most prey face more than one predator species (Schoener 1989; Polis 1991), understanding when and where such non-additive multi-predator effects (MPE) arise is a pre-requisite for understanding community dynamics. In a review of 23 studies, Sih *et al.* (1998) showed that MPEs often combined non-additively (27 of 43 comparisons), and that most often the combined effects were less than expected from their independent effects (20 of 27 comparisons), a result referred to as risk reduction. Risk reduction primarily arose due to predator-predator interactions, such as intraguild predation (Polis *et al.* 1989) or interference, but has also been shown to arise via predator effects on prey traits that reduce the efficacy of a second predator (Peacor & Werner 1997). Interestingly,

nearly all of the studies reviewed by Sih *et al.* (1998) focused on prey that undergo ontogenetic habitat shifts; however, all of these studies focused on interactions of predators of a single life-stage (e.g. Van Buskirk 1988; Fauth 1990; Peckarsky 1991; but see Briggs & Latto 2001). In addition to being exposed to multiple predators within life-stages, species that undergo ontogenetic habitat shifts are exposed to multiple predators across life-stages. Because these predators are unlikely to interact directly, the mechanisms that most commonly give rise to risk reduction are absent. As a result, the apparently predominant occurrence of risk reduction revealed by Sih *et al.* (1998) may simply reflect this bias in the types of multi-predator systems studied. There have been no studies of MPEs across life-history stages and habitats, despite the common occurrence of spatially stage-structured prey populations (Werner & Gilliam 1984; Werner 1986; McPeck & Peckarsky 1998).

Although predators of different prey stages may not interact directly, prey encounters with predators early in their life history may indirectly influence predator-prey interactions in later life-stages via changes in prey density

and traits (e.g. behaviour, morphology, life history). The magnitude and nature of interactions between predators across prey life-stages are not necessarily similar to those observed for predators of the same life-stage. To explore this issue, we examined the effects of sequential stage-specific predators of the African reed frog, *Hyperolius spinigularis*. This is a model system for examining the implications of MPEs that arise from sequential stage-specific predation because the life history of the prey allows us to isolate the density- and trait-mediated indirect effects of an early life-stage predator on subsequent predator efficacy.

Hyperolius spinigularis oviposits on vegetation above water, where eggs are vulnerable to predation from other treefrogs. Upon hatching, larvae drop into the pond, where they are vulnerable to predators such as larval dragonflies. Hence the early life history consists of two stages that are vulnerable to different suites of predators. Two classes of mechanisms may lead to non-additive MPEs in this system: (1) *Density-mediated effects* – Egg-stage predation will decrease aquatic larval densities, potentially reducing larval competition and increasing growth. If mortality risk decreases with prey size, as is often the case with aquatic predators of anuran larvae (reviewed in Alford 1999, Table 10.3), then faster growth can reduce risk to aquatic predators. As a result, larval survival in the presence of larval predators should be greater given previous exposure to egg predators (i.e. risk reduction). In addition, reduced larval density can increase or decrease larval survival depending on the functional response of the aquatic predator (Soluk 1993). (2) *Size/Age-mediated effects* – Egg predation induces surviving eggs in the clutch to hatch earlier at smaller sizes (Warkentin 1995; Vonesh 2000). If younger or smaller hatchlings are more vulnerable to larval predators, then previous exposure to egg predators should lead to lower larval survival (i.e. risk enhancement). Our goal was to test for a non-additive MPE between egg- and larval-stage predators of *H. spinigularis* and to quantify the direction and relative contribution of density- and size/age-mediated effects to the overall MPE.

MATERIALS AND METHODS

Site

Research was conducted at the Amani Nature Reserve Conservation Headquarters in the East Usambara Mountains of northeastern Tanzania (5.06°S and 38.37°E, Elevation: 950 m) from October 2001 to August 2002. Field observations were made at Amani Pond, an artificial permanent shallow pond bordered by submontane rainforest. Pond vegetation is dominated by floating mats of milfoil, *Myriophyllum spicatum*, and marginal patches of emergent cattails, *Typha* sp. *Hyperolius* breeding activity at

Amani Pond in 2002 began on March 4, peaked in April (90% clutches laid in March–April) and continued intermittently through July 17. Our surveys and lab studies revealed that most egg-stage mortality was due to predation by another hyperoliid treefrog, *Afrinoxalus fornasini*, and that larvae of the libellulid dragonfly, *Trapezostigma basilaris*, were the most abundant aquatic predator and were substantially more effective predators than any of the other five most abundant aquatic predators in short-term predation trials (Vonesh unpublished data).

Experimental design

To quantify MPEs and isolate the mechanism leading to emergent effects, we conducted a $2 \times 2 \times 2$ factorial randomized block design (Table 1) in which we mimicked the two types of effects of the egg predator: effects on (1) initial larval density (N_- : a control mimicking density without egg predators; N_+ : density with egg predators) and (2) initial larval size/age (S_- : a control; S_+ : size and age that results from exposure to egg predators). These treatments were crossed with the presence ($+P_A$) or absence ($-P_A$) of aquatic predators. Each treatment was replicated four times. N_- was 38.8 larvae m^{-2} , based on estimates of larval density that would arise without egg predators (37.43 ± 24.2 SD larvae m^{-2}), derived as the product of mean clutch density along two 3×30 m random transects

Table 1 *Experimental design.* N_- is the density of *H. spinigularis* larvae entering the aquatic habitat when no egg predation occurs; N_+ is the density of larvae entering the aquatic habitat with ambient *A. fornasini* egg-stage predation (i.e. reduced). S_- is the age and average body size of tadpoles at hatching when not exposed to egg predation; S_+ is the age and size at hatching of tadpoles surviving an egg predator encounter (i.e. younger and smaller); $+P_A$ is the presence of aquatic predators (libellulid dragonfly larvae) at ambient field densities; and $-P_A$ indicates their absence

		Initial density			
		N_-	N_+	N_-	N_+
	S_-	1. $+P_A$	2. $+P_A$	5. $-P_A$	6. $-P_A$
Initial size/age	S_+	3. $+P_A$	4. $+P_A$	7. $-P_A$	8. $-P_A$
Quantify MPE		Isolate mechanism			
1. Aquatic predation only		2. Aquatic predation + Egg predation density effect			
4. Aquatic predation + Egg predation size and density		3. Aquatic predation + Egg predation size/age effect			
5. No predation		6. Egg predation density effect			
8. Egg predation only		7. Egg predation size/age effect			

between March 4 and the start of the experiment on April 4 (0.49 ± 0.27 SD clutches $\text{m}^{-2} \text{week}^{-1}$), the average clutch size (83.94 ± 21.9 SD embryos clutch $^{-1}$, $n = 123$), and the average expected survival of clutches that were not attacked (0.91 ± 0.20 SD, $n = 29$). N_+ was 11.1 larvae m^{-2} , approximating the field-derived estimate of 14.1 ± 11.3 SD obtained by decrementing N_- by the proportion of clutches attacked by *A. fornasini* (0.73 ± 0.18 SD clutches week^{-1}) and the survival of eggs in attacked clutches (0.14 ± 0.19 SD, $n = 86$). $+P_A$ was 3.13 dragonflies m^{-2} , approximating the mean field density of 3.16 ± 4.78 SD dragonflies m^{-2} . We used late instar *T. basilaris* larvae (Total Length (TL): $18.9 \text{ mm} \pm 2.54$ SD).

To simulate the sublethal effect of egg-stage predation on hatchling size and age we collected fresh (<Gosner stage 10, ~1–12-h old) *H. spinigularis* clutches from the field – 10 each on March 26 and 29. On April 4, clutches were placed in water and gently shaken to induce hatching, yielding large, 10-days-old larvae (TL: 9.33 ± 0.08 mm 95% CI) for Treatments 1, 2, 5, and 6 and smaller, 7-days-old larvae (TL: 7.14 ± 0.06 mm 95% CI) for Treatments 3, 4, 7, and 8 (Table 1). This reflects a decrease in hatching age and size (–3 days and -2.19 ± 0.11 mm 95% CI) consistent with that induced by *A. fornasini* ($\bar{x} \pm 95\%$ CI: -2.6 ± 1.1 days and -2.17 ± 0.77 mm).

The experiment was conducted in plastic tanks ($1.2 \times 0.8 \times 0.4$ m) arrayed in a partially shaded forest clearing near the pond, filled with 220 L of filtered pond water and covered with fine-mesh screens. To each tank we added 200 g of washed and dried pond litter, 10 g of commercial fish food (Hikari brand), 300 g of washed *M. spicatum*, and a 0.5 L inoculum of pond phyto/zooplankton collected using a 80 μm plankton net. The experiment was initiated on April 5, with the addition of newly hatched larvae. Metamorphosed dragonflies were replaced weekly to maintain predator density. After 16 weeks, tanks were emptied and remaining larvae counted. Survival was estimated as the number of metamorphs (removed weekly) plus the number of surviving larvae (proportion of survivors reaching metamorphosis: 0.75 ± 0.09 95% CI).

Detecting MPEs

Testing for a non-additive MPE requires comparison of the proportion of the initial cohort that survived when no predators were present (Treatment 5), only egg predators were present (Treatment 8), only aquatic predators were present (Treatment 1), and both predators were present (Treatment 4). The expected survival from oviposition to metamorphosis assuming independent risks (Billick & Case 1994) in the presence of both egg-stage and aquatic-stage predators, $\hat{\sigma}_{E+A}$, can be derived from their separate effects:

$$\hat{\sigma}_{E+A} = \frac{(\sigma_E)(\sigma_A)}{(\sigma_{NP})} \quad (1)$$

which can be log-transformed into the linear (i.e. additive) form,

$$\ln(\hat{\sigma}_{E+A}) = \ln(\sigma_E) + \ln(\sigma_A) - \ln(\sigma_{NP}) \quad (2)$$

where σ_i is the proportion of eggs that survive from oviposition to metamorphosis in the presence of predator assemblage i ($i = E$: egg predator only; A : aquatic predator only; $E + A$: both the egg and aquatic predator; NP : no predator). Because we simulated effects of egg predators through their effects on density and size/age, the fraction of eggs surviving the egg stage was either 1.0 (in the absence of egg predators) or N_+/N_- (in their presence); the final survival depended on how these effects (of density and size/age) translated through the aquatic stage. We tested for the presence of an emergent MPE with a two-factor ANOVA using log-transformed number (or proportion) surviving to the end of the experiment as the response variable (Billick & Case 1994; Wootton 1994) – a significant interaction indicates non-additivity (i.e. deviation from the model in eqns 1 and 2). Greater survivorship than expected indicates risk reduction; lower survivorship indicates risk enhancement. We then decomposed this overall response into effects attributable to density- and size/age-mediated mechanisms using the remaining treatments.

RESULTS

The overall MPE of *Afrivalus* egg-stage and dragonfly larval-stage predation was non-additive (Fig. 1). The expected survival in the presence of both egg and larval predators was 3.04 frogs (8.6%); however, the observed survival was more than twice that: 7.5 frogs ± 3.05 95% CI (21%; Fig. 1). Despite large effects of dragonflies in the absence of *Afrivalus*, dragonflies had little effect when they acted after *Afrivalus*. Both the density- and size/age-mediated effects of *Afrivalus* egg predation reduced the efficacy of dragonflies to a similar extent (Density: 54% reduction; Size/Age: 44% reduction, Fig. 2). Under combined *Afrivalus* density and size/age effects the efficacy of dragonfly predation was reduced by 70% (Fig. 2).

DISCUSSION

This study represents the first empirical test of whether the aggregate effects of predators that attack different life-stages in different habitats can be predicted from their independent effects or whether density- and/or trait-mediated indirect effects acting across stages lead to a non-additive MPE. We found that the combined effects of arboreal egg-stage and aquatic larval-stage predation were less than

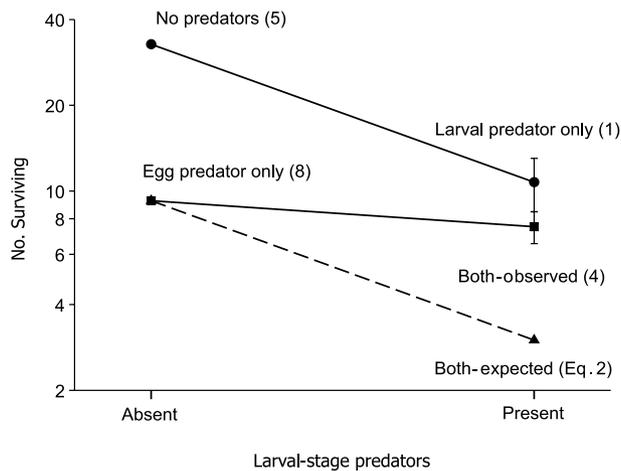


Figure 1 Testing for a non-additive MPE – Effects of egg-stage predation (via *A. formasini*) and larval-stage predation (via dragonfly larvae). “No Predators” (Treatment 5) had no larval-stage predators and an initial density and size/age that occurs in the absence of egg-stage predators. “Egg predators only” (Treatment 8) lacked larval-stage predators and had an initial density and size/age representative of *Afrixalus* egg predation. “Larval predators only” (Treatment 1) had dragonflies and initial density and size representative of no egg-stage predation. “Both” (Treatment 4) indicates treatments with larval predators and initial density and size/age representative of the egg-stage predators. The dashed line indicates the expected effect of both predators, assuming independent effects (Eqns 1 and 2). Values are mean \pm SE. The effects of the two predators are not independent: interaction term, $F_{1,12} = 8.96$, $P = 0.011$.

expected from their independent effects, leading to risk reduction (Fig. 1). Previous studies focusing on MPEs within a particular life-stage have frequently observed risk reduction (Sih *et al.* 1998), but in the majority of these cases risk reduction arose as a result of predator-predator interactions (e.g. intraguild predation leading to reductions in predator density or foraging behaviour). In our study system, where predators are separated in time and space, there is no opportunity for direct predator-predator interactions. Instead, risk reduction resulted from changes in prey (rather than predator) density (Schoener 1993) and prey traits (Peacor & Werner 1997). Only a few studies have directly compared the relative effects of density-mediated indirect interactions (DMIIs) and trait-mediated indirect interactions (TMIIs) within a system (discussed in Werner & Peacor 2003). Our study provides evidence that trait-mediated effects acting across stages (i.e. shifts in the timing of hatching and associated size/developmental consequences) can be of similar magnitude to density-mediated effects across stages (Fig. 2).

Counter to our initial expectations, density and size/age effects acted in the same direction. As expected, reduced

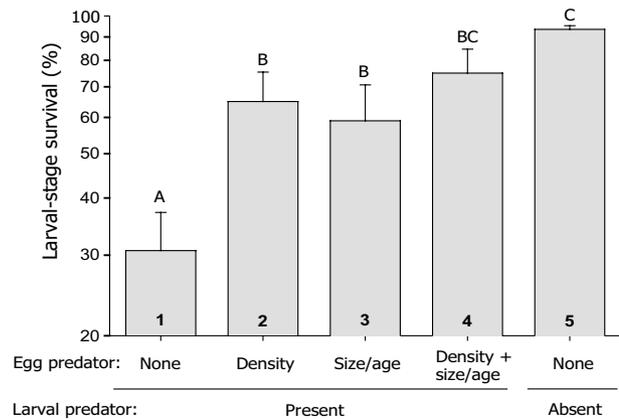


Figure 2 Examining the mechanisms – Effects of the density and size/age effects of *Afrixalus* egg predation on aquatic larval survival in the presence of dragonflies. Numbers within bars correspond to treatment codes in Table 1. The first four bars give the % survival in the presence of larval-stage predators; the last bar gives the survival in the absence of both egg- and larval-stage predators. Treatments are further distinguished by the type of egg-stage predator effect that was included: none, reduced density, reduced size (and age), or reduced density and size/age. Larval survival was similar in all treatments without larval predators (i.e. we present treatment 5, but larval-stage survival in treatments 5–8 was similar; ANOVA, $F_{3,12} = 1.84$, $P = 0.19$). Values are mean \pm SE. Bars with different uppercase letters identify treatments that were statistically different. Responses are not equal across treatments (ANOVA, $F_{4,15} = 7.22$, $P = 0.002$); the independent and combined effects of density and size/age resulted in greater survival relative to the effect of dragonflies alone in the “No Egg Pred” treatment (Post hoc Fisher’s least significant difference (LSD); Density: $P = 0.019$; Size/Age: $P = 0.046$; Density + Size/Age: $P = 0.004$). Survival in the presence of both density and size/age effects and in the presence of dragonflies was not significantly different from survival without dragonflies (Fisher’s LSD, $P = 0.10$).

density led to risk reduction, suggesting that larvae released from intraspecific competition grew more rapidly through vulnerable size classes and/or that libellulid predators were less effective at lower tadpole densities. The egg predator induced size/age effect also led to risk reduction – early hatching/smaller initial size increased larval survival. This is perhaps our most surprising result and is inconsistent with the results from previous short-term studies in similar systems (Warkentin 1995) and the expectations from theoretical studies (Matsuda *et al.* 1993), which indicate that predator-specific defences (e.g. predator-induced early hatching) should result in risk enhancement to other predators. The greater survivorship of younger (and smaller) larvae we observed appears to be driven by the functional form of larval size-specific risk to libellulid predators and compensatory growth in early-hatched larvae (Vonesh unpublished data). Vulnerability to dragonflies

initially increases until *c.* 13 mm TL and then declines as larvae attain a size refuge after 25 mm TL (metamorphosis typically occurs between 40 and 50 mm TL). The initial increase in vulnerability with size is likely driven by changes in activity associated with the shift from reliance upon yolk stores to foraging. Early-hatched larvae exhibit greater growth rates early in ontogeny compared to later-hatched larvae (a trend that is lost after reaching 25 mm) and as a result pass more rapidly through the window of vulnerability. Additional mechanisms may also increase the survival of early-hatched larvae. For example, morphological differences between early- vs. later-hatched tadpoles (of the same age) have been observed in other systems (Warkentin 1999) and may result in differences in their relative vulnerability to aquatic predators (e.g. Van Buskirk & McCollum 1999).

This study represents a first attempt to test for non-additive MPEs across stages and habitats. To increase our power to examine this phenomenon, we used a simplified model system. Because much of our observed response depends upon prey density, our ability to generalize beyond the particulars of this study would be limited if we found that intraspecific competition in our artificial ponds differed substantially from that in natural ponds. Two arguments suggest our results can provide insight into mechanisms that operate in natural systems. First, we established our artificial ponds using conditions very similar to the adjacent natural pond. Second, a recent meta-analysis of intraspecific competition among larval anurans demonstrated that experimental density manipulations conducted in natural ponds yield very similar estimates of intraspecific competition as studies conducted in artificial ponds (Skelly & Kiesecker 2001, mean \ln (response ratios) \pm 95% CI: artificial ponds -0.134 ± 0.014 ; field enclosures -0.117 ± 0.023). Thus, it is unlikely that our results are simply an artifact of our experimental venue. Instead, they highlight the potential for DMIIIs and TMIIIs to act across life-stages and habitats, resulting in non-additive MPEs. Additional work in other systems with stage-structured prey populations will facilitate comparative analyses of non-additive MPEs that will help determine if risk reduction is a general feature of predator-prey systems.

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