Mosquito responses to lethal and nonlethal effects of predation and an insect growth regulator

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Abstract. Lethal and nonlethal effects of predation are likely to impact phenotypic traits of potential prey that elude capture. Nonlethal effects of predation on prey can include alterations in behavior or morphology in response to predation risk which may impair prey growth and fitness. The application of pesticide in mosquito control during aquatic stages can interact with predation stress and alter density and phenotypic traits of prey. Insect growth regulator (IGR) pyriproxyfen is a pesticide that mainly prevents pupal-adult metamorphosis by mimicking juvenile hormone, whereas the larval stage is not targeted. The use of IGR can therefore act in conjunction with natural aquatic predators that target the larval stage to affect population of prey. In this study, we assessed the invasive mosquito prey *Aedes aegypti* responses to lethal and nonlethal effects of a combination of IGR and predatory mosquito larvae of *Toxorhynchites rutilus*. The combination of IGR and *Tx. rutilus* heavily lowered *Ae. aegypti* metamorphosis to adulthood more than the independent effects of IGR or *Tx. rutilus*. Exposing *Ae. aegypti* larvae to the combination shortened life span of adults after metamorphosis for both males and females, whereas control and numerical density reduction “removals” treatments lengthened life span. Our results show strong lethal and nonlethal outcomes of the combination on *Ae. aegypti*. These findings suggest an additional benefit, decreases adult life span, of the use of an IGR when combined with a natural predator of mosquitoes that may be exploited to improve mosquito control strategies to reduce the risk of disease transmission.

Key words: *Aedes aegypti*; insect growth regulator; lethal and nonlethal effect; phenotypic traits; *Toxorhynchites rutilus*.

INTRODUCTION

The interactions between predator and prey have been long studied by ecologists because of the potential consequences of removing prey from ecological systems on the dynamics of prey populations and on the function of ecosystems (Sih et al. 1985, Abrams 2000, Werner and Peacor 2003, Schmitz 2017). In addition to lethal predation effects on prey, exposure to predators may include fear or intimidation resulting in nonlethal effects on prey growth rate, morphology, development, and behavior (Skelly and Werner 1990, Werner and Peacor 2003, Preisser et al. 2005, Relyea and Rosenberger 2018). A growing number of empirical studies observed nonlethal effects on prey in the presence of predators. For instance, individuals of crucian carp *Carassius carassius* developed deeper bodies (longer from back to belly than wide) in sections of a pond with predatory pike *Esox Lucius* than
survival rate in predation, however, produced adults with high reductions of prey that mimicked daily rate of predation for the control of immature stages of 

Aedes aegypti mosquitoes. 

Protecting humans from mosquito-borne diseases has historically been achieved by control of mosquito populations using pesticides (Floore 2006). Insect growth regulators (IGRs) are a common group of pesticides that are used to lethally prevent mosquito metamorphosis to adulthood (Mulla 1995). Numerous studies have shown that IGR-induced stress can cause nonlethal effects on traits of adult mosquitoes that survived IGR exposure (Ohashi et al. 2014, Mbare et al. 2014, Yadav et al. 2019). Reduction of adult Ae. aegypti life span was observed after immature exposure to IGR methoprene (Sawby et al. 1992). Other mosquito species, including Anopheles and Culex, experienced female sterilization when exposed to IGR pyriproxyfen as adults (Mbare et al. 2014). Moreover, behavioral and morphological alterations in mosquitoes have been observed following IGRs exposure (Arias and Mulla 1975, Busvine et al. 1976, Yodbutra et al. 1985, Braga et al. 2005, Fiaz et al. 2019).

Insect growth regulator mechanism of action (e.g., juvenile hormone analog) that acts at late immature stages (i.e., pupae) has potential to exploit the presence of natural aquatic predators to limit targeted mosquito population sizes. This is because predation can cause larval mortality prior to IGR induces pupal death, resulting in higher overall reduction in density of mosquitoes (Juliano 2007). Together, these observations suggest that IGRs and predators may influence mosquito-borne disease transmission directly by decreasing mosquito recruitment to the population of adults and indirectly by lowering the life span of adults related to their ability to transmit diseases. Here, we investigated the lethal and nonlethal responses of invasive mosquito prey Ae. aegypti across multiple life stages to the combination of IGR pyriproxyfen and predatory mosquito larvae of Tx. rutilus. We predicted that the combination would greatly reduce prey metamorphosis to adulthood, growth (dry weight of adult), and adult life span more than IGR or predator treatment alone.

**MATERIALS AND METHODS**

**Rearing and maintenance of prey and predatory mosquitoes**

The F2-generation of Ae. aegypti used in this study was offspring of field-collected larvae from
water-filled containers (e.g., plastic buckets) from Vero Beach, Florida (FL), USA. Mosquitoes were provided with larval food consisting of equal parts lactalbumin and Saccharomyces cerevisiae yeast and maintained in a bio-room chamber under the following conditions: a photoperiod of 14:10-h light: dark diurnal cycle, 60–80% relative humidity, and 28 ± 1°C. Adults were held in insect cages (30 cm³, BugDorm, BioQuip Products, Rancho Dominguez, California, USA) with constant access to 10% sucrose water through cotton wicks. Adult females were blood-fed on restrained chickens on a weekly basis. Chicken care followed the animal use and care policies of the University of Florida’s Institutional Animal Care and Use Committee (IAACUC Protocol 201003892). Newly laid eggs were collected from germination paper (Anchor Paper, St. Paul, Minnesota, USA) and kept in plastic boxes under room temperature (23–25°C) with moist paper towels to maintain humidity for a minimum of one week prior to use, ensuring embryonation of the eggs. Eggs were hatched in deoxygenated water prepared in an insulated vacuum container powered with an electronic pump to synchronize hatching (Zimler and Alto 2019).

The laboratory colony of predatory mosquito Tx. rutilus originated from eggs was obtained from Lee County Mosquito Control in Lehigh Acres, FL. Adults of Tx. rutilus were held in a wooden insect cage (65 × 50 × 37 cm) and maintained in an insectary under natural light and photoperiod. Adults were provided with constant access to 10% sucrose water from cotton wicks as well as black plastic cups with water for oviposition. Females of Tx. rutilus can produce fertile eggs autogenously and so freshly hatched larvae were immediately collected from oviposition cups. Since larvae of Tx. rutilus are cannibalistic, we reared them individually in plastic cell trays filled with water and fed them with a diet of Ae. aegypti larvae (1st to 4th instars) until pupation after which they were transferred to the colony cage for eclosion. We used Toxorhynchites mosquitoes as our predator model since species in this genus are found in the same or similar habitats as Ae. aegypti and Ae. albopictus (Sulaiman and Jeffery 1994, Focks 2007, Nyamah et al. 2011). Inoculative releasing of Tx. rutilus for biological control could be effective in reducing prey population since a single Tx. rutilus larva has ability to consume a few hundred to 5000 larval prey (Focks 2007). Although the use of Tx. rutilus alone is unlikely to provide complete control of mosquito vectors, its combination with other control tools may enhance the control results.

**Experimental design and insect growth regulator**

Two hundred mosquito larvae of Ae. aegypti (~24 h old) were counted and transferred to experimental trays (35 × 25 × 5 cm) with 1.5 L of tap water and 0.1 g of larval food. The experimental trays serving as replicates were assigned to one of the following treatment groups: Control, IGR, Tx. rutilus, IGR + Tx. rutilus, larval removal, IGR + larval removal. Treatment groups were replicated three times. Immediately after adding Ae. aegypti larvae to the experimental trays, a single 1st instar Tx. rutilus larva was added to each of the replicates of the Tx. rutilus and IGR + Tx. rutilus treatments. In our experiment, we opted a low IGR concentration (0.02 ppb) that was approximately causing 30% prevention of pupal-adult metamorphosis in Ae. aegypti with expectation to induce both lethal and nonlethal changes in life-history traits of Ae. aegypti. The maximum recommended applied concentration of IGR in water is 10 ppb (WHO 2008); however, IGR undergoes rapid photodegradation in water and also the use of IGR in autodissemination approaches in mosquito control, where females mechanically transfer IGR to new mosquito oviposition sites, exposes immature stages of mosquitoes to very low concentrations of IGR. For each of the replicates of IGR and IGR + Tx. rutilus treatments, we applied a 0.02 ppb of IGR after larvae of Ae. aegypti reached to 3rd instar. The late application of IGR is due to pyriproxyfen mechanism of action that mimics the juvenile hormone at late instars of immature stages, aiming at interfering mosquito hormonal system which leads to pupal-adult metamorphosis prevention (Seccacini et al. 2008).

In treatments of Tx. rutilus and IGR + Tx. rutilus, we counted and recorded Ae. aegypti prey daily using established methods (Alto et al. 2012, Bellamy and Alto 2018). The daily average mortality rates from treatments with Tx. rutilus (e.g., Tx. rutilus and IGR + Tx. rutilus) were the basis for the number of larvae removed daily for
both the larval removal and IGR + larval removal treatments, as described by Bellamy and Alto (2018). No treatment manipulations were performed for the controls. All treatment trays were examined daily and newly *Ae. aegypti* pupae were transferred to vials with water for eclosion, upon which their number and sex were recorded. Newly eclosed adults were transferred to adult cages and allowed to feed on water only through soaked cotton, but not nutrition. Thus, we forced mosquitoes to rely on their nutrient reserves carried over from immature stages.

Adults were examined daily between 800 and 12 00 h, and dead mosquitoes were counted, sexed, and recorded. At the end of the experiment, dead adults were dried at 75°C for 2 d and then weighed using an Orion Cahn C-33 microbalance (Thermo Fisher Scientific, Waltham, Massachusetts, USA). We used the weight as an approximate of total mosquito size. In our experiment, we determined the total *Ae. aegypti* metamorphosis to adulthood (male and female combined), growth (dry weight of male and female), and life span of male and female (metamorphosis to adulthood to death, expressed in days). All experimental trays and cages were maintained in a bio-room chamber (28 ± 1°C and 60–80% RH and 14:10 L:D).

### Statistical analysis

We ran multivariate analysis of variance (MANOVA) to determine treatment manipulation effects on the response variables, metamorphosis to adulthood (an indicator of suppression of *Ae. aegypti*), and weight of adult male and female. Standardized canonical coefficients (SCC) were used to determine the relative contribution and relationship (positive or negative) of each response variable to the multivariate effect. When the effects of treatment manipulation were significant, multivariate pairwise comparisons with sequential Bonferroni adjustment for experiment wise alpha (0.05) were carried out for treatments’ comparisons. Treatment manipulation effects on adult life span of *Ae. aegypti* were compared using a regression analysis of survival data based on the Cox proportional hazards model. Significant treatment effects were followed by pairwise contrasts of means using Tukey-Kramer adjustment for multiple comparisons. Adult life span calculations were performed separately for male and female mosquitoes. All analyses were run using SAS version 9.22 (SAS Institute Inc., Cary, North Carolina, USA).

### Results

#### Metamorphosis and adult dry weight

The MANOVA for male and female demonstrated significant effects of treatments ($F_{15,36} = 3.29$, Pillai’s trace $15.36 = 1.73$, $P = 0.0017$). Standardized canonical coefficients showed that differences in metamorphosis to adulthood ($SCC = 4.47$) were the largest contributor to the treatment effects, whereas male ($SCC = -0.08$) and female ($SCC = -0.21$) weights were lesser contributing factors to treatment differences, indicating negligible effects on weights. Treatment groups were significantly different from each other except for the following comparisons: IGR + *Tx. rutilus* vs. IGR + larval removal, IGR + *Tx. rutilus* vs. larval removal, IGR + larval removal vs. *Tx. rutilus*, IGR + larval removal vs. larval removal, and *Tx. rutilus* vs. larval removal (Table 1). Metamorphosis to adulthood was highest in control and IGR treatments, and lowest in treatments with reduced density or the presence of *Tx. rutilus* (e.g., IGR + larval removal, larval removal, IGR + *Tx. rutilus*, and *Tx. rutilus* treatments). The combination of IGR and *Tx. rutilus* led to lowest metamorphosis to adulthood than treatments of IGR or *Tx. rutilus* alone (Fig. 1A). The dry weights of male and female adults were heavier in treatments where density was lowest and lighter in the remaining treatments (Fig. 1B, C).

#### Adult life span

The life span of adults was significantly affected by treatment manipulations ($\chi^2 = 756.0$, df = 5, $P < 0.0001$). Adult life span depended on sex ($\chi^2 = 446.3$, df = 1, $P < 0.0001$), with significantly steeper declines in male than female mosquitoes (Fig. 2A, B). Mosquitoes derived from treatments containing IGR, *Tx. rutilus*, or IGR + *Tx. rutilus* had significantly steeper declines in adult life span than from all other treatments for both male and female mosquitoes. The life span was high in IGR + larval removal and larval removal compared with other treatments (Fig. 2A, B). No significant differences were
detected in life span of male mosquitoes between the following treatments: Control vs. IGR + larval removal, control vs. larval removal, IGR vs. *Tx. rutilus*, and IGR + larval removal vs. larval removal (Fig. 2A). For female life span, IGR + *Tx. rutilus* vs. *Tx. rutilus* and IGR + larval removal vs. larval removal treatments had no significant differences between each other (Fig. 2B). There was a significant reduction in life span of male, but not female mosquitoes, in IGR + *Tx. rutilus* treatment compared to IGR or *Tx. rutilus* treatment alone (Fig. 2A).

### Table 1. MANOVA for multivariate pairwise comparisons with sequential Bonferroni adjustment of treatment effects on metamorphosis to adulthood and male and female weight.

| Comparison                              | $P$     | $F$   | Pillai’s trace | df | Metamorphosis to adulthood | Male weight | Female weight |
|-----------------------------------------|---------|-------|----------------|----|---------------------------|-------------|---------------|
| Control vs. IGR                         | 0.0052* | 8.00  | 0.70           | 3,10| 6.15                      | 1.05        | 1.53          |
| Control vs. IGR + *Tx. rutilus*         | <0.0001*| 64.34 | 0.95           | 3,10| 4.46                      | −0.19       | −0.10         |
| Control vs. IGR + Larval removal        | <0.0001*| 51.13 | 0.94           | 3,10| 4.82                      | −0.91       | −0.93         |
| Control vs. *Tx. rutilus*               | <0.0001*| 41.21 | 0.92           | 3,10| 5.50                      | 0.59        | 0.41          |
| Control vs. Larval removal              | <0.0001*| 49.88 | 0.93           | 3,10| 5.02                      | 0.25        | 0.09          |
| IGR vs. IGR + *Tx. rutilus*             | <0.0001*| 36.71 | 0.92           | 3,10| 3.04                      | −0.75       | −0.86         |
| IGR vs. IGR + Larval removal            | <0.0001*| 25.45 | 0.88           | 3,10| 3.39                      | −0.44       | −0.87         |
| IGR vs. *Tx. rutilus*                   | 0.0004* | 15.73 | 0.82           | 3,10| 4.51                      | 0.21        | 0.43          |
| IGR vs. Larval removal                  | <0.0001*| 23.53 | 0.87           | 3,10| 3.73                      | −0.24       | −0.76         |
| IGR + *Tx. rutilus* vs. IGR + Larval removal | 0.3239 | 1.31  | 0.28           | 3,10| 1.15                      | −2.01       | −0.70         |
| IGR + *Tx. rutilus* vs. *Tx. rutilus*   | 0.0079* | 7.05  | 0.68           | 3,10| 0.19                      | −2.02       | −1.31         |
| IGR + *Tx. rutilus* vs. Larval removal  | 0.1446  | 2.25  | 0.67           | 3,10| −0.20                     | 2.24        | 0.99          |
| IGR + Larval removal vs. *Tx. rutilus*  | 0.1287  | 2.40  | 0.42           | 3,10| 0.52                      | 1.99        | 1.73          |
| IGR + Larval removal vs. Larval removal | 0.9159  | 0.17  | 0.04           | 3,10| 2.48                      | 2.60        | 1.68          |
| *Tx. rutilus* vs. Larval removal        | 0.3043  | 1.38  | 0.29           | 3,10| −0.18                     | 1.71        | 1.70          |

*Note:* Asterisks (*) indicate statistically significant differences ($P < 0.05$) between treatment groups.
In aquatic habitats, environmental biotic factors (e.g., competition and predation) have potential to act in conjunction with pesticides to alter mortality and phenotypic traits of prey survivors (Relyea and Mills 2001). To improve our understating regarding the lethal and nonlethal effects of IGR and predation by predatory mosquito Tx. rutilus on Ae. aegypti, we designed our treatments to independently estimate IGR, Tx. rutilus, and their combined effects during mosquito immature stages on subsequent prevention of metamorphosis to adulthood and phenotypic traits of adults. Our treatment manipulations enabled us to assess nonlethal effects of exposure to a pesticide and a predator among adult survivors, especially after accounting for density-mediated changed associated with immature mortality attributable to a pesticide exposure and consumption by a predator. Outcomes from treatments that mimicked mortality in individuals during immature stages produced adults with longest life spans, whereas treatments with an actual pesticide and predator exposure, alone or in combination, resulted in adults with shortest life spans. These results illustrate how the exposure to multiple stressors during mosquito immature stages can carry over to alter traits of adult survivors.

Our study showed that combined effects of IGR and Tx. rutilus achieved higher prevention (~90%) of Ae. aegypti metamorphosis to adulthood compared to other treatments, including exposure to IGR or Tx. rutilus alone. Consistent with these findings, higher population reduction of Ae. aegypti in urban environments in Florida was achieved when pesticide malathion was applied in combination with inoculative releases of predatory mosquito Tx. splendens (Tietze et al. 1993). Collectively, these results suggest that a greater reduction in population size of Ae. aegypti can be achieved when Toxorhynchites spp. predators, naturally occurring or released as part of biological control, are combined with pesticide-induced mortality. Insect growth regulators are extremely effective in preventing Ae. aegypti metamorphosis to adulthood at exceptionally low rates of application and even after short-time exposure (Vasuki and Rajavel 1992, Sihuincha et al. 2005, Darriet and Corbel 2006). In the current study, we exposed mosquitoes to low concentration of IGR at 3rd instar with continuous exposure until metamorphosis to adulthood. This type of exposure reflects the field conditions and is a typical approach used by other...
investigators to determine lethal and nonlethal effects of IGRs on mosquito biology (Sawby et al. 1992, Ritchie et al. 1997).

Nonlethal effects of exposure to predators on feeding activity and size of prey have been documented by several studies. Kohler and McPeek (1989) observed that feeding activity of larval prey mayfly Baetis tricaudatus was reduced due to the presence of predatory sculpin Cottus bairdi. The development rate and size at metamorphosis of dipteran Chironomus tentans was decreased in the nonlethal presence of predatory pumpkinseed sunfish Lepomis gibbosus, an outcome of prey behaviorally avoiding fish predators or their cues (Ball and Baker 1996). Similarly, larval exposure to predatory fish Poecilia sphenops reduced feeding opportunities, extended the development time and reduced adult size of An. pseudopunctipennis (Bond et al. 2005). These studies suggest that exposure to predators during immature stages may influence feeding and growth rates and compromise size at metamorphosis. In our experiment, we did not specifically determine prey feeding and development rates (i.e., immature development rates of mosquito prey exposed to a predator) as part of our manipulations of treatments, which are limitations of this study. Instead, we examined the nonlethal effects of predator stress on prey size, as an approximation of nutrient stores, at metamorphosis (Briegel et al. 2001). We compared mosquito prey in predator treatments to conspecifics in the larval removal treatments which have similar metamorphosis to adulthood rates. If exposure to predators altered feeding rates, we predict that mosquito prey would have lengthened development time (i.e., lower feeding rates necessitate longer time to achieve critical mass for metamorphosis) and/or be smaller in size at metamorphosis compared to conspecifics from the removal treatments. Our results revealed that the weight of mosquitoes exposed to Tx. rutilus had no differences compared to conspecifics in the larval removal treatments, providing weak support for our prediction. Additionally, when mosquitoes were exposed to the combination of IGR and predator, the weights of males and females were similar to those in IGR + larval removal, also providing no support for the prediction of predator-induced reduction in mosquito size. Further, there is little evidence from empirical behavioral studies suggesting that Ae. aegypti adopts anti-predator responses, such as reduced activity in the presence of predators (Grill and Juliano 1996).

Thinning of mosquito prey through consumption by predators or mortality attributable to pesticides is predicted to accelerate development and growth by releasing survivors from competition, especially among species that do not exhibit predator-induced behavioral responses (e.g., reduce time spent foraging; Grill and Juliano 1996, Babbitt and Tanner 1998, LaFiandra and Babbitt 2004, Relyea and Hoverman 2008, Relyea and Rosenberger 2018). Metamorphosis to adulthood of mosquitoes from treatments with Tx. rutilus or removal of prey was approximately four times lower than controls (IGR alone was an exception). Male and female mosquitoes from these former treatments experienced enhanced growth, with an 30–32% increase in weight gain than in controls, suggesting that survivors experienced competitive release from resources, such as microbes that comprise their diet.

Adult mosquito life span is an epidemiologically important parameter that can influence vectorial capacity, an entomological analog of a pathogen’s basic reproductive rate (R0), which measures the efficiency of vector-borne disease transmission (Louinbos and Kramer 2016). In our experiment, we examined the treatment manipulations’ effects on life span of adults under starvation conditions because of the possibility that adult nutrition may ameliorate life-shortening effects of larval multiple stressors, such as the presence of IGR and predator (Bellamy and Alto 2018). Treatments including Tx. rutilus, IGR, or their combination, compromised surviving adult life span, an indicator of deferred costs of immature environment imposed on post-metamorphic individuals. Exposure to Tx. rutilus led to similar or lower adult life span of Ae. aegypti compared with conspecific individuals exposed to IGR, suggesting predatory stress to have more profound effects on life span of adults than exposure to a low concentration of IGR. Control conditions or treatments that mimicked the daily mortality attributable to predation yielded adults with the highest life span, whereas treatments with a predator, IGR, or their combination produced adults with the lowest life span. This observation provides evidence for nonlethal effects of predation and IGR exposure on adult mosquitoes.
Reduction in adult life span due to larval predation stress has been previously demonstrated in interactions between mosquito predators (e.g., *Tx. rutilus* and *C. appendiculata*) and *Aedes* mosquito vectors of pathogens, including *Ae. aegypti* (Costanzo et al. 2011, Alto et al. 2012, Bellamy and Alto 2018). The mechanism of how predatory stress influences adult life span is not fully understood, but it may be related to an energetic cost (Bellamy and Alto 2018). Our observation in declining life span of adults following larval exposure to IGR is in agreement with previous studies that evaluated the sublethal effects of IGRs against mosquitoes (Sawby et al. 1992, Vasuki 1992, Ritchie et al. 1997). Although the mechanism responsible for this declining is unknown, it may relate to alteration in neuroendocrine abnormalities induced by juvenile hormone analogues, like pyriproxyfen (Sawby et al. 1992). Cumulative effects of IGR and *Tx. rutilus* led to similar or steeper declines in adult life span when compared to the *Tx. rutilus* or IGR treatment for males but not females, suggesting stronger effects of multiple stressors in surviving adult males. This variation between two sexes in response to the cumulative effects may relate to sexual dimorphism and energy uptake resulting in different fitness consequences between males and females (Costanzo et al. 2011). Exposure to a higher concentration of IGR may further reduce the life span among surviving adults.

Although the lethal and nonlethal effects of IGR on predatory mosquito *Tx. rutilus* was not evaluated in the current study, all predators successfully metamorphosed to adulthood, suggesting that low concentration of IGR had no lethal effect on *Tx. rutilus*. This observation was not surprising given that the predatory larvae of *Tx. rutilus* were bigger in size and may require higher concentrations of IGR to induce lethal effect comparing to the larvae of *Ae. aegypti* (Amalraj and Velayudhan 1989, Amalraj and Das 1996). Investigations using *Toxorhynchites* spp. in combination with *Bacillus* toxins (Bti; Lacey and Dame 1982) and organophosphates (Rawlins and Ragoonasingsh 1990, Tietze et al. 1993) observed *Toxorhynchites* spp. to be less susceptible to these chemical compounds than *Ae. aegypti*. In some cases, the lethal effective dose of Bti in *Ae. aegypti* had undetectable effects on older instars of *Tx. rutilus* (Lacey and Dame 1982). The distinctive mode of action of IGR that does not kill the larval stage (i.e., predaceous stage) of predatory mosquito *Tx. rutilus* suggests that IGR to be a good candidate for the application with *Tx. rutilus* in an integrated mosquito management approach.

In the current study, we found that a low concentration of IGR was capable of interacting with a dipteran predator to heavily prevent *Ae. aegypti* metamorphosis to adulthood and by association population size. Also, exposure to IGR and predators reduced adult life span which is likely to strongly mitigate disease transmission as measured by alterations in vectorial capacity. The life-shortening effect in females of *Ae. aegypti* is predicted to reduce the number of females that survive the pathogen extrinsic incubation period and therefore should reduce risk of disease transmission. This study underscores the importance of considering environmental stressors on disease ecology.

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