Published in final edited form as:

Ecology. 2018 July ; 99(7): 1660–1670. doi:10.1002/ecy.2375.

How can mortality increase population size? A test of two mechanistic hypotheses

Kristina M. McIntire and Steven A. Juliano

School of Biological Sciences, Illinois State University, Normal IL USA

Abstract

Overcompensation occurs when added mortality increases survival to the next life-cycle stage. Overcompensation can contribute to the Hydra Effect, wherein added mortality increases equilibrium population size. One hypothesis for overcompensation is that added mortality eases density-dependence, increasing survival to adulthood (“temporal separation of mortality and density dependence”). Mortality early in the life cycle is therefore predicted to cause overcompensation, whereas mortality later in the life cycle is not. Another hypothesis for overcompensation is that threat of mortality (e.g., from predation) causes behavioral changes that reduce overexploitation of resources, allowing resource recovery, and increasing production of adults (“prudent resource exploitation”). Behaviorally active predation cues alone are therefore predicted to cause overcompensation. We tested these predictions in two experiments with larvae of two species of *Aedes*. As predicted, early mortality yielded greater production of adults, and of adult females, and greater estimated rate of population increase than did later mortality. Addition of water-borne predation cues usually reduced browsing on surfaces in late-stage larvae, but contrary to prediction, resulted in neither significantly greater production of adult mosquitoes nor significantly greater estimated rate of increase. Thus we have strong evidence that timing of mortality contributes to overcompensation and the Hydra effect in mosquitoes. Evidence that predation cues alone can result in overcompensation via prudent resource exploitation is lacking. We expect the overcompensation in response to early mortality will be common in organisms with complex life cycles, density dependence among juveniles, and developmental control of populations.

Keywords

Aedes albopictus; *Aedes triseriatus*; population dynamics; overcompensation; Hydra Effect; mosquitoes; mortality

Corresponding Author: Steven Juliano, sajuliano@ilstu.edu.
DR. STEVEN A. JULIANO (Orcid ID : 0000-0002-6178-4553)

Data Availability: Data associated with this study are archived at Figshare: <https://doi.org/10.6084/m9.figshare.6139955.v1>

Authorship: KMM collected data and wrote the first draft of the manuscript, SAJ provided funding and designed experiment 1, all authors contributed to design of experiment 2, data analysis and interpretation, and manuscript revisions.

Introduction

When an extrinsic (added) source of mortality impinges on a population, the intuitive expectation is that population size will decrease. Counterintuitively, multiple dynamic models indicate that the “Hydra effect” (Abrams and Matsuda 2005), wherein the added density-independent mortality leads to increased population size, is likely to occur (de Roos et al. 2007, Abrams 2009, de Roos and Persson 2013). The Hydra effect in response to mortality is expected to be associated with production of the next life cycle stage that is greater than (stage-specific overcompensation) or equal to (stage-specific compensation) that which would have resulted without that mortality (Zipkin et al. 2009). These effects may be manifest in number of individuals in a stage or total biomass in a stage, and have been demonstrated in varied systems (Jonzén and Lundberg 1999, de Roos et al. 2007, Zipkin et al. 2008, Pardini et al. 2009, Ohlberger et al. 2011, Huss and Nilsson 2011), and a number of different mathematical models have been shown to result in these counter-intuitive effects by a multiplicity of mechanisms (de Roos et al. 2007, Zipkin et al. 2008, Seno 2008, Abrams 2009, Schröder et al. 2009, Karatayev and Kraft 2015, Cortez and Abrams 2016). Central to all of these forms of population increases in response to added mortality is the action of density dependence on some component of the population (Abrams 2009). Individual fitness of those remaining after increased mortality occurs in the population is increased due to changes in population density, resource availability, or individual behavior (Abrams 2009). This increase in individual fitness brings about an increase in population growth or stage-specific overcompensation (i.e., mortality results in production of more individuals in the next life cycle stage; de Roos et al. 2007). Empirical tests of mechanisms producing stage-specific overcompensation and the Hydra effect have lagged behind models of the conditions producing overcompensation, and in particular a dearth of experimental tests of mechanisms for stage-structured populations with ontogenetic niche shifts (e.g., amphibians, insects) (Schröder et al. 2009).

Abrams (2009) proposed three mechanistic hypotheses for overcompensation and increased population size due to the addition of mortality, two of which we test in this paper, specifically focusing on overcompensation in numbers (as opposed to biomass; de Roos and Persson 2013). Abrams’ (2009) third hypothesis, which we do not test, is that a population with endogenously produced stable fluctuations can yield the Hydra effect when extrinsic mortality increases fluctuation amplitude.

Hypothesis 1: Temporal Separation of Mortality and Density Dependence (Abrams 2009), postulates that the timing of additional mortality relative to the time at which density impacts individuals is the key to whether mortality will have an overcompensatory effect on population size (N) and per capita rate of increase (dN/Ndt). Additional mortality before density dependence makes overcompensation more likely (Seno 2008, Abrams 2009). For this hypothesis to create a Hydra effect, density dependence must be strong (Abrams 2009). Density-dependent constraints on a population could include nutrient limitation (de Roos et al. 2007), or increased developmental time (Karatayev and Kraft 2015) with increasing population density (Gromko et al. 1973). Under this hypothesis, death of a subset of the population reduces density-dependent constraints on the remaining individuals, increasing survival, growth, and development. This mechanism is implicated for Garlic Mustard

(*Alliaria petiolata*), which has cyclic density-dependence wherein additional mortality could induce overcompensation of population size (Pardini et al. 2009).

In mosquitoes, density dependence reduces larval survivorship, increases time to pupation, and reduces female pupae size with increasing density (Hawley 1985b, Walsh et al. 2011, 2012, 2013) whereas density dependence is thought to have limited effects on adults (Juliano 2007). In this way density dependence in the larval stages likely influences population dynamics as described by Abrams (2009), through reduction in survivorship, increased development time, and possibly reduced fecundity as pupal size is positively related to female fecundity (Blackmore and Lord 2000). Density-dependent effects among larvae seem primarily a result of resource depletion (Leonard and Juliano 1995, Juliano 1998). A reduction in population size prior to accumulation of negative effects due to density dependence and resource depletion may more than offset the initial population reduction, as changes in per capita resource availability may produce stage specific overcompensation and the Hydra effect (Abrams and Vos 2003).

Hypothesis 2: Prudent Resource Exploitation (Abrams 2009) postulates that the addition of a mortality source (e.g., a predator) to a consumer population causes a change in individual behavior in the direction of reduction of risky active foraging. This results in more limited resource exploitation, greater resource regeneration, and consequently greater resource dependent growth and development, leading to overcompensation of the consumer population. Threat of predation often causes behavioral shifts in consumers that influence trophic cascades (Beckerman et al. 2014). These behavioral shifts may include changes in resource type consumed (Bernot and Turner 2001, Beckerman et al. 2014) or reduced consumer total foraging effort (Morrison 1999, Matsuda and Abrams 2004, Alexander et al. 2013). This altered resource use can allow increased productivity of the resource (Schmitz 1998, Trussell et al. 2003). The interaction between change in foraging effort and resource growth allows for the potential reversal of the initial population reduction (Abrams 2009). This hypothesis assumes that the resource is overexploited to the point that productivity of the consumer is below maximum, and postulates that the positive effects of a mortality source are not solely due to density changes *per se* but rather include a trait-mediated effect of the threat of mortality (Schmitz 1998, Morrison 1999, Trussell et al. 2003, Preisser et al. 2005).

In mosquitoes, and many other organisms with aquatic juveniles larval behavior affects predation risk such that active behaviors involving feeding and movement increase interaction with predators and predation risk (reviewed by Orrock et al. 2013; also Juliano and Reminger 1992, Grill and Juliano 1996, Juliano and Gravel 2002). Changes in individual behavior have been observed in response to chemical cues present in the residues of damaged victims (Kesavaraju et al. 2007, Costanzo et al. 2011), but potential population level effects of this behavioral change interacting with density dependence have not been investigated.

Organisms

Aedes triseriatus and *Aedes albopictus* were chosen for this experimental test of these hypotheses as the complex life cycle of both species include density-dependent effects in the larval stage that strongly impact survivorship and traits of adults in a manner similar to that described by Abrams (2009). *Aedes triseriatus* in particular exhibits strong behavioral plasticity in response to predation cues (Juliano and Reminger 1992, Grill and Juliano 1996, Juliano and Gravel 2002, Kesavaraju et al. 2007, 2009, Wormington and Juliano 2014). Thus, both of Abrams' (2009) hypotheses for mechanisms of overcompensation and the Hydra effect are plausible for these species. These effects may be important in efforts to control mosquitoes and other pests, wherein a human intervention is the extrinsic source of mortality (Agudelo-Silva and Spielman 1984, Walker and Lynch 2007, Zipkin et al. 2009, Minnie et al. 2016).

These *Aedes* species develop as larvae in water-filled containers, feeding on bacteria, fungi, protozoa, and fine detritus, both from the water column and associated with detritus and container surfaces (Kaufman et al. 1999). For larvae, density dependence is driven by competition for food resources and may impact early larval stages (Southwood et al. 1972, Dye 1984, Legros et al. 2009) and compensatory mortality has been observed in some field experiments with container *Aedes* (Washburn et al. 1991, Nannini and Juliano 1998). Adults are terrestrial, and reproduction is dependent on blood-feeding from vertebrates (Westby et al. 2015); thus, the life cycle is consistent with two-stage models of stage specific overcompensation with development control of dynamics (de Roos et al. 2007).

Larval behavior, including feeding behavior, changes in response to water-borne cues from predation, with reduced foraging in the presences of predation cues, particularly for *Aedes triseriatus* (Juliano and Reminger 1992, Kesavaraju et al. 2007) which is a likely mechanism for the prudent resource exploitation hypothesis (Abrams 2009). Predation cues have also been observed to produce larger adults (Copeland and Craig 1992). The size of the adult female is strongly affected by growth rate during larval stages, and is directly related to fecundity (Hawley 1985a, Washburn et al. 1991, Edgerly and Livdahl 1992, Leonard and Juliano 1995, Frankino and Juliano 1999, Blackmore and Lord 2000, Leisnham et al. 2009).

Our goal is to test Abrams' (2009) two hypotheses of Temporal Separation of Mortality and Density-Dependence and Prudent Resource Exploitation as non-mutually exclusive mechanisms contributing to overcompensatory effects on production of adults and estimated population growth rate. We did this in two experiments. The first experiment manipulated timing of controlled mortality of larval *A. albopictus*, and tested the prediction that earlier mortality is more likely to cause overcompensation in adult production and to enhance population growth. The second experiment manipulated cues from simulated predation, larval density, and presence or absence of early controlled mortality in a factorial design using larval *A. triseriatus*, a species well known to change its behavior in response to predation cues (Kesavaraju et al. 2007) and tested the predictions that: 1) early mortality by itself should induce overcompensation in adult production, at least at some densities; 2) addition of cues from simulated predation, without early mortality, should also induce overcompensation in adult production associated with changes in behavior that reduce

resource exploitation; and 3) combination of early mortality with cues from simulated predation should produce at least the same degree of overcompensation in adult production as either early mortality or predation cues alone. We expect this because effects of mortality and cues should not cancel one another.

Methods

Experiment 1: Timing of mortality

Oak leaves (*Quercus virginiana*) and crickets (*Gryllodes sigillatus*) were oven dried at 50°C for 24 hours, and used as detritus resources to support microorganisms that are the food sources of larvae. Natural containers typically contain a mix of leaf and insect detritus (Yee et al. 2007). Cylindrical, white plastic 500mL containers were prepared 4 days before addition of larvae, each containing: 400mL reverse-osmosis (RO) water, 1.0g dried oak leaves, and 0.05g dried cricket. Prepared containers were housed in an environmental chamber at 24°C with a 14h:10h (light:dark) photoperiod. Eggs from two *Aedes albopictus* populations were used in separate trials, originating from Tampa, Florida and Tyson Research Center, Eureka, Missouri. Eggs were placed in 15mL vials and submerged in nutrient broth (Difco) solution (0.4g/L RO) water) for 24h in the environmental chamber. All first instar larvae were rinsed with RO water and cohorts of 250 individuals were added to each prepared container (day 0). All containers were maintained at 24°C with a 14h:10 h (light:dark) photoperiod for the duration of the experiment. Leaf and insect detritus were supplemented with 0.50g and 0.025g, respectively, on days 16 and 32 of the experiment.

Cohorts were assigned to one of four mortality treatments: Early, Repeated, Late, or Control. Early mortality cohorts were reduced to 128 individuals (i.e., a 48.8% reduction) on Day 2. Repeated mortality cohorts were reduced to 200 individuals on Day 2, 160 individuals on Day 4, and 128 individuals on Day 6 (20% every 2 days, resulting in the same 48.8% cumulative mortality). Late mortality cohorts were reduced to 128 individuals on Day 8. Control cohorts received no additional mortality. There were 15 replicate containers per treatment. On days when any cohort received additional mortality, larvae in all cohorts were removed from the treatment container, counted, and returned. For Late and Repeated cohorts, any cohorts containing less than the assigned number of individuals for that day were brought up to that assigned number by adding individuals reserved from the initial hatch, and maintained under the same conditions as the Control cohorts. No individuals were added to Control cohorts, or to any cohort after it reached the target of 128 individuals. In this way, our manipulated cohorts followed as closely as possible the mortality schedules we planned and effects of intrinsic mortality (i.e., due to density) are minimized until after the action of our experimental extrinsic mortality.

Non-traumatic extrinsic mortality was implemented on manipulated cohorts by use of a set of 6 3cm diameter PVC pipe rings (Kesavaraju and Juliano 2009). Rings were numbered 1–6 and connected in a 2×3 grid. Rings were lowered in this formation into a 30cmX15cm shallow pan, containing the larval cohort, isolating individuals in each ring. A random number generator was used to select one of the six rings from which larvae were removed and returned to the larval habitat via pipette. Rings were then removed from the pan and the process repeated until the designated number of survivors was returned to the larval habitat.

Remaining individuals were removed via pipette, and water and detritus were returned to the rearing container. This method of removal should reduce density without producing cues from damaged larvae, which can be a cue to the presence of predation (Costanzo et al. 2011).

Pupae were removed daily and placed in 1mL shell vials, labelled with cohort and treatment. At emergence, water was removed from the vial via syringe, and sex and day of emergence recorded. Adults were dried at 50°C, and wing length was determined for females. Livdahl and Sugihara's (1984) composite index of performance (r') was calculated for each cohort. The composite index synthesizes data from each experimental cohort on number of females surviving to adulthood, their sizes as predictors of fecundity (using a regression eggs vs. wing length in mm for *A. albopictus*; Lounibos et al. 2002), and their development times to adulthood as an indication of age at first reproduction. These experimental data are combined in a way analogous to life table calculations to produce an index that estimates per capita population rate of change (i.e., dN/Ndt) for each cohort (Livdahl 1984, Livdahl and Sugihara 1984). This composite index is often used for cohort studies of when it is impractical to follow individual survival and reproduction beyond age at adulthood (Livdahl 1984, Livdahl and Sugihara 1984, Juliano 1998). Previous work has shown that the composite index of performance for mosquitoes (r') is strongly correlated with actual values of r estimated from full life tables (Chmielewski et al. 2010). Full details on the logic and calculation of r' are provided by Livdahl and Sugihara (1984). Surviving numbers of adults, mean female wing length, median male and female development times, and the composite index r' , all quantified for the cohorts that constituted the experimental units, were analyzed by factorial mixed model ANOVA, with temporal batches as the random effect.

2) Experiment 2: Behavior, Density, Mortality, and Predation Cues

Oak leaves (*Quercus virginiana*) and crickets (*Gryllobates sigillatus*) were prepared as in experiment 1. Containers received 400 mL RO water, 1.5g dried oak leaves and 0.075g dried cricket 4 days prior to egg hatch and were maintained at 24°C with a 14h:10h (light:dark) photoperiod for the duration of the experiment. We used a greater amount of detritus in this experiment because in our experience, this experiment's test species, *A. triseriatus*, requires more resources to complete development than does *A. albopictus*. For that reason, leaf and insect detritus was supplemented more frequently: beginning on day 2, we added 0.25g dried leaf and 0.0125g dried cricket material every 6 days, ending on Day 44. Containers were assigned one of eight combinations of levels of Density (120 larvae=low, 240 larvae=high), Mortality (0% or 50% reduction on day 2), and Predation cue (present, absent), as the treatment group. There were 4 replicate containers per Density-Mortality-Predation cue combination. Newly hatched *A. triseriatus* larvae were added in appropriate numbers to each container on day 0.

On Day 2, Mortality cohorts were reduced to 50% of the initial density. Random non-traumatic removal of larvae was implemented as in experiment 1 (Kesavaraju and Juliano 2009). Day 2 was chosen for removal because experiment 1 indicated this timing is likely to cause overcompensation (see Results).

Predation cue water was created as described (Costanzo et al. 2011), by holding 10 larvae of *A. triseriatus* in 20mL water for 24 h, crushing the larvae with a small metal spatula, and replacing with 10 additional conspecific larvae. This process is repeated for 5 days. Control water was created by rearing 20 conspecifics in 20 m L water for 5 days. Both predation cue water and control water were poured through a sieve prior to addition to larval containers. Cohorts received either Predation cue or Control water (larvae removed) twice, on days 2 and 8 (Costanzo et al. 2011). Larval behavior was video recorded for 30 minutes on days 3 and 9, approximately 24 h after addition of Predation/Control cues (Costanzo et al. 2011). Costanzo et al. (2011) found significant behavioral effects of similar predation cues ~48–72 h after preparation; hence we are confident our predation cues were active at our time of recording. Behavior of randomly chosen larvae on the video was recorded at each minute for 30 minutes, or until the larva was lost from view, for two criteria as described by Juliano and Reminger (1992): activity (Resting, Browsing, Filtering, Thrashing), and location (Surface, Wall, Bottom, Middle). For each cohort, behavior of multiple larvae was recorded until observations totaled at least 10 larvae and 300 minutes per cohort. Locations of larvae within the habitat results in differential risk of predation, with the surface least risky and the bottom most risky; larval activity similarly results in differential risk of predation, with resting least risky than feeding behaviors, and thrashing (movement to change location within the habitat) most risky (Juliano and Reminger 1992).

Pupae and adults were handled as in experiment 1. Composite index of performance (Livdahl 1984, Livdahl and Sugihara 1984, Juliano 1998, Chmielewski et al. 2010) was calculated using predicted fecundity from a regression of eggs vs. wing length in mm for *A. triseriatus* (Livdahl 1984), survivorship, and days to emergence for each cohort. Data were analyzed by factorial mixed model ANOVA on production of adults, mean female wing length, median male and female development times and the composite index of performance, with temporal blocks as the random effect. Given the large number of tests conducted (see Table 1) we adhere consistently to the standard $P < 0.05$ to declare an effect significant. Behavior data were analyzed by first converting proportion of time at each location and activity to principal component (PC) scores, and analyzing PC scores by separate MANOVAs for Days 3 and 9 (Kesavaraju et al. 2007). PCs with Eigenvalues > 1.5 were retained (Juliano and Gravel 2002, Kesavaraju et al. 2007).

Results

Experiment 1: Timing of mortality

Survivorship data for *A. albopictus* indicated no significant effects of population origin and treatment-population interaction. The effect of mortality treatment was significant for all adults, males, and females (Table 1). Although the overall mortality treatment effect was significant, pairwise comparisons yielded no significant differences among treatments for production of males. The Early Mortality treatment yielded significantly more adults and more females than any other treatment. No other treatment pairs differed significantly (Fig. 1A).

Estimated cohort rate of increase (r' , the composite index) was significantly affected by mortality treatment but not by population or treatment-population interaction (Table 1).

Least-squares means comparison among treatment groups indicated that r' for Early Mortality treatment cohorts differed significantly from all other groups, and that there were no differences among the other groups (Fig. 1B).

Female mean wing length was not significantly affected by mortality treatment (Table 1, Fig. 1C). Median female development time was significantly affected by mortality treatment (Table 1). Least-squares means comparison among treatment groups indicated that development time for Early Mortality treatment significantly differed from the Repeated and Late Mortality treatments, but not from the Control treatment (Fig 1D). Median male development time was significantly affected by mortality treatment (Table 1); least-squares means comparison among treatment groups indicated that development time for Early Mortality treatment differed from Repeated and Late treatments and Late Mortality treatment differed from Control and Early treatments (Fig 1D).

2) Experiment 2: Behavior, Density, Mortality, and Predation Cues

For *A. triseriatus* adult production, there was a significant interaction of Predation Cue and Mortality for all adults and for males, but not for females (Table 1, Fig. 2A). Despite these interactions, pairwise comparisons indicated no significant differences in adult production due to either Mortality (when holding Predation cues constant), or Predation cues (when holding Mortality constant). There was a tendency for greater production of adults and of males with mortality in the absence of cues, and similarly, for greater production of adults and of males with predation cues in the absence of mortality (Fig. 2A). All other interactions and main effects were not significant (Table 1). Thus for *A. triseriatus*, at both densities, compensation in adult production (rather than overcompensation) seems to be the response to early mortality. The addition of predation cues did not significantly increase production of adults either with or without mortality.

Estimated cohort rate of increase (composite index r') was significantly affected by Density and by Mortality*Cue interaction (Table 1). Low density yielded greater r' (least squares mean $\pm$ SE=0.038 $\pm$ 0.005) than did High density (0.015 $\pm$ 0.005). There were no significant pairwise differences in r' among Mortality*Cue combinations (Fig. 2B).

Mean female wing length was significantly affected by the interaction of Density*Mortality (Table 1); however, no pairwise comparisons of means for Density*Mortality combinations were significant (Fig 2C). Significant main effects of both Density and Mortality treatment were found for median male development time (Table 1). Low density or mortality yielded shorter male development time than did high density or no mortality, respectively (Fig. 2D). Median female development time was significantly affected by Density*Mortality interaction (Table 1). Pairwise comparisons of means for Density*Mortality combinations indicated that Low Density/Mortality treatments differed significantly from all others (Fig 2D).

Day 3 PCs (Appendix S1) summarized 51.6% of the total behavioral variation with a rotated factor pattern in which Day 3 PC1 yielded large positive coefficients for surface and resting and large negative coefficients for middle and thrashing. PC1 thus quantifies allocation of time between resting at the surface vs. thrashing through the water column. Day 3 PC2

positive scores indicate more time allocated to browsing at the wall and bottom, and less time allocated to thrashing in the water column (Appendix S1). For day 3 MANOVA indicated that all two-way interactions were significant, but that the three-way interaction was not (Table 2). The interactions involving predation cues are central to testing the hypothesis of trait-mediated effects contributing to overcompensation; hence we focus on comparisons among means for the effects of presence/absence of predation cues (filled vs. open symbols of the same shape and color in Fig. 3A) at different levels of the other factors. Standardized canonical coefficients (SCCs, Scheiner 2001) indicated that PC2 contributed more to these interactions than did PC1 (Table 2). Predation Cue yielded greater PC1 scores (that is, greater time resting at the surface) for Low Density groups (Table 2, Fig. 3A). In contrast, Predation Cue yielded greater PC2 scores (that is, greater time browsing on walls) in the High Density group. (Table 2, Fig. 3A). Multivariate comparisons of group means indicated no significant effect of Predation Cue on behavior in Mortality groups, but that Predation Cue yielded greater PC1 (surface, resting) and PC2 (wall, browsing) scores in No Mortality groups (Table 2, Fig. 3A).

Day 9 PC analysis (Appendix S1) yielded two factors summarizing 56.8% of the total behavioral variation and rotated factor pattern indicated that high scores on Day 9 PC1 were associated with more time allocated to browsing at the wall and less time allocated to resting and filtering at the surface, whereas high scores on Day 9 PC2 were associated with more time allocated to thrashing in the middle of the water column, and less time resting or browsing at the surface. MANOVA yielded a significant three-way interaction with PC1 contributing to most of the variation (Table 3). We concentrate our pairwise comparisons of behavior PCs on the differences between presence/absence of Predation Cues at each combination of Density and Mortality. In Fig. 3B each of the following four comparisons involves filled vs. open symbols of the same shape and color. Multivariate comparisons of group means (Table 3) indicate that Predation Cue shifted behavior away from browsing and towards resting at the surface and thrashing in the middle (i.e., from lower right to upper left in Fig. 2B) for the groups with High Density/Mortality, Low Density/Mortality, and Low Density/No Mortality. In contrast Predation Cue shifted behavior in the opposite direction in the group with High Density/No Mortality.

Discussion

Mortality impacting early stage larvae led to overcompensation in production of adults and greater r' for *A. albopictus*, suggesting greater population rate of increase relative to no-mortality control. Late or repeated mortality treatments resulted in compensation in production of adults and no difference in r' relative to no-mortality control (Fig. 1B). This indicates that density-dependence throughout the larval stage of this species creates sufficient pressure that relief, via density reduction, at any point allows for improved performance of the remaining individuals. Our observation of overcompensatory production of adults with early mortality is consistent with the hypothesis that temporal separation of additional mortality and density-dependent effects is a mechanism contributing to overcompensation and the Hydra effect in this system: mortality acting on the early larval stages is most likely to cause overcompensation and to enhance population growth rate. In a short-term laboratory experiment, we cannot directly observe the effect of treatments on

equilibrium population; however, the combination of overcompensatory production of adults and greater estimated rate of increase with early mortality suggests that populations subject to early mortality are likely farther from their zero-growth equilibrium than are controls, or populations subject to later mortality. Thus, the effect on rate of increase is at least consistent with early mortality yielding a greater equilibrium population size than controls, or later-acting mortality. Overcompensation due to early mortality was stronger for females than for males for *A. albopictus* (Fig. 1). Thus, our experiment 1 provides strong support for Abrams' (2009) hypothesis, when applied to *A. albopictus*, that temporal separation of mortality and density dependence is a critical mechanism for both overcompensation and the Hydra effect.

Our analyses of multiple components of population rate of increase (survivorship, female size as a correlate of fecundity, and development time) suggest that the effect of mortality timing seen on estimated rate of increase in experiment 1 is primarily a result of the increased survival of female *A. albopictus* to adulthood. We detected no effects of our treatments on mean sizes of adult females from the experimental cohorts. Mean female development time was consistently correlated with both estimated rate of increase and number of surviving females (compare Fig. 1A, B, D), but the strength of this effect on development time was less clearly related to estimated rate of increase compared to the effect on female survival. Previous experiments on *Aedes* using a similar experimental approach have also showed that effects of inter- and intraspecific competition on estimated rate of increase correlate most strongly with effects on survival (Juliano 1998). In our present experiment the release from intraspecific density effects produced by early mortality follows this same pattern of a strong effect on survival to adulthood as the most influential effect on estimated rate of increase.

In contrast to *Aedes albopictus* in experiment 1, *A. triseriatus* in experiment 2 showed a pattern consistent with compensation in response to early mortality, with the number of adults produced with early mortality and no cues not significantly different from that produced by the no mortality – no cues treatment. These results suggest these species differ in their survival responses to density. Our results for these two *Aedes* also suggest the possibility that container *Aedes* in general are likely to show patterns of compensation or overcompensation in response to early mortality. These results provide experimental support for the hypothesis that the responses of container mosquitoes in general to mortality of larvae is likely to fall somewhere along the spectrum of compensation or overcompensation (Washburn 1995, Nannini and Juliano 1998, Juliano 2007) and suggests that different species differ in their responses to similar conditions. Differences in population overcompensation in adult production in response to mortality may be indicative of a difference between populations in development (=maturation) vs. reproduction limitation (de Roos et al. 2007, de Roos and Persson 2013). We suspect that for both of the species we examined, development limitation is likely, but that the species differ in their responses to the specific densities and mortalities in our experiments. Further experiments to resolve this question would be desirable.

Results from experiment 2 did not provide evidence that predation cues alone could induce overcompensation via changes in behaviors. Predation cues also yielded no significant

increase in estimated rate of population change, suggesting all cohorts receiving cues were not farther from equilibrium density than cohorts not receiving cues. Thus, experiment 2 is inconsistent with Abrams' (2009) prudent resource exploitation hypothesis as a mechanism yielding overcompensation and the Hydra effect. This was true despite evidence that cues changed behavior, and that some of these changes seem likely to reduce exploitation of the food resource (in this case, bacteria, fungi, and other microorganisms) and could allow resource regeneration particularly of leaf-associated microorganisms.

Addition of Predation Cues shifts behavior on day 3 toward resting and browsing and away from thrashing (Fig. 3A; note positions of filled symbols relative to open symbols). This change does not directly suggest reduced exploitation of food resources in the presence of predation cues. At day 9, Predation Cues shifted behavior of larvae in three of four density-mortality combinations toward greater resting at the surface and thrashing, and less time browsing (Fig. 3B; note positions of filled symbols relative to open symbols). Thus, elements of the behavioral changes induced by predation cues on day 9 may be consistent with reduced resource exploitation particularly because *A. triseriatus* typically depletes microorganism numbers and productivity on leaf surfaces, which is often the location of browsing (Kaufman et al. 1999, 2001). Despite these changes in behavior, predation cues did not induce overcompensation, suggesting that the Prudent Resource Exploitation hypothesis is unlikely to be the mechanism producing overcompensation in *A. triseriatus*. Because *A. triseriatus* is the container mosquito exhibiting the most dramatic changes in behavior in response to water-borne cues to predation (Juliano and Reminger 1992, Grill and Juliano 1996, Juliano and Gravel 2002, Kesavaraju et al. 2007, 2009, Costanzo et al. 2011, Wormington and Juliano 2014) it seems relatively unlikely that Prudent Resource Exploitation is generally important for overcompensatory effects on mosquitoes. One limitation of our experiment is that we did not quantify the food resources (microorganisms in the containers) available to *A. triseriatus*. It may be that in our experimental setting, the change in behavior that we observed is insufficient to produce the predicted rebound of the microorganisms fed upon by *A. triseriatus*, perhaps because of the short term nature of our experiment, or because the behavioral changes came too late in development of the experimental cohort. Longer-term experiments may be needed for tests of the prudent predation hypothesis.

Abrams (2009) and de Roos and Persson (2013) have shown in theory that multiple mechanisms can result in the Hydra effect and overcompensation in numbers or biomass. Because of both the diversity of mechanisms and the diversity of organisms, no experimental study can definitively establish that one postulated mechanism (e.g., temporal separation of mortality and density dependence) is the general explanation for the Hydra effect or overcompensation. Likewise, we cannot rule out the prudent exploitation hypothesis as a mechanism for these effects in other systems. Empirical understanding of how the Hydra effect and overcompensation arise requires experimental investigations of multiple systems that fit the assumptions of the models that predict these counterintuitive outcomes. We expect that many populations with ontogenetic niche shifts and strong density dependence acting on juveniles are likely to show overcompensation and the Hydra effect in response to mortality. This description likely fits many aquatic insects, amphibians, and fish.

Establishing that timing of mortality is crucial for determining how extrinsic mortality impacts populations of mosquitoes or any other pest. Knowing that different species respond to the same timing and level of mortality differently improves our understanding of the population ecology of the target organism. Because *A. triseriatus* and *A. albopictus* are medically important vectors of viral human disease (Paupy et al. 2011, Grard et al. 2014, Westby et al. 2015) this understanding may also have practical consequences for efforts to control mosquitoes via imposing mortality on larvae. Field experiments at natural densities of two species of container *Aedes* have shown that enemies can induce compensatory mortality, yielding no net reduction of adult production compared to the absence of the enemy (Washburn et al. 1991, Nannini and Juliano 1998). Other approaches to controlling mosquitoes by killing larvae also differ in timing and extent of mortality (e.g., Harris et al. 2011, Alphey et al. 2010), and these approaches may thus differ in their likelihood of producing overcompensation.

We have demonstrated that timing of extrinsic mortality imposed on mosquito larvae can induce overcompensation or compensation through relief from density dependence, and that increased equilibrium population with mortality is likely for some mosquitoes. Though our study system involves mosquitoes, we expect the basic principle, that early mortality acting on a population regulated by density dependent survival, growth, and development can increase production of adults and potentially increase equilibrium density, is likely to apply to many kinds of organisms under many natural conditions where density dependent effects are strong. A wide array of models shows that this is possible (de Roos et al. 2007, Abrams 2009) and in multispecies communities, many different mechanisms can result in the Hydra effect and overcompensation (Cortez and Abrams 2016). Identifying the natural conditions under which species' ecologies make this effect likely presents an important and challenging problem for empirical ecology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank K.M. Westby, J. Oremus, J.A. McIntire, and G.A. McIntire for aid in the laboratory and field; P.A. Abrams, V.A. Borowicz, J.T. Neale, G.D. Ower, and two referees for helpful comments on the manuscript. Support came from NIAID grants 1R15AI094322-01A1 and 1R15AI124005-01 to SAJ, and undergraduate research fellowships from Illinois State University's office of Research and Sponsored Programs to KMM.

Literature cited

- Abrams PA. When does greater mortality increase population size? The long history and diverse mechanisms underlying the hydra effect. *Ecology Letters*. 2009; 12:462–74. [PubMed: 19220393]
- Abrams PA, Matsuda H. The effect of adaptive change in the prey on the dynamics of an exploited predator population. *Canadian Journal of Fisheries and Aquatic Sciences*. 2005; 62:758–766.
- Abrams PA, Vos M. Adaptation, density dependence, and the responses of trophic level abundances to mortality. *Evolutionary Ecology Research*. 2003; 5:1113–1132.
- Agudelo-Silva F, Spielman A. Paradoxical effects of simulated larviciding on production of adult mosquitoes. *American Journal of Tropical Medicine and Hygiene*. 1984; 33:1267–1269. [PubMed: 6507734]

- Alexander ME, Dick JTA, O'Connor NE. Trait-mediated indirect interactions in a marine intertidal system as quantified by functional responses. *Oikos*. 2013; 122:1521–1531.
- Alphey L, Benedict MQ, Bellini R, Clark GG, Dame DA, Service MW, Dobson SL. Sterile-insect methods for control of mosquito-borne diseases: an analysis. *Vector Borne and Zoonotic Diseases*. 2010; 10:295–311. [PubMed: 19725763]
- Beckerman AP, Uriarte M, Schmitz OJ. Experimental evidence for a behavior-mediated trophic cascade in a terrestrial food chain. *Proceedings of the National Academy of Sciences*. 2014; 94:10735–10738.
- Bernot R, Turner A. Predator identity and trait-mediated indirect effects in a littoral food web. *Oecologia*. 2001; 129:139–146. [PubMed: 28547061]
- Blackmore MS, Lord CC. The relationship between size and fecundity in *Aedes albopictus*. *Journal of Vector Ecology*. 2000; 25:212–7. [PubMed: 11217219]
- Chmielewski MW, Khatchikian C, Livdahl T. Estimating the per capita rate of population change: how well do life-history surrogates perform? *Annals of the Entomological Society of America*. 2010; 103:734–741.
- Copeland RS, Craig GB. Interspecific competition, parasitism, and predation affect development of *Aedes hendersoni* and *Aedes triseriatus* (Diptera Culicidae) in artificial treeholes. *Annals of the Entomological Society of America*. 1992; 85:154–163.
- Cortez MH, Abrams PA. Hydra Effects in Stable Communities and Their Implications for System Dynamics. *Ecology*. 2016; 97:1135–1145. [PubMed: 27349091]
- Costanzo KS, Muturi EJ, Alto BW. Trait-mediated effects of predation across life-history stages in container mosquitoes. *Ecological Entomology*. 2011; 36:605–615.
- de Roos, AM., Persson, L. Population and community ecology of ontogenetic development. Princeton University Press; Princeton, NJ, USA: 2013. p. 535
- de Roos AM, Schellekens T, van Kooten T, van de Wolfshaar K, Claessen D, Persson L. Food-dependent growth leads to overcompensation in stage-specific biomass when mortality increases: the influence of maturation versus reproduction regulation. *The American Naturalist*. 2007; 170:E59–76.
- Dye C. Models for the population dynamics of the yellow fever mosquito, *Aedes aegypti*. *Journal of Animal Ecology*. 1984; 53:247–268.
- Frankino WA, Juliano SA. Costs of reproduction and geographic variation in the reproductive tactics of the mosquito *Aedes triseriatus*. *Oecologia*. 1999; 120:59–68. [PubMed: 28308054]
- Grard G, Caron M, Mombo IM, Nkoghe D, Mboui Ondo S, Jiolle D, Foutenille D, Paupy C, Leroy EM. Zika Virus in Gabon (Central Africa) - 2007: A New Threat from *Aedes albopictus*? *PLoS Neglected Tropical Diseases*. 2014; 8:e2681. [PubMed: 24516683]
- Grill CP, Juliano SA. Predicting species interactions based on behaviour: predation and competition in container-dwelling mosquitoes. *Journal of Animal Ecology*. 1996; 65:63–76.
- Gromko MH, Mason FS, Smith-Gill SJ. Analysis of the crowding effect in *Rana pipiens* tadpoles. *Journal of Experimental Zoology*. 1973; 186:63–71.
- Harris AF, Nimmo D, McKemey AR, Kelly N, Scaife S, Donnelly CA, Beech C, Petrie WD, Alphey L. Field performance of engineered male mosquitoes. *Nature Biotechnology*. 2011; 29:1034–1037.
- Hawley WA. A high fecundity Aedine: Factors affecting egg production of the western treehole mosquito, *Aedes sierrensis* (Diptera: Culicidae). *Journal of Medical Entomology*. 1985a; 22:220–226.
- Hawley WA. The effect of larval density on adult longevity of a mosquito, *Aedes sierrensis*: Epidemiological consequences. *Journal of Animal Ecology*. 1985b; 54:955–964.
- Huss M, Nilsson KA. Experimental evidence for emergent facilitation: Promoting the existence of an invertebrate predator by killing its prey. *Journal of Animal Ecology*. 2011; 80:615–621. [PubMed: 21323922]
- Jonzén N, Lundberg P. Temporally structured density-dependence and population management. *Annales Zoologici Fennici*. 1999; 36:39–44.
- Juliano SA. Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? *Ecology*. 1998; 79:255–268.

- Juliano SA. Population dynamics. *Journal of the American Mosquito Control Association*. 2007; 23:265–275. [PubMed: 17853611]
- Juliano SA, Gravel ME. Predation and the evolution of prey behavior: an experiment with tree hole mosquitoes. *Behavioral Ecology*. 2002; 13:301–311.
- Juliano SA, Reminger L. The relationship between vulnerability to predation and behavior of larval treehole mosquitoes: Geographic and ontogenetic differences. *Oikos*. 1992; 63:465–476.
- Karatayev VA, Kraft CE. Racing through life: maturation rate plasticity regulates overcompensation and increases persistence. *Ecosphere*. 2015; 6:1–12.
- Kaufman MG, Bland SN, Worthen ME, Walker ED, Klug MJ. Bacterial and fungal biomass responses to feeding by larval *Aedes triseriatus* (Diptera: Culicidae). *Journal of Medical Entomology*. 2001; 38:711–719. [PubMed: 11580044]
- Kaufman MG, Walker ED, Smith TW, Merritt RW, Klug MJ. Effects of larval mosquitoes (*Aedes triseriatus*) and stemflow on microbial community dynamics in container habitats. *Applied Environmental Microbiology*. 1999; 65:2661–2673. [PubMed: 10347058]
- Kesavaraju B, Alto BW, Lounibos LP, Juliano SA. Behavioural responses of larval container mosquitoes to a size-selective predator. *Ecological Entomology*. 2007; 32:262–272. [PubMed: 18185841]
- Kesavaraju B, Juliano SA. No evolutionary response to four generations of laboratory selection on antipredator behavior of *Aedes albopictus*: potential implications for biotic resistance to invasion. *Journal of Medical Entomology*. 2009; 46:772–781. [PubMed: 19645279]
- Legros M, Lloyd AL, Huang Y, Gould F. Density-dependent intraspecific competition in the larval stage of *Aedes aegypti* (Diptera: Culicidae): revisiting the current paradigm. *Journal of Medical Entomology*. 2009; 46:409–19. [PubMed: 19496407]
- Leonard PM, Juliano SA. Effect of Leaf-Litter and Density on Fitness and Population Performance of the Hole Mosquito *Aedes triseriatus*. *Ecological Entomology*. 1995; 20:125–136.
- Livdahl TP. Interspecific interactions and the r-K continuum: laboratory comparisons of geographic strains of *Aedes triseriatus*. *Oikos*. 1984; 42:193–202.
- Livdahl TP, Sugihara G. Non-Linear Interactions of Populations and the importance of Estimating Per Capita Rates of Change. *Journal of Animal Ecology*. 1984; 53:573–580.
- Lounibos LP, Suárez S, Menéndez Z, Nishimura N, Escher RL, O'Connell SM, Rey JR. Does temperature affect the outcome of larval competition between *Aedes aegypti* and *Aedes albopictus*? *Journal of Vector Ecology*. 2002; 27:86–95. [PubMed: 12125878]
- Matsuda H, Abrams PA. Effects of predator–prey interactions and adaptive change on sustainable yield. *Canadian Journal of Fisheries and Aquatic Sciences*. 2004; 61:175–184.
- Minnie L, Gaylard A, Kerley GIH. Compensatory life-history responses of a mesopredator may undermine carnivore management efforts. *Journal of Applied Ecology*. 2016; 53:379–387.
- Morrison L. Indirect effects of phorid fly parasitoids on the mechanisms of interspecific competition among ants. *Oecologia*. 1999; 121:113–122. [PubMed: 28307879]
- Nannini MA, Juliano SA. Effects of the Facultative Predator *Anopheles barberi* on Population Performance of its Prey *Aedes triseriatus* (Diptera Culicidae). *Annals of the Entomological Society of America*. 1998; 1:33–42.
- Ohlberger J, Langangen Ø, Edeline E, Claessen D, Winfield IJ, Stenseth NC, Vøllestad LA. Stage-specific biomass overcompensation by juveniles in response to increased adult mortality in a wild fish population. *Ecology*. 2011; 92:2175–2182. [PubMed: 22352155]
- Orrock JL, Preisser EL, Grabowski JH, Trussell GC. The cost of safety: Refuges increase the impact of predation risk in aquatic systems. *Ecology*. 2013; 94:573–579. [PubMed: 23687883]
- Pardini EA, Drake JM, Chase JM, Knight T. Complex population dynamics and control of the invasive biennial *Alliaria petiolata* (garlic mustard). *Ecological Applications*. 2009; 19:387–397. [PubMed: 19323197]
- Paupy C, Kassa F, Caron M, Nkoghe D, Leroy EM, Kassa FK. A chikungunya outbreak associated with the vector *Aedes albopictus* in remote villages of Gabon. *Vector-Borne and Zoonotic Diseases*. 2011; 12:167–169. [PubMed: 22141733]
- Preisser E, Bolnick D, Benard M. Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*. 2005; 86:501–509.

- Schmitz O. Direct and indirect effects of predation and predation risk in old-field interaction webs. *The American Naturalist*. 1998; 151:327–342.
- Schröder A, Persson L, de Roos AM. Culling experiments demonstrate size-class specific biomass increases with mortality. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:2671–2676. [PubMed: 19193850]
- Seno H. A paradox in discrete single species population dynamics with harvesting/thinning. *Mathematical Biosciences*. 2008; 214:63–9. [PubMed: 18602928]
- Southwood TR, Murdie G, Yasuno M, Tonn RJ, Reader PM. Studies on the life budget of *Aedes aegypti* in Wat Samphaya, Bangkok, Thailand. *Bulletin of the World Health Organization*. 1972; 46:211–26. [PubMed: 4537483]
- Trussell GC, Ewanchuk PJ, Bertness MD. Trait-mediated effects in rocky intertidal food chains: Predator risk cues alter prey feeding rates. *Ecology*. 2003; 84:629–640.
- Walker K, Lynch M. Contributions of *Anopheles* larval control to malaria suppression in tropical Africa: Review of achievements and potential. *Medical and Veterinary Entomology*. 2007; 21:2–21. [PubMed: 17373942]
- Walsh RK, Aguilar CL, Facchinelli L, Valerio L, Ramsey JM, Scott TW, Lloyd AL, Gould F. Regulation of *Aedes aegypti* population dynamics in field systems: Quantifying direct and delayed density dependence. *American Journal of Tropical Medicine and Hygiene*. 2013; 89:68–77. [PubMed: 23669230]
- Walsh RK, Bradley C, Apperson CS, Gould F. An experimental field study of delayed density dependence in natural populations of *Aedes albopictus*. *PLoS ONE*. 2012; 7(4):e35959. <https://doi.org/10.1371/journal.pone.0035959>. [PubMed: 22563428]
- Walsh RK, Facchinelli L, Ramsey JM, Bond JG, Gould F. Assessing the impact of density dependence in field populations of *Aedes aegypti*. *Journal of Vector Ecology*. 2011; 36:300–7. [PubMed: 22129401]
- Washburn JO. Regulatory factors affecting larval mosquito populations in container and pool habitats: implications for biological control. *Journal of the American Mosquito Control Association*. 1995; 11:279–283. [PubMed: 7595462]
- Washburn JO, Mercer DR, Anderson JR. Regulatory role of parasites: impact on host population shifts with resource availability. *Science (New York, NY)*. 1991; 253:185–8.
- Westby KM, Fritzen C, Paulsen D, Poindexter S, Moncayo AC. La Crosse encephalitis virus infection in field-collected *Aedes albopictus*, *Aedes japonicus*, and *Aedes triseriatus* in Tennessee. *Journal of the American Mosquito Control Association*. 2015; 31:233–241. [PubMed: 26375904]
- Yee DA, Kaufman MG, Juliano SA. The significance of ratios of detritus types and micro-organism productivity to competitive interactions between aquatic insect detritivores. *Journal of Animal Ecology*. 2007; 76:1105–1115. [PubMed: 17922707]
- Zipkin EF, Sullivan PJ, Cooch EG, Kraft CE, Shuter BJ, Weidel B. Overcompensatory response of smallmouth bass population to harvest: release from competition? *Canadian Journal of Fisheries and Aquatic Sciences*. 2008; 65:2279–2292.
- Zipkin EF, Kraft CE, Cooch EG, Sullivan PJ. When can efforts to control nuisance and invasive species backfire? *Ecological Applications*. 2009; 19:1585–1595. [PubMed: 19769105]

Figure 1.

Survivorship and Performance Index analysis results for Experiment 1. A: Mean ($\pm$ SE) adult production for each timing of mortality. B: Mean ($\pm$ SE) composite performance index r' , as a surrogate for per capita rate of change by mortality treatment. C: Mean female wing length in mm ($\pm$ SE) for each timing of mortality treatment. D: Median development time in days ($\pm$ SE) for each timing of mortality treatment. In A, B, and C asterisks indicate means that differ significantly from all others in the group; In D means associated with the same letter are not significantly different in pairwise comparisons. ANOVA results summarized in Table 1

Figure 2.

Survivorship, Performance Index, Wing Length, and Development Time analysis results for Experiment 2. A. Mean (+SE) adult production for combinations of Mortality*Predation Cue. B. Mean (+SE) composite performance index, r' , as a surrogate for the per capita rate of change for combinations of Mortality*Predation Cue. C. Mean female wing length in mm (+SE) for combinations of Density*Mortality. D. Median development time in days (+SE) for each Density*Mortality combination. In all panels, asterisks indicate means that differ significantly from all others in the group. ANOVA results summarized in Table 1.

Figure 3. Bivariate least-squares means ($\pm$ SE) for behavioral Principal Component (PC) scores for combinations of initial density, mortality, and predation cues for A. Day 3 and B. Day 9. PC Analysis results summarized in Appendix S1.

Table 1

Results of Mixed Model ANOVA for Experiments 1 and 2. Effects that yielded significant F-tests are indicated in **bold**.

	Survivors						Development Time					
	Adults			Males			Females			r'		
	F	Pr>F	F	F	Pr>F	F	F	Pr>F	F	F	Pr>F	F
D.F.	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F
Experiment 1												
Population	1,49	0.26	0.6107	1.09	0.3007	0.16	0.7024	1.09	0.3022	0.20	0.6599	1.04
Treatment	3,49	7.66	0.0003	3.65	0.0186	13.00	<.0001	13.00	<.0001	1.50	0.2260	6.98
Population*Treatment	3,49	0.67	0.5742	0.29	0.8353	0.70	0.5549	1.59	0.2040	0.32	0.8096	0.64
Experiment 2												
Cue	1,23	0.43	0.5161	0.26	0.6124	0.32	0.5794	0.11	0.7388	0.02	0.8841	4.51
Density	1,23	0.53	0.4755	0.46	0.5059	0.26	0.6159	20.21	0.0002	0.18	0.6743	17.1
Mortality	1,23	0.43	0.5161	0.09	0.7689	0.60	0.4455	0.57	0.4575	1.05	0.3158	5.09
Mortality*Cue	1,23	4.74	0.0400	5.03	0.0348	1.72	0.2026	4.68	0.0412	0.04	0.8421	3.58
Density*Mortality	1,23	2.30	0.1429	1.23	0.2792	1.86	0.1854	0.66	0.4253	6.64	0.0169	0.14
Density*Cue	1,23	1.26	0.2738	0.26	0.6126	1.72	0.2026	1.27	0.2707	1.01	0.3263	0.17
Density*Mortality*Cue	1,23	0	1.0000	0.01	0.9361	0.01	0.9367	0	0.9648	0.31	0.5834	0.05

Table 2

MANOVA results for larval behavior for Day 3 and Day 9. Significant effects based on Pillai's trace in **boldface**. SCC=Standardized Canonical Coefficient. Note: PC1 and PC2 differ between Day 3 and Day 9 as they are derived from separate analysis.

Observation day and effect	DF	P	SCC	
			PC1	PC2
Day 3				
Replicate	6, 618	0.1903	0.024	1.050
Density	2, 308	<0.0001	0.687	0.951
Mortality	2, 308	0.1818	0.834	0.808
Predation Cue	2, 308	<0.0001	0.821	0.825
Density*Mortality	2, 308	<0.0001	0.499	1.063
Density*Predation Cue	2, 308	0.0046	0.252	1.123
Mortality*Predation Cue	2, 308	0.0002	0.587	1.020
Density*Mortality* Predation Cue	2, 308	0.2108	0.374	1.101
Day 9				
Replicate	6, 618	0.1037	0.683	0.787
Density	2, 308	<0.0001	1.229	-0.503
Mortality	2, 308	0.1532	0.547	0.898
Predation Cue	2, 308	<0.0001	-0.973	0.999
Density*Mortality	2, 308	<0.0001	-0.480	1.214
Density*Predation Cue	2, 308	<0.0001	-1.016	0.956
Mortality*Predation Cue	2, 308	<0.0001	-1.005	0.968
Density*Mortality*Predation Cue	2, 308	<0.0001	1.231	-0.492

Table 3

Multivariate pairwise contrasts of bivariate group means of larval behavior PCs from experiment 2. For all comparisons, DF=2, 308. SCC=Standardized Canonical Coefficient. Effects significant based on Pillai's trace, after Bonferroni adjustment in boldface.

Observation day and contrast	P	SCC	
		PC1	PC2
Day 3			
Effect of Density with Cue	<0.0001	0.539	1.042
Effect of Density without Cue	0.1013	0.990	0.528
Effect of Density with Mortality	<0.0001	0.599	1.010
Effect of Density without Mortality	0.6434	1.039	0.005
Effect of Mortality with High Density	<0.0001	0.595	1.012
Effect of Mortality with Low Density	0.0567	0.268	1.121
Effect of Mortality with Cue	0.1930	0.361	1.104
Effect of Mortality without Cue	0.0001	0.666	0.967
Effect of Cue with Mortality	0.2088	1.041	0.308
Effect of Cue without Mortality	<0.0001	0.732	0.915
Effect of Cue with High Density	<0.0001	0.654	0.975
Effect of Cue with Low Density	0.0158	1.037	0.340
Day 9			
Effect of Cue in High Density/Mortality	<0.0001	1.091	−0.862
Effect of Cue in High Density/No Mortality	<0.0001	1.148	−0.766
Effect of Cue in Low Density/Mortality	<0.0001	−0.913	1.048
Effect of Cue in Low Density/No Mortality	<0.0001	1.102	−0.846
Effect of Relief in High Density/Cue	<0.0001	1.216	−0.109
Effect of Relief in High Density/No Cue	<0.0001	−0.880	1.071
Effect of Relief in Low Density/Cue	<0.0001	−0.662	1.176
Effect of Relief in Low Density/No Cue	0.8069	1.221	−0.132
Effect of Density in Mortality/Cue	<0.0001	−0.861	1.084
Effect of Density in Mortality/No Cue	0.0046	1.158	−0.744
Effect of Density in No Mortality/Cue	<0.0001	1.240	−0.383
Effect of Density in No Mortality/No Cue	0.0046	−0.771	1.133