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Oviposition site selection is a critical fitness enhancing decision for container breeding insects. Predators have typically been shown to repel gravid females whereas conspecifics have been shown to be attractive at low-intermediate densities but repellent at high densities resulting in hump-shaped relations. The interaction of these two factors has, unfortunately, rarely been studied. In this study, I addressed this question by testing the effect of dragonfly nymphs as larval predators, conspecifics, and their combination on the oviposition response of *Aedes albopictus* mosquitoes. I expected a negative effect of predators, a hump-shaped effect of conspecifics, and a rightward shift in the peak of the hump in the presence of larval predators. I used three levels (0, 1, 3) of caged *Odonata* (dragonfly) nymphs and a range of predetermined conspecific larvae numbers (0, 10, 50, 100, 300, 500). I used two experimental designs: (1) Six 3-by-6 oviposition traps grids each containing all 18 predator-by-larvae combinations; (2) Three transects containing 12 pairs of oviposition traps with both cups containing a similar number of larvae, but one containing a given level (0, 1, 3) of caged nymphs. In the latter, I also cultured a sample of the water medium to evaluate bacterial concentration. Hump-shaped relations of egg number with conspecifics was observed at the grid design for the one nymph level and for the transect design at nymph level zero. The effect predator level on oviposition response was either non-

significant or, unexpectedly positive. Due to increased larval mortality in the predator cups, I could not evaluate the third hypothesis concerning the combined effect of conspecifics and predators. Bacterial concentration was negatively associated with number of eggs laid. The absence or positive effect of dragonfly nymphs on *Ae. albopictus* oviposition response is encouraging in terms of its usage as a biocontrol agent for container breeding mosquitoes which in combination with low-intermediate levels of conspecifics could be attractive to gravid female mosquitoes. Their offspring, in turn, will be decimated by the control agent.

EFFECT OF DRAGONFLY NYMPH PRESENCE AND CONSPECIFIC LARVAE DENSITY ON OVIPOSITION RESPONSE OF THE INVASIVE ASIAN TIGER MOSQUITO (*AEDES ALBOPICTUS*)

by

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CHAPTER I

INTRODUCTION

Brood care in insects is mostly confined to species of orders Hymenoptera and Isoptera. Eusocial ants, termites, and some bees practice varying degrees of caste systems, where reproduction is restricted to one female queen, and morphologically specialized nest members secure fitness via kin selection; by rearing their younger siblings rather than producing their own offspring. With few notable exceptions, such as male giant water bugs (*Belostomatinae)* that carry their mates' eggs on their back until hatching (Smith and Larsen 1993), direct parental care in insects is rare. However, egg placement is far from haphazard. Eggs and developing larvae are vulnerable life stages, and relatively immobile. They are subject to proximate environmental fluctuations, such as desiccation and temperature extremes, also contending with resource competition and predation. Prolific offspring production is one method to buffer high immature stage mortality; but fitness is largely dependent on parental oviposition site selection. Gravid female insects must select suitable larval habitat to optimize reproductive success via maximal larval survivorship (Peckarsky et al. 2000, Honório et al. 2003, Kiflawi et al. 2003).

Most female container breeding mosquitoes are hematophagous; requiring blood protein in order to develop eggs. Once a blood meal is obtained, the female deposits her eggs in suitable locations, often tree holes or similar isolated containers of water. Since developing larval mosquitoes are completely aquatic, they are unable to leave their natal body of water. The cues driving the oviposition site selection of gravid females is hypothesized to indicate the suitability of potential oviposition sites (Bentley and Day 1989, Navarro-Silva et al. 2009). Suitable oviposition sites for mosquitoes should typically indicate, among others, appropriate abiotic conditions (e.g., temperature, pH, site permanence), absence of natural enemies, low competition, and food abundance. Microhabitat conditions are often indicated by semiochemicals, which are a broad spectrum of chemicals which cause behavioral changes in living organisms. For example, pheromones are semiochemicals that influence behavioral responses among members of the same species, while kairomones and allomones are inter-specific semiochemicals benefiting either the receiving or transmitting individuals of the other species, respectively. In conjunction with other environmental cues, gravid female mosquitoes recognize pheromones released by other females that previously oviposited in a location, or by younger stages such as eggs or larvae, indicating an oviposition site suitable for larval development (Navarro-Silva et al. 2009).

Effects of Predation Risk

Predation risk effects on mosquito oviposition are extensively documented. Mosquitoes preferentially lay eggs in sites with fewer or no predators, versus sites with high levels of predation, providing greater parental fitness (Silberbush and Blaustein 2008). Presence of predatory *Anax imperator* nymphs, an *Odonata* species, resulted in substantially lower egg rafts than oviposition sites without nymphs (Stav et al. 1999). Mosquito larval densities are lower in oviposition sites containing *Odonata* predators, compared to sites lacking predators, due to direct effects of predation (Fincke et al. 1997). Modeling mosquito oviposition choice in response to predation suggests gravid female mosquitoes should preferentially seek out oviposition sites without predation, over sites containing predators, even when accounting for the cost of time and mortality risk to the female (Kershenbaum et al. 2012). *Culiseta longiareolata* avoid oviposition in artificial pools containing *Notonectidae* predators; decreased egg numbers not due to predation, indicates oviposition avoidance mediated by semiochemical cues (Blaustein et al. 2004). Gravid females consistently prefer sites lacking predation when presented with pairwise oviposition sites with or without *Notonectidae* predators, suggesting ability to determine predation risk (Silberbush and Blaustein 2011).

A recent study by Wasserberg, et al. (2013) found that when gravid *Aedes albopictus* (Skuse, 1894) females were presented with four choices of varying larval food concentrations, they preferentially oviposited in higher nutrient load containers. This

effect was independent of predation risk, indicating a positive selection of larval resource availability independent from predation risk avoidance. This same study also recorded 18.7% decrease in the number of eggs oviposited in containers with a caged *Odonata* nymph predator (the same used in this experiment), compared to containers without predation, suggesting selection for low predation risk oviposition sites. These effects of food resource availability and predation risk were shown to be independent of one another. Lastly, when oviposition trap water samples were cultured for the presence of bacteria, Wasserberg et al. were able to quantify the energetic cost of predation, showing a relatively weak (18.7%) depression of oviposition (Wasserberg et al. 2013).

Effect of Conspecific Immature Stages

Since microhabitat isolation can develop the potential for overcrowding, the presence of preexisting immature conspecifics is especially influential for container-breeding mosquitoes (Lord 1998). *Ae. aegypti* oviposition behavior varies in presence of pre-existing eggs; females preferentially oviposit in sites containing lower conspecific larval densities, as higher densities incur costs of intraspecific competition, decreasing individual larval fitness (Benzon and Apperson 1988, Williams et al. 2008). *Ae. albopictus* preferentially oviposits in sites containing conspecific or *Ae. aegypti* larvae, rather than sites with no larvae (Allan and Kline 1998). Too many conspecifics incur cost of

competition via resource depletion and physical overcrowding (Bédhomme et al. 2005). Density-dependent oviposition responses are thus expected to form a hump-shaped curve with individual peak larval fitness at intermediate densities of conspecifics (*Fig. 1*).

The Allee effect describes the positive relationship between increasing population density and individual fitness. This could evolve by a number of mechanisms such as foraging, defending territories, deterring predation, finding a mate, or group protection of offspring, especially when a species is rare (Allee 1931). The Allee effect is observed in numerous animal species, and this trend exists until optimum per capita fitness is achieved (Kramer et al. 2009). Limitations on resources such as food, space and mate availability restrict population density, but group living offers the advantage of increased individual fitness through safety in numbers (Berec et al. 2007). Wasserberg et al. (2014) linked five studies to indication of mosquito oviposition following a hump-shaped relationship (HSR) in the presence of preexisting conspecifics (Benzon and Apperson 1988, Zahiri and Rau 1998, Sumba et al. 2008, Williams et al. 2008, Wachira et al. 2010). Despite a current lack of published support, the author suggests this is a general trend for mosquito oviposition. I based my experiment on this supposition.

Combined Effect of Predation Risk and Conspecific Immature Stage Density

Predation risk could be influenced by group size. As group size increases, individual risk of predation decreases, known as victim dilution effect (Inman and Krebs

1987, Dehm 1990, Mooring and Hart 1992). According to Hamilton's selfish herd theory, individuals on aggregation perimeters have higher risk of predation than the interior, thus when animals aggregate, they compete for safety of center place (Hamilton 1971). Fiddler crabs (*Uca pugilator*) consistently form aggregations when threatened by shorebird and humans (Viscido and Wethey 2001, Viscido et al. 2008). Aquatic organisms, such as European toad tadpoles (*Bufo bufo*), form aggregations in response to fish predation, which results in decreased per capita risk, but incur increased risk to the group as a whole (Watt et al. 1997). Predator presence similarly alters mosquito larvae behavior, second instar *Ae. albopictus* and *Ae. triseriatus* become less active and aggregate when in presence of predatory midges, *Corethrella appendiculata* (Kesavaraju et al. 2007, Kesavaraju and Juliano 2008). This may suggest that increased larval density confers protection against individual predation. *Ae. triseriatus* larvae decrease foraging activity and time spent below the surface of water in containers containing the predator *Toxorhynchites rutilus.* Decreased activity and foraging behavior decrease competition between conspecifics and increase larval development time (Juliano and Gravel 2002). Gravid *Cu. longiareolata* females avoid ovipositing in sites with more predators (Silberbush and Blaustein 2008), and high densities of preexisting conspecific density (Benzon and Apperson 1988, Williams et al. 2008). The interaction of both factors may change the point where competition costs are negated by benefit of larval aggregation,

and cause Allee threshold shift towards oviposition site selection of higher conspecific densities when predation risk is present (*Fig. 1*).

A field study of *Anopheles quadrimaculatus*, deployed buckets containing nutritive leaf litter and *Notonectidae* predators in combination with three resource competitors (zooplankton, snails, and tadpoles). Results indicated that conspecific larval competition and predation risk negatively affect survivorship and developmental time, but larval survivorship analysis suggested effects of competition and predation are independent (Knight et al. 2004). *Anopheles gambiae* oviposition patterns indicate avoidance under *Xenopus* tadpole predation risk, with increased oviposition avoidance in higher larval densities, suggesting female selection against predation or larval competition (Munga et al. 2006). *Bufo* tadpole predators in combination with conspecific larval densities, reveal *C. longiareolata* oviposition is positively influenced by larval food availability, and deterred in sites with predation risk (Blaustein and Kotler 1993).

Although oviposition site selection responses to predation risk and conspecific density are well documented, this experiment is innovative in that it explores combined interactive effect on an *Aedes* species, using a spectrum of conspecific densities, and *Odonata* nymphs as predators. The purpose of this study is to investigate the effects of conspecific larval competition, predation risk, and their interaction on *Ae. albopictus* oviposition site selection.

Public Health Implications

Ae. albopictus, a mosquito species native to Southeast Asia, is an significant vector of numerous human diseases such as dengue, Chikungunya, Yellow fever, West Nile Virus, La Crosse, eastern equine encephalitis, and the heartworm nematode *Dirofilaria immitis* (Schmidt and Roberts 1977). *Ae. albopictus* habitat preferences consist mainly of forest edges with thick understory vegetation, allowing the formation of small water-holding basins where eggs are deposited, and larvae develop. This aquatic microenvironment is mimicked by stacked automobile tires, which likely led to introduction and establishment of this species to the United States during the 1980s (Hawley 1988, Lord 1998). Females oviposit eggs at water surface interfaces and usually apply "skip oviposition" strategy, which means distributing eggs individually across numerous oviposition sites (Chadee et al. 1993, Corbet and Chadee 1993). This is in contrast to other mosquitoes like *Culex* and *Culiseta* species which oviposit all their eggs in a single location, in raft-like structures (Mogi and Mokry 1980, Reiter 2007). This egg distribution method spreads out predation risk and environmental threats for each offspring (Edman et al. 1998), and increases dispersal potential.

Although egg developmental time is highly dependent on environmental temperature (Ratte 1985), recorded time from oviposition to egg hatching between two and six days (Hawley 1988). Once larvae hatch, pupation occurs within seven days at 32°C and twenty-eight days at 12°C (Briegel and Timmermann 2001). This equates to a

potential a new generation of disease carrying adults in as little as nine days, making population control essential for human health. Since its introduction, this species has spread in range and number throughout much of the eastern U.S., along with many mosquito-born diseases (Burkett-Cadena 2013).

Increased public awareness of environmental and human health effects of traditional pesticides (Davies 1990, Weisenburger 1993), lead to increasingly negative public perception of chemical-based arthropod control (Dunlap and Beus 1992, Ray 2000, Rose 2001). Most pesticides indiscriminately target both beneficial and pest species (Pimentel et al. 1992, Desneux et al. 2007, Gill et al. 2012), and contributes to pesticide resistance (Brown 1986, Mouches et al. 1986). Integrated pest management (IPM) seeks to resolve these issues, implementing ecosystem-level combinations of alternative solutions, including biocontrol via predator species (Elliott and Dent 1995). In order to devise effective IPM strategies, it is important to recognize ecological susceptibilities by researching population dynamics and predation control systems in natural habitats.

Study Goal, Study Questions, Hypotheses, and Predictions

As described above, in the absence of natural enemies many mosquito species are hypothesized to exhibit a density-dependent oviposition response that follows a humped-shaped relationship (HSR) model (Wasserberg et al. 2014). Figure 1 displays the

relationship (solid line) where maximal fitness occurs at the point of highest benefit of conspecifics and lowest perceived costs of competition. Preexisting conspecifics may provide reassurance that a potential oviposition site is suitable for successful development of larvae. However, higher densities may incur competition costs for resources, indicating an unsuitable oviposition site. In the presence of predation, I expected to observe a rightward shift (dotted line) in peak oviposition rate towards greater conspecific densities. This shift is based on the interaction of overall fitness suppression due to enhanced predation risk, and the decreased perceived cost of competition associated with behavioral changes in the presence of predation (Kesavaraju et al. 2007, Kesavaraju and Juliano 2008). Hence, in this study I used the *Ae. albopictus* system to study the combined effect of conspecific larval density, and predator risk on the oviposition response of a container breeding organism.

I asked the following questions:

1. How does conspecific larval density affect the oviposition response of container breeding organisms?

2. How does oviposition response of a container breeding organism change with increasing level of predation risk?

3. Does predation risk affect the nature of the functional relationship between conspecific density and oviposition response?

Hypotheses and Predictions

1. Effect of conspecifics. Gravid females are expected to select oviposition sites based on tradeoff between aggregation benefits and competition costs, resulting in HSR between larvae density and oviposition rate (*Fig. 1*). I predict *Ae. albopictus* oviposition response to be consistent with this pattern.

2. Effect of predator risk. Gravid females are assumed to be able to detect the presence of larval predator kairomones in the water and therefore avoid oviposition there. The degree of avoidance is expected to be dose dependent and proportional to the density of predator present. I predict the oviposition rate of *Ae. albopictus* to decrease with the number of caged *Odonata* nymphs.

3. The combined effect of predation risk and conspecific density. In the presence of predation risk, the benefit of higher density should extend to higher intraspecific densities. As a consequence, the tipping point where the cost of competition overweighs the benefit of aggregation should occur at higher intraspecific densities (*Fig. 1*). I therefore predict that for *Ae. albopictus*, the hump-shaped oviposition response curve should rightward shift (to higher intraspecific densities) with a lower peak related to reduced effects of competition as the number of caged dragonfly nymphs increase (*Fig. 1*).

CHAPTER II

MATERIALS AND METHODS

Experimental Strategy

This study consisted of a series of experiments studying effects of conspecific larvae, predation risk, and the combination of both on oviposition behavior of *Ae. albopictus*. In this study I manipulated predation risk level using caged larval predators and deployed predetermined number of *Ae. albopictus* larvae and measured *Ae. albopictus* oviposition response under natural conditions. Predation risk was mediated using caged *Odonata* nymphs, obtained from Carolina Biological Supply.

Study Site

I conducted this study entirely within Peabody Park, a thirty-four acre deciduous forest used for research and recreation, located in the northern portion of The University of North Carolina at Greensboro campus. Buffalo Creek, a system of several streams, part of the Haw River Basin, flows throughout the park, providing high ambient humidity relative to adjacent developed urban environment. Average elevation is 241 m, with loamy soil texture. Between first deployment on 6/30/2012 and last collection on 9/27/2012, mean daily temperature was 24.14°C, average soil temperature 28.0°C,

average relative humidity 74.21%, and precipitation averaged 7.11 mm. Weather data were obtained from the North Carolina Climate Retrieval and Observations Network of the Southeast Database (CRONOS), via the State Climate Office of North Carolina in Raleigh.

Experimental Predators

Predator species in this study were local species of Dragonfly nymphs (*Odonata, Libellulidae*), obtained from Carolina Biological Supply Company. The following *Libellulidae* species collected in the Piedmont region of North Carolina were confirmed in a previous study (Wasserberg et al. 2013) by a random sampling of fifteen nymphs verified by David L. Stephan at North Carolina State University; *Erythemis simplicicollis* Say (54%), *Plathemis Lydia* Drury (38%), and *Pachydiplax longipennis* Burmeister (8%). *Odonata* nymphs are aquatic predators of numerous insect species, demonstrated as effective in controlling mosquito populations (Fincke et al. 1997, Saha et al. 2012). *Odonata* nymphs of the *Libellulidae* family cause an 18.7% decrease in *Ae. albopictus* oviposition rate, compared to sites without predation risk (Wasserberg et al. 2013). I used *Libellulidae* nymphs with medium body size to permit ease in handling and containment in mesh cages during field experiments. I also evaluated *Toxorhynchites amboinensis* mosquito larvae as potential larval predators (supplied by New Orleans Mosquito, Termite, and Rodent Control Board). *Toxorhynchites* larvae are well

documented as effective biocontrol agents in the control of pest species such as *Ae. aegypti* (Trpis 1973, Focks et al. 1980), *C. quinquefasciatus* (Miyagi et al. 1992), and *Ae. albopictus* (Miyagi et al. 1992, Toma and Miyagi 1992). *Odonata* nymphs were secured in cages constructed from aluminum screen mesh, 1.41 x 1.59 mm, then cut, folded, secured by staples, resulting in average final cage size of 6.5 x 6.0 x 1.0 cm (*Fig. 3*).

Mosquito Larvae

Conspecific larvae were obtained from eggs collected in Peabody Park, prior to and during the experiments, and reared in the laboratory. Additional eggs were graciously supplied by the laboratory of Charles Apperson. Larval densities used were based on previous studies indicating *Ae. aegypti* oviposition behavior markedly decreases at a conspecific density of 2.0 larvae per mL (Benzon and Apperson 1988, Zahiri and Rau 1998, Bédhomme et al. 2005).

Oviposition Sampling Methods

Oviposition traps consisted of 480 mL black stadium cups containing seed germination paper secured by a single small binder clip to the top rim. A drainage hole drilled two-thirds up on both sides of the cup prevented rain overflow and resulting in total water volume of 350 mL per cup. Germination paper remained half-way submerged in water with half continuously moist yet exposed, functioning as oviposition

substrate. Traps were secured to wooden stakes by a single black screw. Stakes were hammered into the ground so the bottom of each trap rested approximately 5 cm above ground level, facilitating trap rotation during sample collection (*Fig. 2*). Oviposition papers were collected, and water was discarded. New papers were introduced to each trap, which were filled with fresh dechlorinated water at each deployment. Egg number was recorded for each oviposition paper. Oviposition papers were collected, and water discarded, except for a portion of Experiment 3. Eggs and final larvae numbers were counted in the laboratory under microscopes using Tally Hand Counters, and data collected, entered into Microsoft Excel spreadsheets.

Pilot Experiment 1A

To determine typical *Ae. albopictus* oviposition rate in the study location, I established six grids comprised of three-by-six grid oviposition traps (*Fig. 4*), with one-meter distance between each trap, and each grid spaced at least 100 m apart, in sites selected for similarity between groundcover and proximity to local sources of freshwater. Two sampling sessions were conducted with deployment taking place on 7/7/2012 and 7/14/2012. Sampling site locations depicted in Figure 5A.

Pilot Experiment 1B

In a pilot study aimed at determining which predator is to be used in this study, I compared the relative effect odonate nymph and *T. amboinensis* on the oviposition response of *Ae. albopictus* mosquitoes. On 7/31/2012, three grids (A, B, E), shown in Figure 5, received randomized predator treatment of a single caged *Odonata* nymph, single caged *T.* amboinensis, or an empty cage. Oviposition papers were collected from all grids, and eggs recorded.

Experiment 2: Effect of Conspecific Larvae and Odonate Nymphs, Grid Design

Experiment 2 used the same oviposition trap grid formation as in Experiments 1 (*Fig. 5A*), but measured oviposition rate in response to three predation risk levels, followed by a combination of predation risk and five different conspecific larval densities, summarized in Table 1.

Experiment 3: Effect of Conspecific Larvae and Odonate Nymphs, Transect-Cup Transect Design

To account for potential effects of multiple stimuli at a relative close distance during the grid layout of Experiment 2 (Blaustein et al. 2004), and obtain higher resolution of predation effect, a separate design strategy was implemented using a straight-line transect configuration of paired oviposition traps. Experiment 3 consisted

of three 120m transects (A, B, and C), spaced 200 m apart (*Fig. 5B*), which is more than the maximum average distance traversed by an average *Aedes* female (Harrington and Edman 2001, Colton et al. 2003, Harrington et al. 2005). Each station contained a single pair of oviposition traps, one treatment and one control. Both traps contained the same number of larvae, but the treatment traps also contained three caged predator levels: 0, 1, 3. Deployment dates, collection dates, conspecific larvae, and predation risk level configurations are presented in Table 2.

Lab Methods

Eggs collected from 275 additional oviposition traps between four collection sites 200 m from any experiment locations provided mosquito larvae for the experiment. The eggs were reared in white plastic trays (35.56 x 27.94 x 7.62 cm), and provided with a food source mixture of bovine liver powder (obtained from MP Biomedicals, LLC) and oak leaf infusion (prepared in advance by soaking oak leaves in tap water for several weeks). Larvae were reared to second instar, counted and separated into vials. Complete trap contents of randomly selected traps were reared to adulthood in the lab, to confirm the exclusivity of *Ae. albopictus* mosquitoes and the absence of other local mosquito species. Incubator settings for egg hatching were 28°C at 70% relative humidity with twelve/twelve hour light/dark cycle (Gerberg et al. 1994).

Bacterial Analysis

To account for potential confounding effect of *Odonata* nymph excrements as a potential attractant for mosquitoes, bacterial levels were quantified for some predator presence experiments. Bacterial content of oviposition site water provides developing larvae with nutrients (Trexler et al. 2003). Water samples were collected from all traps of collection dates 9/6, 9/13, 9/20, and 9/27, which included traps containing larvae, predators and the combination of both. Each sample was prepared by aqueous dilutions of 10^{-1}, 10^{-2}, and 10^{-3} for spiral plating (Gilchrist et al. 1973) on trypticase soy agar (TSA) media and incubated at 37°C for 24 hours, and the number of colony forming units (CFU) were counted individually, and recorded (Breed and Dotterrer 1916).

Data Reduction and Statistical Analysis

Analysis of variance (ANOVA) and multiple linear regressions analyses were used to test the effect of predator level, initial larvae, and their interactions on egg deposition. For ANOVAs, Tukey honest significant difference (HSD) post-hoc tests were run subsequently in order to evaluate the significance of individual treatment levels, using location of experimental sites as a control variable. Second-order polynomial regressions were run for all nymph predation risk levels on Experiments 2 and 3 to test for hump-shaped relations between larval number and egg deposition with site and plot

location as control variables. A potential effect of bacteria on oviposition was tested by linear regression analysis.

Data analysis was performed with R version 3.0.1 (2013-05-16), (R Development Core Team) RStudio version 0.97.551, ©2009-2012 RStudio, and tables were configured in Microsoft® Excel® 2010 (version 14) ©2010, Microsoft Corporation.

CHAPTER III

RESULTS

Pilot Experiment 1A: Effect of Grid Location

ANOVA results supported strong location effect on mean egg number (*P=<0.0001, F=10.196, DF=5, N=72*) (*Table 3*). This was followed by a HSD test, which indicated that difference in mean egg number is due to grid location. Grid F produced the least number of mean eggs. Therefore, it was replaced by an identical grid in a different location.

Pilot Experiment 1B: Effect of Predator Type

One-way ANOVA show no significant effect of predator type on the number of eggs laid (*Table 4*). However, a marginally significant effect suggests a counter-intuitive outcome of larger egg number in the predator treatments compared with the control, with highest number of eggs at the nymph treatment, followed by the *Toxorhynchites* treatment (*Fig. 6*). Hence, due to mainly practical convenience (*Libellulidae* dragonfly nymphs were commercially available from Carolina Biological Supply) and, partly, based on the results of Wasserberg et al. (2013) who showed negative effects of such dragonfly nymphs, I chose nymphs for the subsequent experiments.

Experiment 2: Grid Design

Experiment 1B tested the effect of predator level only (*Table 1*). After controlling for the effect of grid location, the effect of predator level was suggestive (*P=0.09*), suggesting counter-intuitively, a positive effect of predator level on oviposition rate when no conspecific larvae were present (*Fig. 7A, Table 5*).

In the last three sessions, I tested the combined effect of predators and conspecifics (*Table 1*). While controlling for the effect of location, I tested for the effect of nymph level, initial larval number and the respective interactions thereof.

A multiple regression analysis evaluated the effect of final larvae, predator level, and interaction of both factors on mean egg count, revealing a significant effect of predation risk (*P=0.0245*) (*Table 6*). Figure 7B illustrates an increasing mean egg number corresponding to increasing nymph predation risk level in the presence of conspecific larvae treatments.

I tested for HSR at each nymph level by fitting a second-order polynomial regression to egg number with location as a control variable (*Table 7 A, B, C*). Evidence for such HSR was suggested only in the case of one nymph (*Table 7B, Fig. 8C*) with a marginally significant positive linear term and a significant second-order polynomial term. A significant effect of location was found at all three nymph levels (*Table 7*) with grid D consistently the most productive plot.

Experiment 3: Transect Paired Design

Multiple regression analysis tested for the combined effect of second-order polynomial effect of conspecifics, predator level, and controlled for the effect of bacterial concentration, location, and date of egg deposition (*Table 8*). A significant HSR was evident with significant positive and negative linear and second-order terms, respectively (*Fig. 9A, Table 8*). The effect of nymphs was not significant. The effect of bacterial concentration was, surprisingly, negative (*Fig. 11*). I tested for HSR at each nymph level (*Fig. 9*). Significant HSR was found only at the zero nymph level (*Fig. 9B*). At one nymph, HSR was not significant but a rough trend could possibly be suggested (*Table 8C, Fig. 9C*). At the one nymph, but especially at the level of three nymphs, larval numbers were substantially reduced compared with zero nymphs (*Fig. 10*). A significant effect of location was found with transect B having lower than average and C having more than average egg deposition (*Table 8*).

Effect of Larvae and Dragonfly Nymphs on Bacterial Concentration

I speculated that the positive effect of nymph level on egg deposition was mediated through the effect of nymph excrements on bacterial growth as potential attractors. Similarly, I expected positive effect of larval level on bacterial growth. I tested these effects using multiple regression analysis with site and date as control variables (*Table 10*). None of these factors had a positive effect (*Table 10, Figure 11*). The linear

analysis did not support a significant correlation between egg number and bacterial colony count (*P=0.305*). Welch Two Sample t-tests performed on the data concluded no significant effect of nymphs on the difference in eggs between the paired Experiment 3 zero nymph control versus either the one nymph (*P=0.787*) or three nymph (*P=0.780*) levels.

CHAPTER IV

DISCUSSION

Based on previous theory (Allee 1931, Wasserberg et al. 2014), I expected oviposition site selection preference to follow a HSR, with greatest egg numbers occurring at intermediate levels of conspecific larvae when presented within a range of density choices. In the presence of predation lethality, I expected an overall suppression of oviposition rate, based on predation lethality. With the interaction of conspecific densities and predation risk, I expected a rightward shift of peak oviposition towards higher conspecific densities, due to the greater per capita fitness conferred by larval aggregations (*Fig. 1*).

Effect of Conspecifics

HSR was observed in Experiment 2, at the one nymph level, and in Experiment 3 in the control traps (zero nymphs). At the predator presence treatments, a clear depression in larval number was apparent, which precluded the evaluation of the putative competitive effect at high densities. Support for HSR of oviposition was evident in Experiment 3. However, this is driven mainly due to patterns at zero and one nymph levels. At the three nymph level, larval number was depressed. Only a non-significant positive trend was suggested at the one nymph level (*Table 9, Fig. 9 A, B, C, D*).

Effect of Predation Risk

The prediction to test Hypothesis 2 was that gravid females would avoid oviposition traps containing increasing levels of predation risk, with traps devoid of predation risk receiving the greatest number of oviposited eggs. The predator type pilot study exhibited a marginally significant increase in egg deposition at the nymph treatment. Egg numbers in the *T. amboinensis* cups did not appear to differ from the control. Oviposition rate increased successively with each nymph level in the grid design of Experiment 2 (*Fig. 7*). In the transect design, however, no significant effect of predator was found. I evaluated the possibility that bacterial concentration might be positively correlated with predator numbers and in turn would attract gravid mosquitoes due to abundance of food resources (Trexler et al. 2003, Ponnusamy et al. 2008, Ponnusamy et al. 2010). This explanation was not supported as the effect of nymphs on bacterial density was non-significant (*Table 10*). None of the water samples from Experiment 2 were evaluated for bacteria.

Combined Effect of Predation Risk and Conspecific Density

The prediction for Hypothesis 3 was the interaction of the previous factors, conspecific density and predation risk, would cause a shift in the oviposition response of gravid females, favoring traps containing greater conspecific densities when also containing predators. Unfortunately, the fact that final larval range decreased

substantially in the predator treatments (both grid and transect designs) precluded the direct evaluation of this hypothesis. In the transect design, a clear HSR was observed at zero nymphs, but a non-significant suggestive trend was observed at the one nymph level. It appears that in the latter case the peak of the HSR curve is actually shifted towards lower larval densities.

Bacterial Analysis

I collected water samples for bacterial plating only during Experiment 3, with the expectation that bacterial load will increase according to the number of conspecifics or predators, with high bacterial load in traps with greatest numbers of conspecifics and predators. My results seem to suggest the opposite, as mean egg number decreases as bacterial colony count increases (*Fig.11*). The presence of more living organisms should produce more organic waste, therefore increasing bacterial load, and leading to greater bacterial colonies resulting from samples plated from those traps. Although bacterial load is often correlated with the presence of organic matter, an excess of bacteria in oviposition traps may cause an inhospitable environment for larval development, and inhibit larval survivorship by presence of anoxic conditions and toxic compounds. I suggest that as the number of nymphs or conspecific larvae increased, so did organic waste levels, including kairomones, as a result of the excretion of larval meals consumed

prior to deployment (nymphs were provided larvae in laboratory holding tanks), as well as a result of intra-nymph cannibalism in the holding tanks.

Temporal and Spatial Effects

Grid D of Experiment 2 produced greater numbers of oviposited eggs than any other grid locations, and it was also the grid location with the densest foliage. Although *Ae. albopictus* is documented as an edge habitat ovipositor species in its native environment (Hawley 1988), the differences in North American temperate forests may place selection pressure for greater oviposition site protection provided by thick vegetation of interior dense foliage.

Ae. albopictus is a container-breeding mosquito, and thus strongly susceptible to microenvironmental conditions within a gravid female's accessible oviposition range. Within Peabody Park, there are numerous small streams, human footpaths, adjacent automotive roads, and variation within forest floor flora composition, which all influence the suitability of individual grid location appeal for gravid female oviposition. Additionally, individual traps within grids for the pilot and grid experiments (1 and 2), offered additional microenvironmental variance of edge, corner, or interior trap location effect (*Fig. 4*), although the randomization of treatments attempted to minimize this aspect.

Overall, weather conditions of temperature, humidity, wind, and sunlight were varied throughout the course of this research. Water level of oviposition traps was subject to evaporation during warmer periods without rain. Conversely, rainwater probably caused larval density dilution and loss through the drainage holes. In addition, predator cages and trap liquid contents were missing in several instances, but all data was included in the analysis. Mosquitoes, like all insects, are heavily dependent on seasonal fluctuations, and oviposition behavior is no exception (Hawley 1988, Kitron et al. 1989). Greatest mean egg numbers were recorded between 8/30/2012 and 9/27/2012, but this time period also had the greatest mean egg number variability.

Synthesis

The Allee effect suggests that animal aggregations could be positively correlated with individual fitness (Allee 1931). Predation risk could induce an Allee effect due to decreasing per capita risk of predation correlated with increasing aggregation size, and exert additional pressures on individual fitness that may be offset by sharing the risk with group members (Foster and Treherne 1981). For container-breeding mosquitoes such as *Ae. albopictus*, oviposition site selection is a heavily weighted choice concerning offspring fitness. My hypotheses suggested optimal aggregation size increases in the presence of predation, causing a shift in oviposition site selection towards higher conspecific larval densities. Unfortunately, due to reduced range of conspecifics in the

presence of predators precluded the possibly of evaluating this hypothesis. Yet, comparing the assessed peak of Figures 8C and 9B suggest a shift in the opposite direction. This may suggest predation risk poses a greater cost to larval fitness than the benefits of larger group size can offer, as overcrowding can negatively impact life history (Zahiri and Rau 1998, Agnew et al. 2000, Bédhomme et al. 2005), but predation is lethal. The large numbers of lost larvae during the experiment might be due to behavioral changes in the presence of *Odonata* nymph predation risk, loss through oviposition trap drainage holes during periods of heavy rain or other environmental disturbances.

Effect of Conspecifics

Conspecific larval density was expected to influence oviposition site selection to follow a HSR with peak mean egg number respective to intermediate densities. Examination of previous literature pertaining to *Aedes* species, I found support for our predictions. There is evidence that larval environmental conditions affect developmental time, and adult life history. *Aedes sierrensis* larval density does not have any negative effects on larval mortality, however density does affect weight of female pupae, and adult size does positively correlate with longevity (Hawley 1985). As larval density increased, *Ae. albopictus* larval mortality and developmental time both increased, and adult size decreased (Lord 1998). Food availability had a greater effect on developmental time of *Ae. triseriatus* than of *Ae. albopictus*, which had greater effects

of larval density, suggesting costs of competition are not strictly limited to food availability (Teng and Apperson 2000). *Ae. aegypti* larval density negatively effects developmental time and emergent adult size, resulting from resource depletion and possibly environmental pollution caused by overcrowding (Bédhomme et al. 2005). These life history effects are more pronounced on females than males, due to the increased female body size required for egg gestation and gravid flight energetics (Hawley 1985, Bédhomme et al. 2003).

Effect of Predation Risk

Most research supporting oviposition site selection due to predation avoidance is based on *Culex* species. Although two *Culex* species were documented to actively avoid oviposition in artificial containers without regard to *Notonecta* predation density, the survivorship of the resultant larvae was dependent on the number of predators present (Eitam and Blaustein 2004). *Cu. longiareolata* oviposit preferentially in containers without *Notonecta* predation risk, pairwise comparison between low (one) and high (four) predation risk confirmed the ability to quantify and select for sites offering less risk (Silberbush and Blaustein 2011). *Ae. albopictus* larvae demonstrate behavioral modification of varying degrees based on instar development phase in the presence of *Corethrella appendiculata* predator cues. Larger forth instar larvae had less of a behavioral change than second instar larvae (Kesavaraju et al. 2007, Kesavaraju and

Juliano 2008). This could explain Experiment 3 larval reduction at the one and three nymph levels, when compared to the zero nymph level. There is evidence from Experiment 2 that gravid females are able to quantify risk of predation between potential oviposition sites (*Figure 7B*), but results are inconclusive for Experiment 3.This ability to not only detect, but quantify predation risk, could be due to the detection of kairomones by the gravid female (Silberbush and Blaustein 2008).

The Combined Effect of Predation Risk and Conspecific Density

The large loss of final larvae, particularly at the one and three nymph levels, prevented proper analysis of the interaction of conspecific density and predation risk (*Fig. 10*). I speculate that the loss of larvae may be due to increased mortality resulting from behavioral changes in the presence of predation risk, leading reduced foraging and surface respiration (Kesavaraju et al. 2007, Kesavaraju and Juliano 2008, Alto et al. 2009). Since encounter dilution theory offers a reduced chance of predation for each individual as aggregation size increases (Hamilton 1971, Mooring and Hart 1992), gravid female mosquitoes should select oviposition sites based on a number of preexisting larvae to confer protection from predation, but avoid larval densities with too much costs of competition. Two *Culex* species *(Cu. quinquefasciatus* and *longiareolata*) display evidence of oviposition site selection based on conspecific density, and predation risk (tadpoles), a mechanism triggered by both chemical and biological cues (Blaustein and

Kotler 1993, Mokany and Shine 2003). This experiment could not test the interaction effect but possible shift was actually suggested towards decreased densities (*Fig. 8B, C, D, Fig.9, B, C, D*), the opposite of my expectations. Experiment 3 had such a large reduction in larval density numbers, that we cannot rely on the data to either support or reject the hypothesis.

Bacteria

The unexpected result of bacterial load negatively correlated with egg number is significant (P=0.0369), but highly variable. This suggests a weak inhibitory effect of bacteria load on oviposition rate (*Fig. 11*). We did not evaluate the bacterial species composition, which is likely relevant for understanding this inhibitory relationship. The presence of two bacterial species, *Acinitobacter calcoaceticus* and *Enterobacter cloaca*are, are confirmed to positively correlate with *Ae. aegypti* oviposition rates, possibly through chemical cues (Benzon and Apperson 1988).

Although the multiple regression results of predator, conspecific, or location effects on bacterial colony count were not statistically significant (*Table 10*), we know from previous studies that bacteria content of oviposition sites has important effects on mosquito life history. *Ae. aegypti* eggs may be prompted to hatch due to the presence of bacteria, or bacterial compounds (Ponnusamy et al. 2011), which may indicate organic nutrient availability. The water of a potential oviposition site must contain

sufficient organic matter to allow for successful larval development. However, too much organic matter decreases the available dissolved oxygen. Although mosquito larvae aspirate via a modified posterior spiracle as a siphon at the water surface (Gullan and Cranston 2009), the ideal larval environment is not brackish or putrid. Water preferred by gravid females does not contain NH_4OH, and pH range of 3 to 10 is preferred (Gubler 1971, Hawley 1988). The presence of high densities of conspecifics can contribute to unfavorable oviposition conditions by excess organic waste (Bédhomme et al. 2005).

Application

This experiment did not offer enough evidence to form any conclusive decisions regarding the use of *Odonata* nymph predators for invasive *Ae. albopictus* biocontrol. Although the use of native species to control invasive populations is potentially ecologically sound and sustainable (Messing and Wright 2006), it may not offer the most efficient solution in removing established and prolific species such as *Ae. albopictus*. *Odonata* nymphs are successful larval predators of *Cu. quinquefasciatus* (Saha et al. 2012), but skip oviposition could complicate control of *Ae. albopictus*. This tactic spreads their offspring among numerous locations, versus dumping all their offspring in one site, reducing mortality risk for their entire brood, and enhancing their own fitness (Colton et al. 2003). This ensures that at least some of their offspring survive, but makes biocontrol

difficult, as larvae mature quickly and require only a small volumes of water (Burkett-Cadena 2013).

Alternatives to traditional non-specific chemical pesticides continue to increase in demand as the public becomes more aware of the potential human and environmental health effects (Davies 1990, Dunlap and Beus 1992, Pimentel et al. 1992). Integrated Pest Management (IPM) is a combination of environmentally aware methods of controlling pest populations of pest target species, focused on life histories, understanding that total eradication is not a viable goal and setting threshold population limits, prevention of new pest introductions, systematic monitoring and observation, the use of non-chemical controls such as physical barriers, encouraging populations of natural predators and parasites to reduce or weaken target populations, and when chemical based pesticides are used, they are applied correctly and sparingly with strong emphasis on appropriate timing and dosage (USEPA 2012). Through this combination of tactics, IPM addresses public concerns about pesticide use and over use, while more efficiently targeting pest species with minimal harm to beneficial insects.

My findings suggest that the presence of *Odonata* nymphs serve as a counter intuitive attractant to ovipositing *Ae. albopictus* (*Figure 7B*). This mechanism, here coined as the Attractive Predator Effect (APE), has also been observed as a positive oviposition response of *Ae. aegypti* to the presence of a copepod predator (*Mesocyclops longisetus*) (Torres-Estrada et al. 2001). The limited time period since

initial introduction of *Ae. albopictus* in the United States (Hawley 1988) may prompt oviposition in response to nymph presence through misinterpretation of local *Odonata* kairomones. The ephemeral water hole preference of *Ae. albopictus* is probably the most limiting factor for biocontrol with odonates, as the *Odonata* nymph life stage may last several years, requiring a more permanent habitat (Corbet 1980). However, the APE of oviposition site selection containing *Odonata* nymph predators could facilitate population control of *Ae. albopictus* at the larval life stage at small scale applications, such as gardens and backyards.

Table 1. Experiment 2: Design and Deployment Combinations. Grid Location, Conspecific Larval Densities, and Predator Type for Each Deployment Date.

DEPLOYMENT DATE	GRID	CONSPECIFIC LARVAE NUMBERS	PREDATOR TYPE
7/14/2012	B, C, D, E, F	0	0, 1, 3 NYMPH
7/21/2012	A, D	0	0, 1, 3 NYMPH
7/21/2012	B, C, E, F	0, 10, 50, 100, 300, 500	0, 1, 3 NYMPH
8/7/2012	A, B, C, D, E, F	0, 10, 50, 100, 300, 500	0, 1, 3 NYMPH
8/14/2012	A, D	0, 10, 50, 100, 300, 500	0, 1, 3 NYMPH

Table 2. Experiment 3: Transect Design. Transect Location, Conspecific Larval Densities, and Predator Type for Each Deployment Date.

DEPLOYMENT DATE	PAIRED TRANSECT	CONSPECIFIC LARVAE NUMBERS	PREDATOR TYPE
8/21/2012	C (stations 1-12)	0, 50, 200, 400	0, 1, 3 NYMPH
8/26/2012	A (stations 1-12)	0, 50, 200, 400	0, 1, 3 NYMPH
9/2/2012	B (stations 1-12)	0, 50, 200, 400	0, 1, 3 NYMPH
9/9/2012	A (stations 1-12)	0, 50, 200, 400	0, 1, 3 NYMPH
9/9/2012	B (stations 1-12)	0, 50, 200, 400	0, 1, 3 NYMPH
9/9/2012	C (stations 1-9)	0, 50, 200, 400	0, 1, 3 NYMPH
9/16/2012	A (stations 1-12)	0, 50, 200, 400	0, 1, 3 NYMPH
9/23/2012	C (stations 1-4)	0, 50, 200, 400	0, 3 NYMPH

Table 3. ANOVA for Pilot Experiment. Effect of Location on Mean Egg Count.

	D.F	Sum of Squares	Mean Square	F value	P value
Location	5	46107	9221.50	10.196	0.0001
Residuals	210	189924	904.40		

Table 4. One-Way ANOVA for Pilot Experiment. Effect of Predator Type on Mean Number of Eggs.

	D.F	Sum of Squares	Mean Square	F value	P value
Predator Type	2	3874	1936.86	2.696	0.0770
Residuals	50	35918	718.37		

Table 5. Experiment 2: ANOVA for Effect of Predator Level, No Larvae Present.

	D.F	Sum of Squares	Mean Square	F value	P value
Location	5	40280	8055.9	18.1324	0.0001
Nymphs	1	1255	1254.9	2.8244	0.0949
Residuals	154	68420	444.3		

Table 6. Experiment 2: Multiple Regression of Effect of Final Larvae Number, Predator Level, and Interaction of Both on Mean Egg Count. Location Serves as a Control Variable.

	Estimate	Standard Error	T value	P value
(Intercept)	30.2058	5.7869	5.2200	0.0001
Nymphs	3.6613	1.6111	2.2730	0.0245
Final Larvae	0.0331	0.0224	1.4780	0.1415
Location B	-9.5349	6.1608	-1.5480	0.1239
Location C	1.5405	6.1009	0.2530	0.8010
Location D	30.3520	6.1835	4.9090	0.0001
Location E	-18.4379	7.2362	-2.5480	0.0119
Location F	2.3139	7.0959	0.3260	0.7448
Nymphs:Final	-0.0089	7.0959	-0.3060	0.7603

Table 7. A, B, C, D. Experiment 2: Test of Second-Order Polynomial for All Three Nymphs Predation Levels, Using Location as Control Variable. A: All nymph levels, B: zero nymphs, C: one nymph, D: three nymphs.

7A: All nymph levels	Estimate	Standard Error	T value	P value
Intercept	36.5814	5.2187	7.01	<0.0001
Final Larvae	-0.0171	0.0616	-0.28	0.7819
Final Larvae^2	0.0001	0.0002	0.51	0.6106
Location B	-9.5809	6.267	-1.53	0.1285
Location C	1.5124	6.2071	0.24	0.8078
Location D	30.1943	6.2886	4.8	<0.0001
Location E	-18.6192	7.2752	-2.56	0.0115
Location F	1.6991	7.1685	0.24	0.8130

7B: 0 Nymphs	Estimate	Standard Error	T value	P value
Intercept	32.7863	8.2534	3.97	0.0003
Final Larvae	-0.039	0.08	-0.49	0.6286
Final Larvae^2	0.0002	0.0002	1.02	0.3139
Location B	-12.5447	9.5362	-1.32	0.1955
Location C	-2.1913	9.3962	-0.23	0.8167
Location D	31.7612	9.3996	3.38	0.0016
Location E	-17.0477	11.3212	-1.51	0.1396
Location F	-2.9434	10.7717	-0.27	0.7860

7C: 1 Nymph	Estimate	Standard Error	T value	P value
Intercept	30.7397	8.4107	3.65	0.0007
Final Larvae	0.2363	0.123	1.92	0.0612
Final Larvae^2	-0.001	0.0005	-2.04	0.0479
Location B	-9.6602	9.7499	-0.99	0.3272
Location C	10.5878	10.1669	1.04	0.3034
Location D	27.1996	9.8797	2.75	0.0085
Location E	-12.7969	11.2767	-1.13	0.2626
Location F	6.6336	11.2448	0.59	0.5583

7D: 3 Nymphs	Estimate	Standard Error	T value	P value
Intercept	34.6843	11.2906	3.07	0.0036
Final Larvae	0.4692	0.5793	0.81	0.4224
Final Larvae^2	-0.0041	0.0058	-0.71	0.4829
Location B	-2.8262	13.2514	-0.21	0.8321
Location C	5.3818	13.0287	0.41	0.6816
Location D	34.7556	13.4282	2.59	0.0130
Location E	-23.8374	15.7342	-1.52	0.1369
Location F	5.6937	15.6451	0.36	0.7177

Table 8. Experiment 3: Mean Egg Count in Response to Combined Effect of Final Larvae, Nymphs, and Bacteria.

	Estimate	Standard Error	T value	P value
(Intercept)	77.880	181.0	0.430	0.6678
Final Larvae	0.369	0.1163	3.172	0.0020
Final Larvae^2	-0.0001	0.0004	-2.087	0.0392
Nymphs	3.768	2.836	1.329	0.1867
Bacteria	-1.074	0.5083	-2.112	0.0369
Location B	-22.12	7.861	-2.814	0.0058
Location C	43.02	8.056	5.340	0.0001
Date	-3.434	19.70	-0.174	0.8619

Table 9. A, B, C, D. Transect Experiment 3: Second-Order Polynomial, for Each Nymph Level. A: All nymph levels, B: zero nymphs, C: one nymph, D: three nymphs.

9A: All Nymph Levels	Estimate	Standard Error	T value	P value
(Intercept)	43.850	13.730	3.1930	0.0018
Larvae	0.247	0.950	2.6010	0.0103
Larvae^2	-0.001	0.0003	-1.9580	0.0523
Location B	-0.529	10.110	-5.2290	0.0001
Location C	1.936	10.880	0.1780	0.8591
Date 8/30/2012	38.870	14.850	2.6170	0.0099
Date 9/13/2012	31.850	10.080	2.8750	0.0047
Date 9/20/2012	-21.260	15.040	-1.4140	0.1598
Date 9/27/2012	61.290	13.330	4.5980	0.0001
Date 9/6/2012	32.890	15.060	2.1840	0.0307

9B: 0 Nymph Level	Estimate	Standard Error	T value	P value
(Intercept)	48.1600	18.2900	2.6350	0.0102
Larvae	0.3089	0.1176	2.6260	0.0105
Larvae^2	-0.0008	0.0001	-2.0730	0.0416
Location B	-59.0600	13.3100	-4.4380	0.0001
Location C	-9.0290	14.2900	-0.6320	0.5293
Date 8/30/2012	30.3600	19.4400	1.5620	0.1224
Date 9/13/2012	28.5300	14.5600	1.9590	0.0538
Date 9/20/2012	27.1900	19.8100	-1.3730	0.1739
Date 9/27/2012	77.2200	18.8200	4.1020	0.0001
Date 9/6/2012	29.8300	19.8000	1.5060	0.1362

9C: 1 Nymph Level	Estimate	Standard Error	T value	P value
(Intercept)	-0.3595	30.9800	-0.0120	0.9909
Larvae	0.2557	0.2284	1.1190	0.2777
Larvae^2	-0.0004	0.0008	-0.5340	0.5999
Location B	-66.6800	22.0000	-3.0310	0.0072
Location C	16.4800	23.4000	0.7050	0.4901
Date 8/30/2012	100.6000	33.1500	3.0350	0.0071
Date 9/13/2012	78.4500	25.7100	3.0510	0.0069
Date 9/20/2012	10.0300	33.4700	0.3000	0.7678
Date 9/6/2012	89.9100	34.8300	2.5810	0.0188

9D: 3 Nymph Level	Estimate	Standard Error	T value	P value
(Intercept)	56.4669	32.1108	1.7580	0.0932
Larvae	0.9114	1.3111	0.6950	0.4946
Larvae^2	-0.0153	0.0235	-0.6510	0.5218
Location B	-18.1702	23.9128	-0.7600	0.4558
Location C	25.9217	26.0314	0.9960	0.3307
Date 8/30/2012	6.4561	34.6311	0.1860	0.8539
Date 9/13/2012	-1.9444	26.0532	-0.0750	0.9412
Date 9/20/2012	-25.0418	34.0601	-0.7350	0.4703
Date 9/27/2012	12.4700	23.9806	0.5200	0.6085
Date 9/6/2012	-9.8075	34.7547	-0.2820	0.7806

Table 10. Experiment 3: Multiple Regression of the Effect of Initial Larvae Number and Nymph Predation Risk Level on Bacterial Count, with Location and Date as Control Variables.

	Estimate	Standard Error	T value	P value
(Intercept)	29.884	33.235	0.899	0.3700
Initial Larvae	-0.004	0.004	-0.944	0.3470
Nymphs	0.281	0.493	0.571	0.5690
Location B	0.215	1.433	0.150	0.8810
Location C	1.137	1.480	0.768	0.4440
Date	-2.268	3.624	-0.626	0.5330

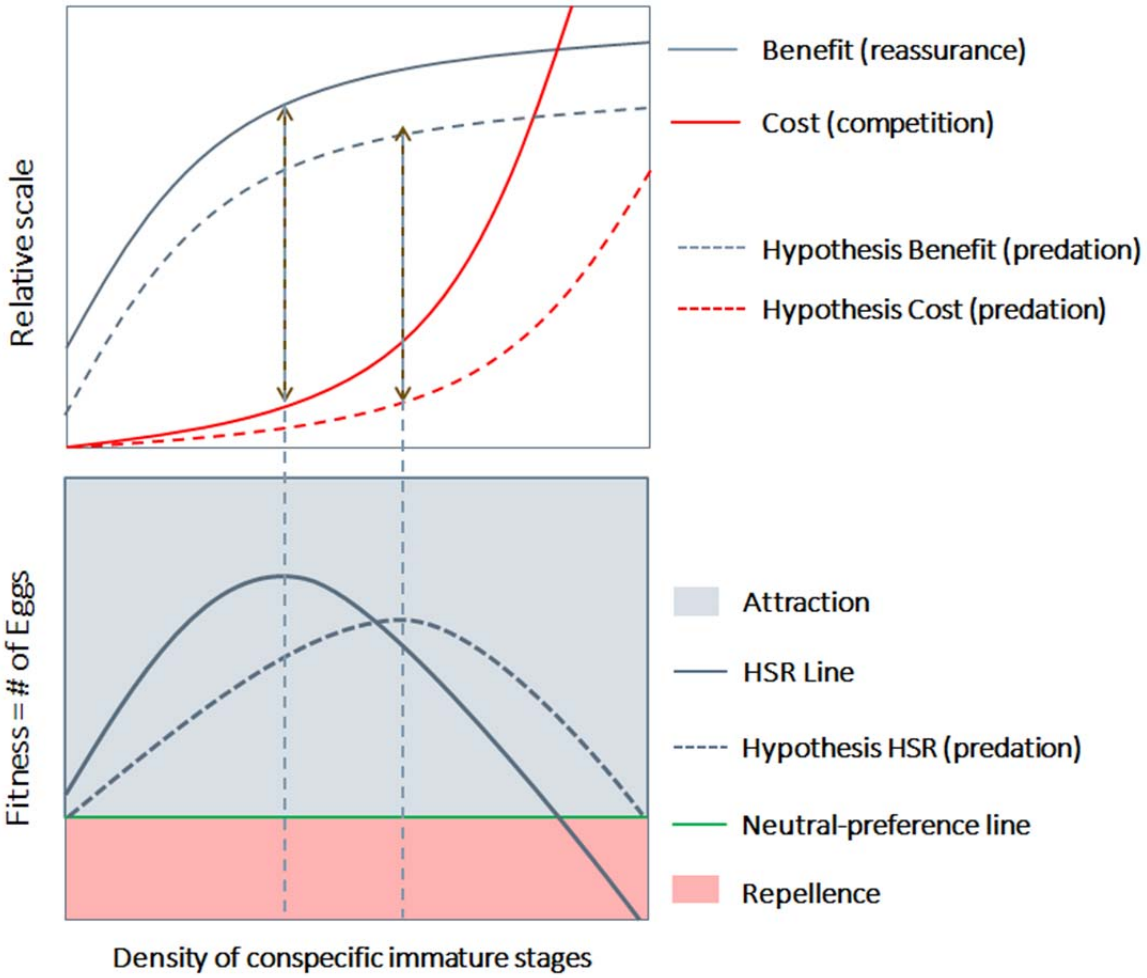

Figure 1. Theoretical Hump-Shaped Relationship (HSR) Between Oviposition Site Selection in Response to Pre-Existing Conspecific Density and Predation Risk. Modified from Wasserberg et al. 2014.

Figure 2. Oviposition Traps in Field Location. Traps were Attached to Wooden Stakes and Contained a Single Germination Paper Secured with Binder Clip.

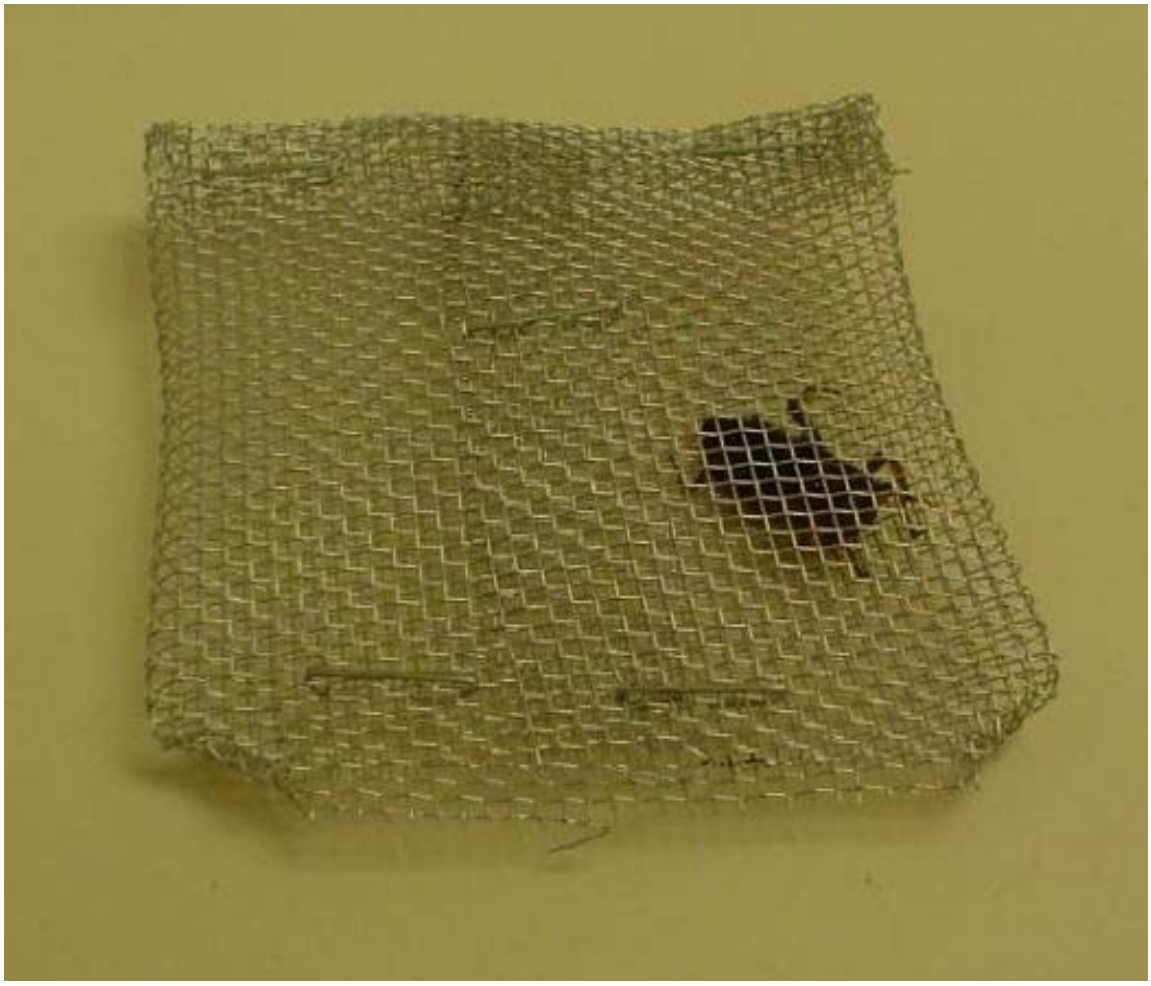

Figure 3. Predator Cage Constructed out of Aluminum Wire Mesh, Containing One *Odonata* Nymph.

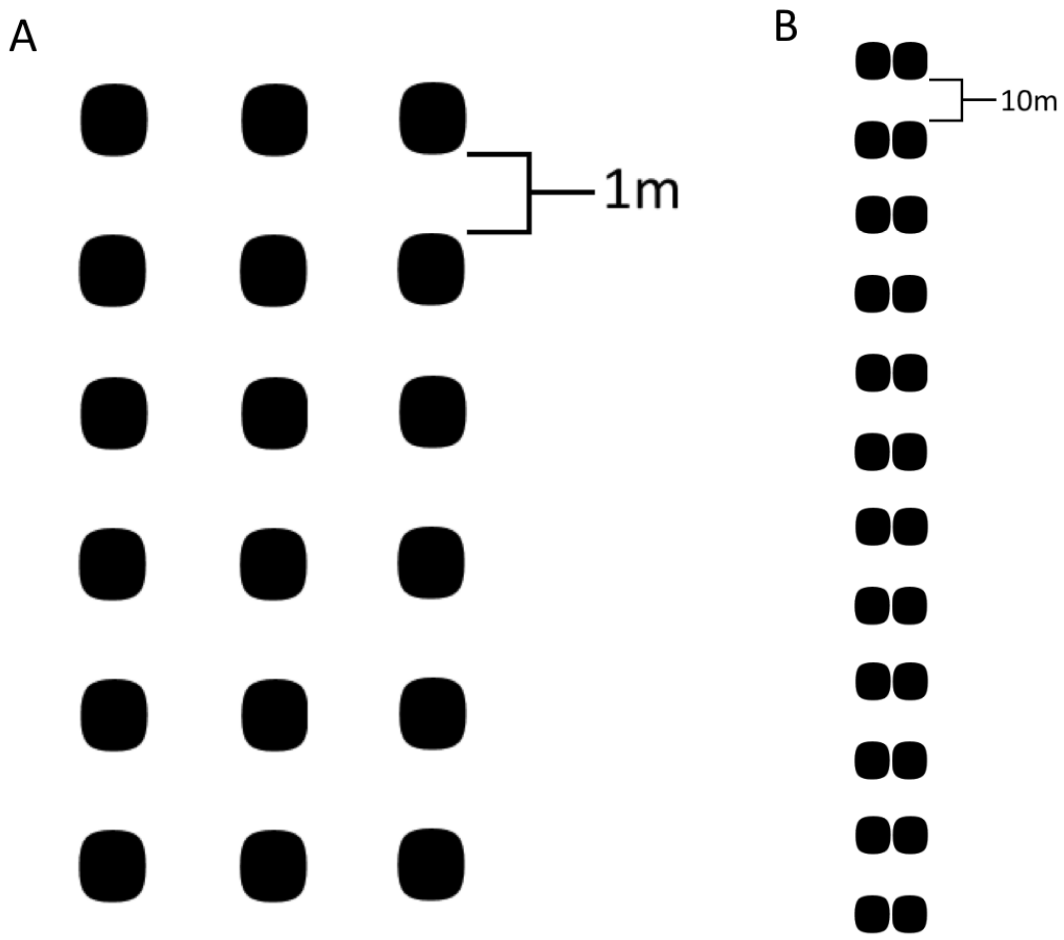

Figure 4. A. Study Design for Grid Layout of Experiment 1 and Experiment 2. B. Study Design for Transect Layout of Experiment 3. Dots Represent Single Oviposition Traps, Not to Scale

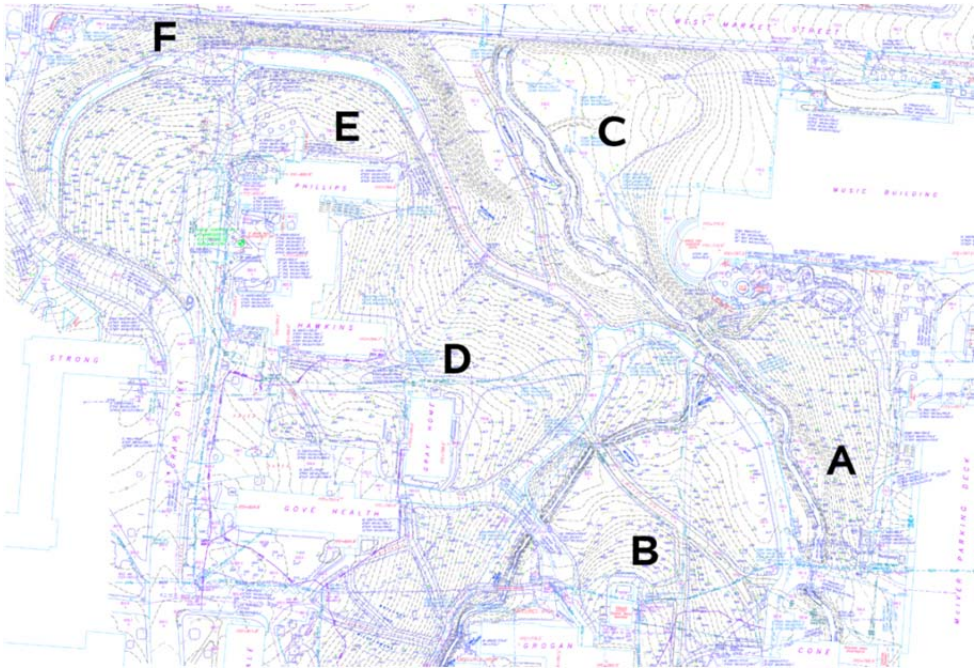

Figure 5. A. Peabody Park Grid Design Experiment Locations.

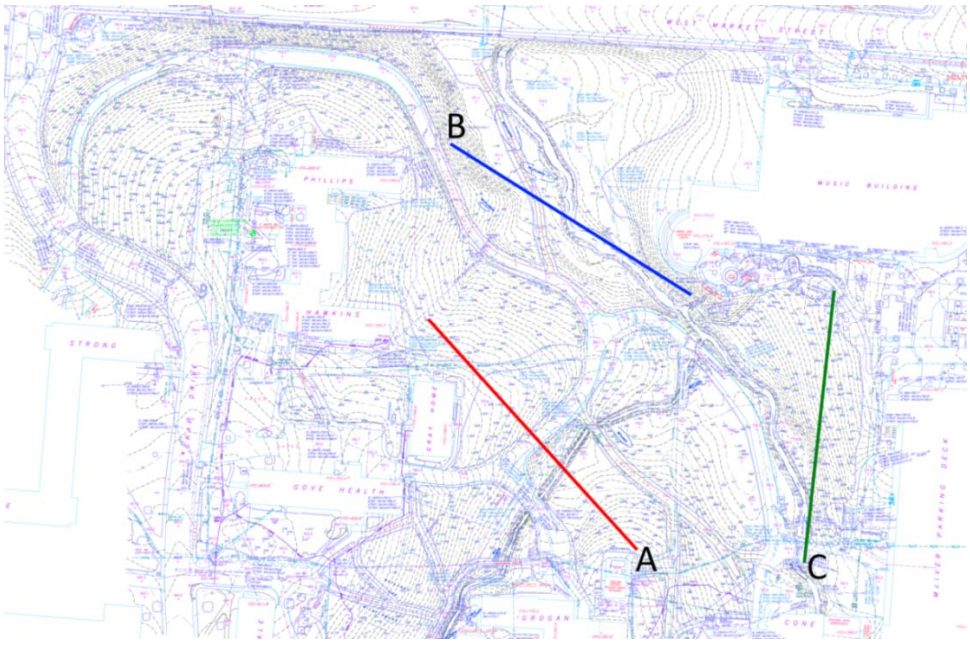

Figure 5. B. Transect Experiment Locations.

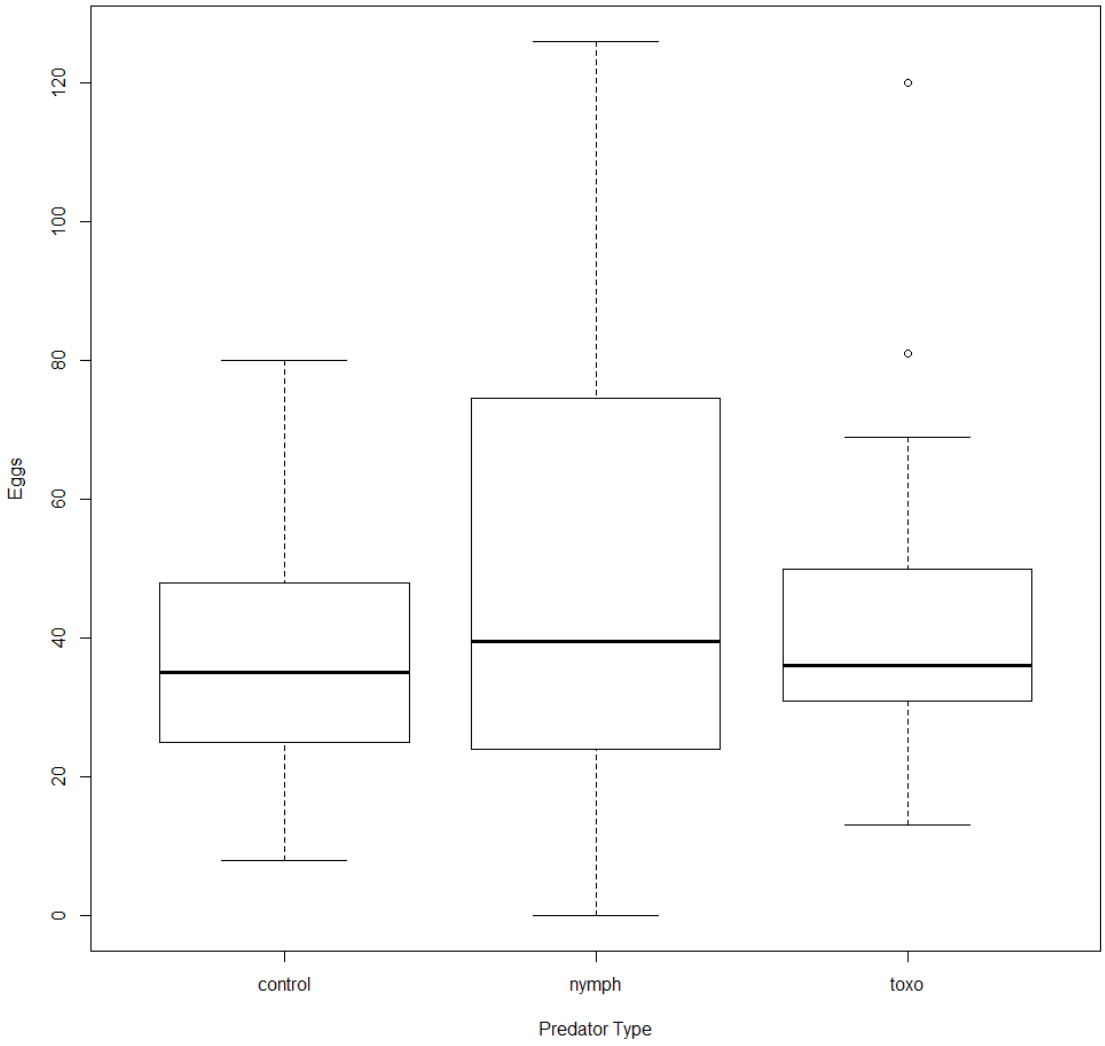

Figure 6. Comparison of Mean Egg Number Between Predator Types. Control: Empty Predator Cage, Nymph: One Caged *Odonata* Nymph, Toxo: One Caged *Toxorhynchites* Larva.

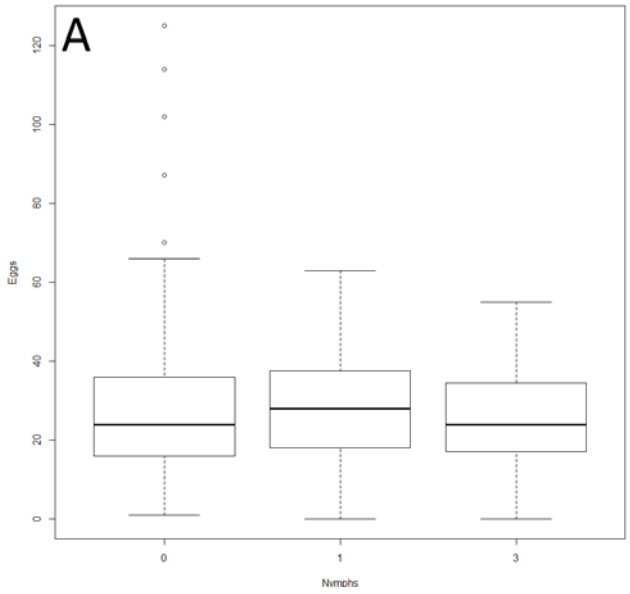

Figure 7. A. Experiment 2: Mean Egg Number for Three Predation Risk Levels (0, 1, 3), Without Conspecific Larvae.

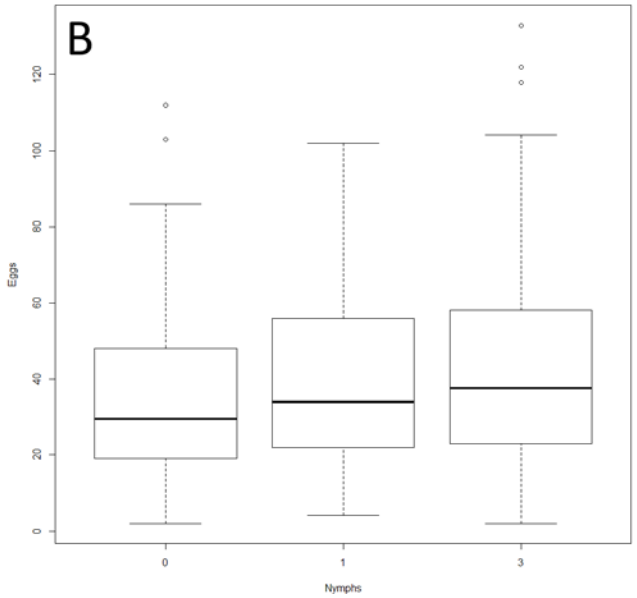

Figure 7. B. Experiment 2: Mean Egg Number for Three Predation Risk Levels (0, 1, 3), With Conspecific Larvae.

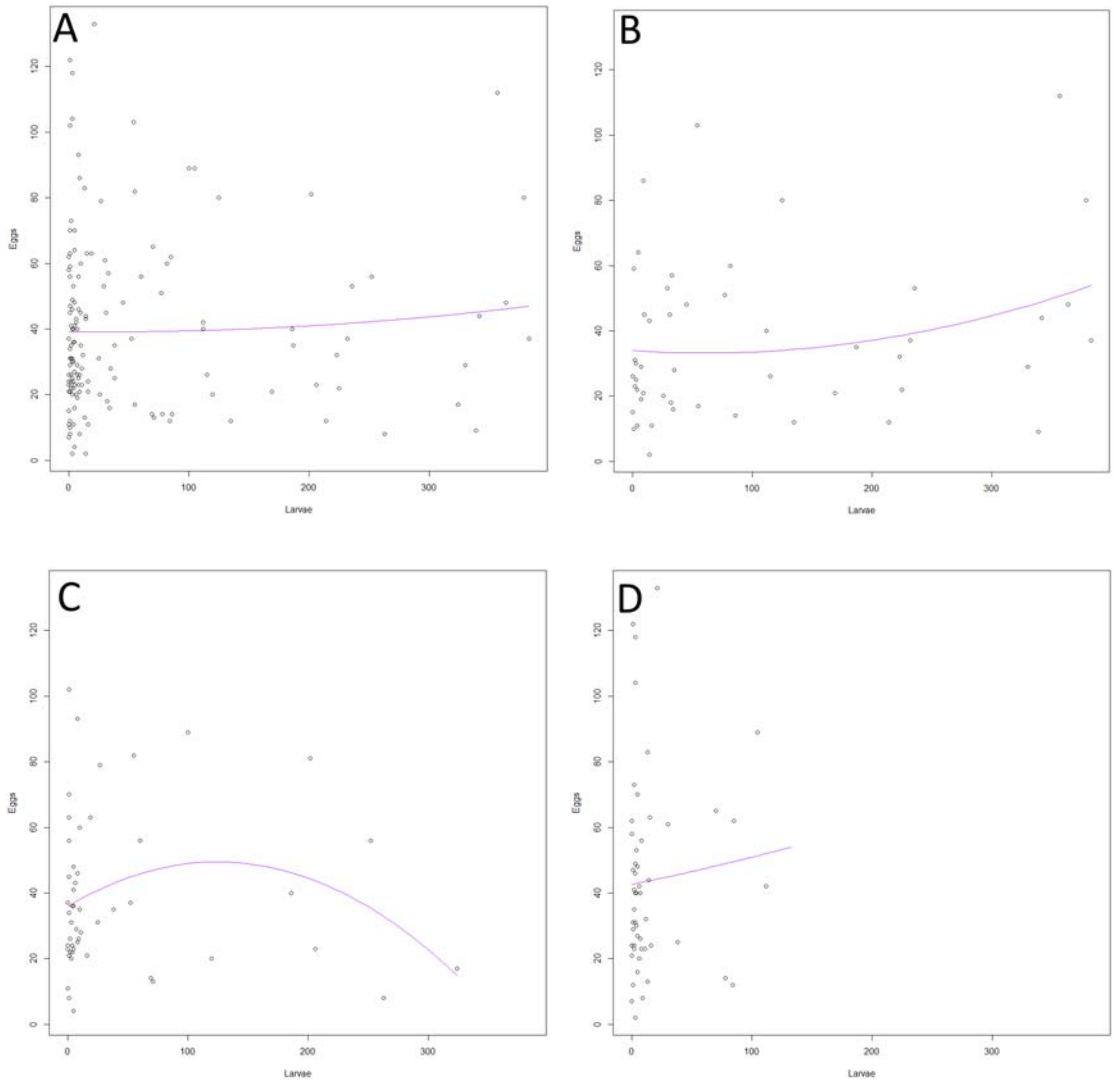

Figure 8. A, B, C, D. Grid Experiment 2 Second-Order Polynomial Regression for All Three Nymph Levels. A: All Levels, B: Zero, C: One, D: Three.

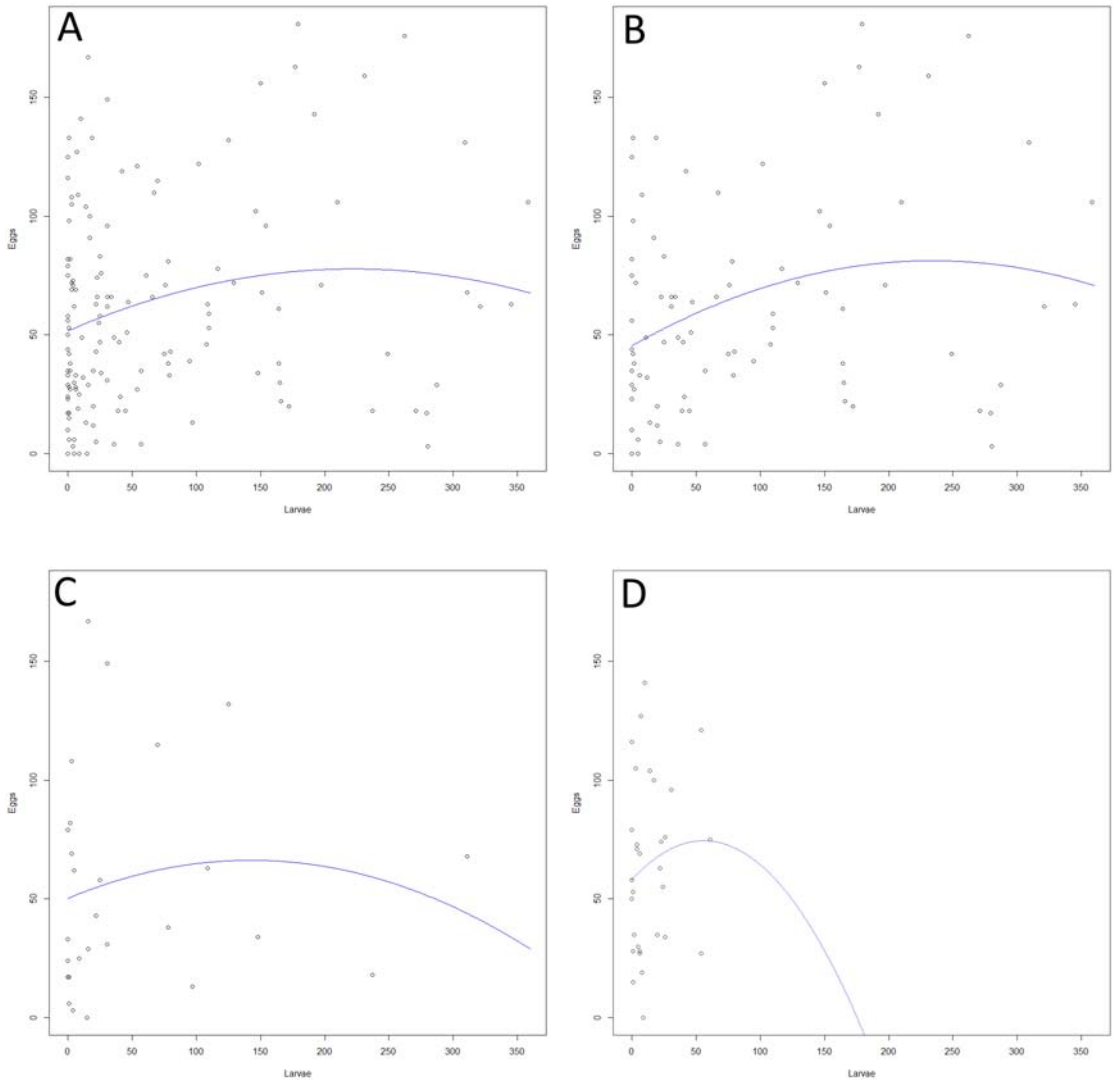

Figure 9. A, B, C, D. Transect Experiment 3 Second-Order Polynomial Regression for All Three Nymph Levels. A: All Levels, B: Zero, C: One, D: Three.

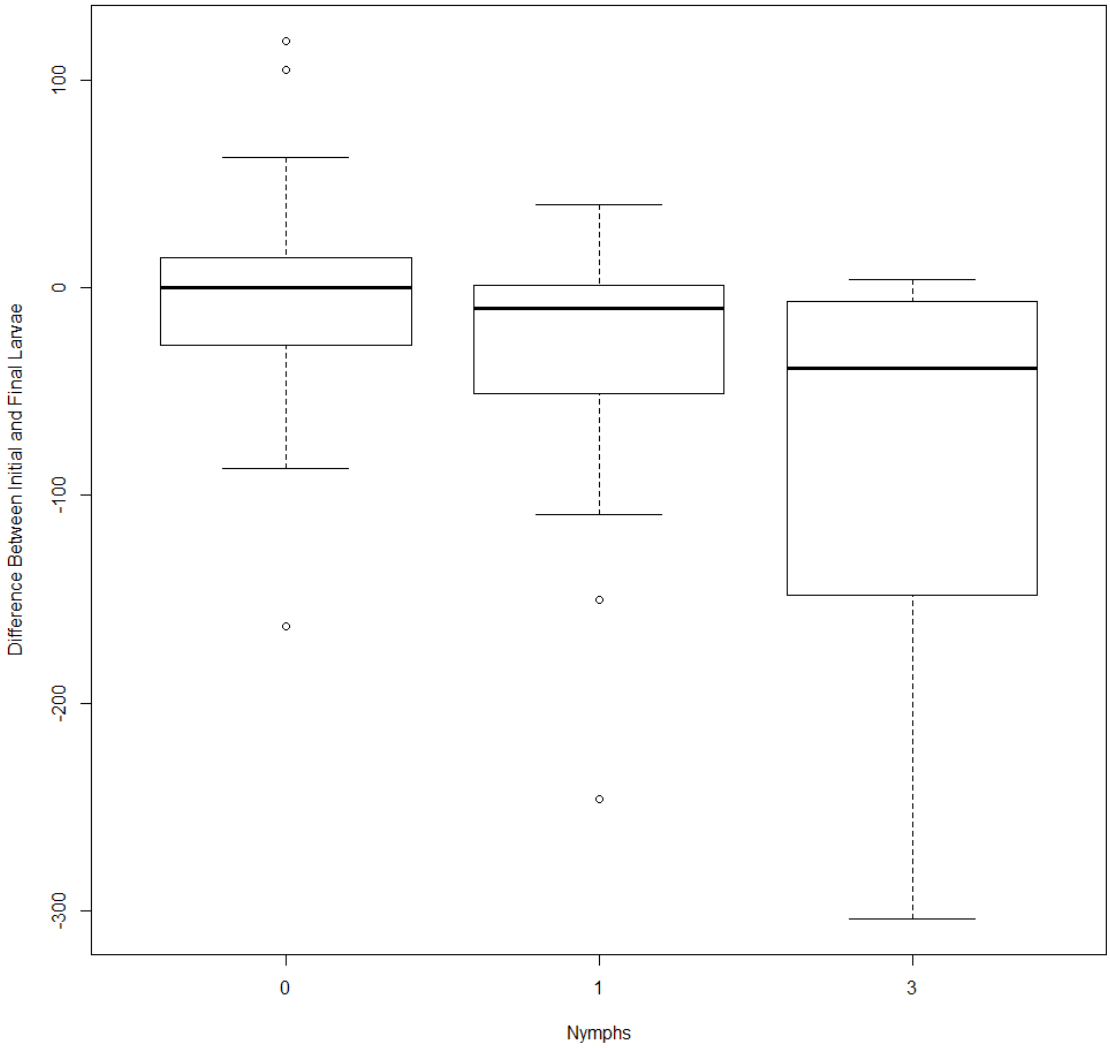

Figure 10. Difference Between Initial Number of Larvae and Final Larvae for Each Nymph Predation Risk Level (0, 1, 3).

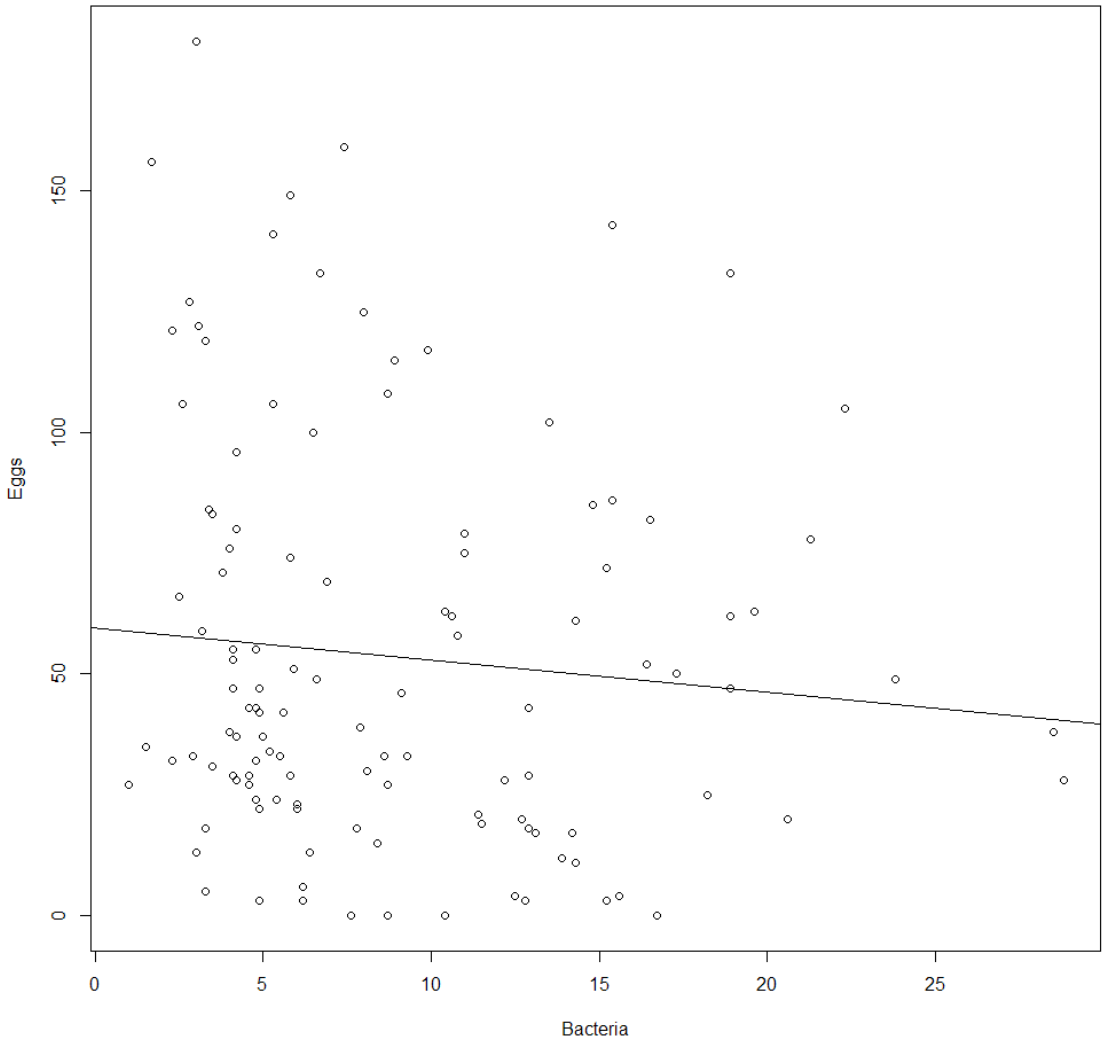

Figure 11. Transect Experiment 3. Linear Regression of Mean Egg Number Versus Bacteria Colony Count.

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