

**THE INFLUENCE OF SOCIAL CUES ON OVIPOSITION DECISIONS IN
THE MOSQUITO *AEDES AEGYPTI***

Thesis submitted in partial fulfilment of the requirements of the
BS-MS Dual Degree Program at IISER, Pune



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M.S. Thesis

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CERTIFICATE

This is to certify that this dissertation entitled “The influence of social cues on oviposition decisions in the mosquito *Aedes aegypti*” towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents the research carried out by Ashwini Ramesh at the Indian Institute of Science, Bengaluru under the supervision of Dr. Kavita Isvaran, Assistant Professor, Centre for Ecological Sciences, during the academic year 2015-2016.



Dr. Kavita Isvaran
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Date :28.03.2016

DECLARATION

I hereby declare that the matter embodied in the report entitled “The influence of social cues on oviposition decisions in the mosquito *Aedes aegypti*” are the results of the investigations carried out by me at the Centre for Ecological Sciences, Indian Institute of Science, under the supervision of Dr. Kavita Isvaran and the same has not been submitted elsewhere for any other degree.



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Abstract

Animals use a variety of social and non-social cues in evaluating the habitat quality of a patch. Assessing social information and incorporating it into future decisions can translate into important fitness consequences for an individual. Local adult-density, among other social cues, can heavily influence individual adult decisions. In particular, adult-density can play a crucial role in affecting maternal decisions like oviposition site-selection that can potentially set-off a cascade of responses in both the parent and the offspring. In this study, I used the mosquito *Aedes aegypti* as a model system to investigate the influence of adult-density on oviposition responses. Specifically, I measured oviposition responses of individual adult females to patches differing in quality when present singly (solitary) or in the presence of non-breeding adult individuals (social setting). In a series of binary choice experiments, patch quality was represented by pools differing in larval predation risk in one set of experiments and by pools differing in larval competition risk in another. My study indicates that social cues appeared to substantially modify female oviposition decisions. In response to competition risk, while social status did not influence several measures of oviposition, social females displayed great selectivity by ovipositing in pools with cues indicating high conspecific larval densities. In contrast, social females showed a higher preference and increased fecundity in predator treatments. Social females also distributed eggs more often in this treatment than solitary adults. In addition, social and solitary females alike were more likely to bet-hedge when the contrast between pools was less pronounced than when it was stark. Also, social and solitary females exhibited a large variation in egg-laying choices across varying risks of predation and competition. Overall, my study indicates that individual adult females are able to assess the presence of other individuals and in response modify their behaviour during oviposition events. Adult females seem to respond to the presence of other females by adopting a bet-hedging strategy, sometimes withholding eggs perhaps to distribute eggs further across multiple pools while exhibiting preference for riskier patches. I suggest that adult female density may favour a shift in preference of individual females towards seemingly riskier patches and discuss potential evolutionary explanations for this shift.

List of Figures

Figure No.	Title	Page No.
01	Comparison of propensity to oviposit in social and solitary individuals in response to varying levels of larval competition	25
02	Comparison of mean total fecundity in social and solitary individuals in response to varying levels of larval competition	26
03	Comparison of oviposition activity in social and solitary individuals in response to varying levels of larval competition	27
04	Relative frequency distribution of oviposition activity index in social and solitary individuals in response to varying levels of larval competition	28
05	Relationship between mean total fecundity and oviposition activity index in social and solitary adults in response to varying levels of larval competition	29
06	Comparison of total eggs deposited in control pool in social and solitary adults in response to varying levels of larval competition	30
07	Comparison of propensity to oviposit in social and solitary individuals in response to varying levels of larval competition at early time period	31
08	Comparison of propensity to oviposit in social and solitary individuals in response to varying levels of larval predation	35
09	Comparison of mean total fecundity in social and solitary individuals in response to varying levels of larval predation	36
10	Comparison of oviposition activity in social and solitary individuals in response to varying levels of larval predation	37
11	Relative frequency distribution of oviposition activity index in social and solitary individuals in response to varying levels of larval predation	38
12	Relationship between mean total fecundity and oviposition activity index in social and solitary adults in response to	39

	varying levels of larval predation	
13	Comparison of total eggs deposited in control pool in social and solitary adults in response to varying levels of larval predation	40
14	Comparison of propensity to oviposit in social and solitary individuals in response to varying levels of larval predation at early time period	41
15	Comparison of propensity to oviposit in social individuals in response to varying levels of larval competition and predation	42
16	Comparison of mean total fecundity in social individuals in response to varying levels of larval competition and predation	43
17	Comparison of oviposition activity in social individuals in response to varying levels of larval competition and predation	44
18	Comparison of total eggs deposited in control pool in social individuals in response to varying levels of larval competition and predation	45

List of Tables

Table No.	Title	Page No.
01	Protocols to prepare cue-water for control and treatment	17
02	Results from model-comparison approach to reach final model for larval competition risk	23
03	Parameter estimates of predictors of oviposition measures for larval competition risk	24
04	Results from model-comparison approach to reach final model for larval competition risk	33
05	Parameter estimates of predictors of oviposition measures for larval competition risk	34

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Introduction

Animals use an array of cues from the environment to assess the quality of resource patches. Cues that are directly derived from the environment, primarily comprising of abiotic factors, are referred to as non-social cues. In contrast, a social cue is a type of information that is acquired from the behaviour of other individuals in the environment (Miller et al., 2013). While most animals rely on the integration of social and non-social cues to assess a resource patch, it has been reported that social cues can override the effects of the environment and can be crucial in decision-making processes (Betts et al., 2008). Social information can be used by an organism to gather information about the quality of the resource, its location and ways to obtain the resources (Bonnie and Early., 2007). Individuals can also signal the stability of a resource (or the degree of stochasticity in the environment), thus relaying social information that may be used by other individuals to modulate plasticity of traits or behaviour towards the resource. In addition, using social information may also reduce an individual's cost of independently assessing their environment (Bonnie and Early., 2007). More importantly, social information obtained from individuals can be reflective of how the individual utilized the resource in the past or how it may use the resource in the future.

Social information can be derived from stimuli produced by conspecifics (Levin et al., 2009). Individuals can use this stimulus to both move towards conspecifics and make choices similar to those made by them (often referred to as conspecific cuing), or avoid choices made by conspecifics, or more broadly modify behaviour in the presence of conspecifics. This type of behavioural modification can result in important fitness consequences for an individual by affecting its breeding status, mate choice and body condition. For example, in choosing between habitats, conspecific cuing among settlers (at low population densities) can be indicative of habitat quality (Greene and Stamps., 2001). Individuals can also avoid choices made by conspecifics by moving to different resources to avoid intraspecific competition. Phytophagous and parasitic females avoid ovipositing in hosts where conspecific eggs or larvae are present, and thus avoid exposing their brood to intraspecific competition (Goulson et al., 1998). Templeton and Giraldeau conducted a series of experiments to discern if the presence of other

individuals affected individual decisions in a foraging context (Templeton and Giraldeau., 1995, 1996). Individual adult starlings were provided with two types of foraging patches containing probe sites for food. One patch contained only empty probes, while the other contained probes with the food source. Starlings were required to poke through the probe sites to check for food availability. The study found that in the presence of a foraging companion, if the companion probed more holes, the focal bird probed less and vice-versa. Thus, the decision to leave a patch was based on personal and public information. In addition, when opaque barriers were used during the trials between partners, focal birds probed more and relied primarily on personal information, thus showing how withdrawal of social information can modify behavioural choices. While there is evidence that heterospecifics can also influence breeding patch choice, indicate nest site-quality and provide information on foraging risks (Seppanen and Forsman., 2007), here I focus on the influence of conspecifics alone.

Behaviour modifications in the presence of conspecifics can have different effects on individuals, based on the type of resources to be exploited. For example, in the utilisation of immediate and perishable resources in events like foraging, focal individuals are perhaps more inclined to be concerned about their foraging success alone, than of those around them. A key difference that distinguishes these events from reproductive episodes like oviposition is that here, focal individuals are more likely to be concerned about the choice and success of surrounding conspecifics who in future may utilise the same resource for egg-laying. Thus, assessing the presence of other individuals can provide gravid females social information that would enable her to weigh her choices before ovipositing.

The life-history trait of an organism is largely dictated by maternal choices, amongst other environmental variables. These maternal choices include the quality of mate-choice, spatial and temporal distribution of her broods, investment per offspring and nest site-selection. Oviposition, the act of egg-laying, is a behavioural decision made by an adult female that can have important fitness consequences. Oviposition site selection (OSS) is a type of habitat selection where females choose a site for egg-laying. In choosing a suitable habitat for egg-laying, the female sets off a cascade of responses in both the parent and the offspring (Bernardo., 1996). Thus, studying

conditions which favour this OSS and consequences of choosing one site over another can help one gain insight into the reproductive fitness of an individual.

Several hypotheses have been suggested for patterns in OSS. The sites chosen can be a result of female adult choosing to maximize egg-survival or her own survival (Refsnider and Janzen., 2010). Further, sites can be chosen that alter offspring traits, or are close to resource-patches that increase offspring fitness. Alternatively, oviposition sites can be a consequence of mating behaviour where sites proximal to mating sites are chosen for oviposition or an inherited trait where adults return to site of hatching to lay eggs (Refsnider and Janzen., 2010).

Several terrestrial and aquatic species show a strong link between oviposition habitat selection and offspring performance. Adaptive choice for certain oviposition sites over others has been documented in birds, fishes and butterflies. Many passerine birds select nesting sites that are masked by high-vegetation cover to protect their eggs from predators (Liebezeit and George., 2002). If this predation risk cannot be mitigated by nest-concealment alone then parents use additional strategies like increased incubation period in the nest, plumage camouflage or anti-predatory behaviour. Phytophagous insects like butterflies show strong preference for specific parts of the host plant that are highly correlated with larval performance on these host patches. Along with clear preferences for a host, there exists a large intraspecific variation in oviposition choices within adults from the same population (Thompson., 1988).

Among the several biotic and abiotic factors that influence oviposition site-selection, larval predation and competition risks are two of the key drivers that affect this maternal behaviour. The risk of predation can be influenced by predator presence, detection of prey, the probability and success of attack, and conspecific and heterospecific density. Similarly, competition risk is a function of conspecific and heterospecific density, spatial and temporal availability of resources, hydro period length etc. Thus, predation and competition risks can elicit a wide range of oviposition responses (Carter et al., 2008).

Organisms like anurans, water-striders and mosquitoes that are semi-aquatic with respect to reproduction have also been reported to exhibit strong OSS. Semi-aquatic organisms exhibit a biphasic cycle, where they remain aquatic as juveniles and

terrestrial as adults. This constructs a two level (aquatic and terrestrial) interaction, each of which may have independent dynamics. A female has to make fine choices before oviposition, as following this act no parental care can be extended to the offspring. There exists a vast literature documenting the effects of predation and competition on these semi-aquatics. For instance, several anuran species avoid laying eggs in sites with high predation risk and high inter- and intra-specific competition (Resetarits and Wilbur., 1989). In water-striders, OSS can be a direct function of adult mortality due to predation risk and females may want to maximize future reproductive events by choosing pools that are sub-optimal for juveniles (Hirayama and Kasuya., 2013). High predation and competition risks also affect oviposition choices in mosquitoes (Munga 2006). In a multiple choice experiment, where adult females (*Anopheles gambiae*) were presented with ovi-cups of varying larval competition risk (varying larval densities), low competition treatment was highly favoured. In addition, in a binary choice experiment between control and low competition treatment, females showed no preference between either pool. These results indicate that females avoid exposing her offspring to the high intra-specific competition posed by pools with high larval densities. Furthermore, recent studies indicate that certain mosquito species respond to absolute levels of predator cues (Silberbush and Blaustein., 2011). When a series of binary choices were presented in the wild, adult females always laid a particular number of eggs in a treatment, irrespective of the alternate option in the set-up. New findings also indicate that predation can result in increased bacterial content in water that may pose as an oviposition attractant (Albeny-Simões et al., 2014). The emphasis on understanding oviposition decisions in context is because of the direct consequences of predation and competition on larval performance. In *A. gambiae*, intraspecific competition increased developmental time and reduced adult body size that could lower adult survivorship and fecundity (Gimnig et al., 2002). Similarly, the presence of its native predator decreased the survivorship and growth of anuran larvae (Blaustein and Kotler., 1993)

Given that oviposition decisions have important fitness consequences, what do we know about influence of conspecifics on egg-laying behaviour? The empirical evidence from several non-social insects like moths, maggots, mites and fruit-flies suggests that eggs laid per female is greater when individuals are in groups than when they are

solitary, highlighting the role of social facilitation in these events (Prokopy and Bush., 1973; Chess et al., 1990; Abernathy et al., 1994, Le Goff et al., 2010). In addition, conspecific cueing at oviposition sites may not only increase the propensity to oviposit at the site but may also result in earlier egg-laying. The benefits of temporal social facilitation may include effective resource exploitation (Prokopy and Duan., 1998). Literature also indicates that adult conspecifics can compete when in groups leading to differences in egg-laying than when they are solitary. In competitive environments, adult female parasitoids laid fewer eggs on hosts and instead allocated greater resources (time and energy) in female-female encounters, increased guarding of hosts against competitors etc. (Irvin and Hoddle., 2005; Goubault et al., 2007). Competition also influenced host choices of adult parasitoids, where individuals in groups favoured parasitized and less profitable hosts than solitary adult (Visser., 1994). However, these studies exhibit several drawbacks or limitation in study-design and fail to incorporate other findings in literature that may affect their inferences. For instance, the idea in several of these studies is to compare fecundity of solitary individuals and individuals held in varying group sizes. Thus, while the overall fecundity of groups is known, fecundity of individuals within a group is not tracked. Literature also indicates that individuals are known to exhibit high intra- and inter-individual variation in egg-laying as a result of distributing their eggs across space and time (Clements., 1992; Gibbs 2005). Hence, concluding that individual fecundity in groups is greater (or lesser) than that of solitary females may be incorrect. Moreover, when females are grouped, it is quite possible that the first female to lay eggs deposits the largest proportion of eggs and the last female the least. In this case, although the mean fitness per individual in groups may be higher than that of solitary females, there may be a large variation in fitness per individual in a group than when present alone (Godfray., 1994). For non-social insects, where inclusive fitness benefits due to genetic relatedness are low, this large variation in egg-laying can be costly. Literature survey also indicates that some studies have tried to modify the above design to determine influence of competition or facilitation on behaviours like foraging and oviposition. The study-designs here involve exposing focal adults to conspecifics prior to tracking individual performance in the foraging or oviposition patch versus when present singly (Visser., 1995; Goubault et al., 2007). However, a major-drawback of this study-design is that one cannot assess if change in

behaviour is due to influence of conspecifics competition (or facilitation), or as a result of release from conspecific competition (or facilitation).

In this study, I investigated the role of social cues on oviposition decisions in individual adult females using *Aedes aegypti* as a model system. The mosquito, *A. aegypti*, is a common species which oviposits in small temporary pools of tropical regions. The adults of these species may be present in different densities at these local pools and gravid females have the opportunity to use social cues provided by local individuals to assist in oviposition decisions. Adult females are exposed to a mosaic of pools of varying risks, amid which they must choose one or more to oviposit in. Amongst these risks, larval predation and competition threats have been most widely reported to influence growth and survival of their progeny in several related mosquito species (Gimnig et al., 2002). Females can chemically detect both conspecific larval and predatory cues associated with these risks to aid in oviposition decisions (Bentley and Day., 1989).

I examined the influence of social cues on an individual female's oviposition response to pools varying in risk. I examined two major types of risk, namely larval competition and larval predation. In a series of binary choice experiments, adult gravid mosquitoes, housed either individually (solitary) or in the presence of adult females (social), were presented with oviposition options varying in either larval predation or competition risks. Females were presented with two pools, a control pool and a treatment pool with a particular level of risk (either predation or competition). I assumed that females viewed the conspecific females present in their environment as potential ovipositors in the available pools, and I made broad predictions about how an individual female's pattern of oviposition should vary between the solitary and social conditions. When presented with a control pool and a pool with larval competition risk, I expected social females to display a lower propensity to oviposit and to withhold more eggs when compared with solitary females. This is because a social female would expect other conspecifics to oviposit in the same environment and whose clutch would be in direct competition with her offsprings. Following the same reasoning, social females were also predicted to distribute their eggs across pools and lay fewer eggs in control pools than solitary females who were expected to be choosier and favour control pools. In contrast, when

presented with a control pool and a pool with larval predation risk, I expected social females to exhibit greater propensity to oviposit and increased egg-laying than solitary females. I also expected females to oviposit greater proportion of eggs in predator pools when present in a social-setting. Laying large number of eggs can result in faster predator satiation, or dilution effect, especially since in the presence of other adult females this dilution effect is likely to be contributed by additional unrelated larvae. Furthermore, since an individual female's larvae are expected to face higher larval competition in social than in solitary settings, predation could potentially rescue larvae from intense competition, ultimately increasing the reproductive value of surviving larvae. For both types of risk, I predicted that in anticipation of competition from conspecific females, social females can decide to oviposit early on. I expect this because *A. aegypti* females are known to lay eggs just above the water-line and eggs closer to this line have greater hatching probability with increase in pool-volumes. Thus, ovipositing earlier than conspecific females can provide social females the opportunity effectively use resources by allowing them to oviposit closer to the water-line and ensure higher hatching success of their broods

Methodology

Overview

To test if social status influences oviposition responses to habitats differing in larval predation and competition risks, two types of assay environments were created: social and solitary. In the solitary assay condition, a single blood-fed female was present and her oviposition behaviour was measured. In the social assay condition, a single blood-fed female was caged with 3 other non-blood fed females. Since non blood-fed females typically do not invest in egg-laying behaviour, one could safely track the oviposition behaviour of an individual female in a social setting and compare her behaviour to that of an individual female in the solitary condition.

Females in each of these assay environments were provided with binary choices that consisted of a control (risk-free patch) pool and a treatment pool. The treatment pool contained either larval predation chemical cues of different concentration, representing varying degrees of larval predation risk, or larval conspecific chemical cues of different concentration, representing differing degrees of larval competition risk. The egg-laying behaviour of an individual female in a replicate was tracked over a period of three days. An individual female was used only once in the study.

Study System

In this study, I used a lab-bred colony of *A. aegypti* mosquitoes. Mosquitoes caught from the wild were reared under laboratory conditions and colony sizes of around 700 – 1000 mosquitoes per cage have been maintained over the last three years. Its relatively short life-span (~ 30 days on average) and even shorter breeding period rendered it easy to evaluate reproductive success and female behaviour. Other features include short aquatic life-cycle, ability to distribute eggs spatially and temporally, super-oviposition (ovipositing on same surface where other conspecific females have deposited eggs) behaviour and easy rearing in laboratory setting. In addition, *Bradinopyga* nymphs, a common predator of *A. aegypti* larvae were also easily accessible from tanks maintained in the nurseries on IISc campus. These dragon fly nymphs, whose aquatic phase lasts for about 30 days before they metamorphose as

terrestrial adults, have been documented to inhabit and oviposit in the same set of natural pools as do several species of mosquitoes. These predators are generally benthic or found on walls of the pools and found to heavily predate upon larvae of mosquitoes (Clements., 1992).

Experimental Design

All experiments were carried out in a laboratory setting using colony mosquitoes between May 2015 – February 2016. The following study-design was used in order to carry out the experiments.

A] Cue water preparation

A. aegypti females are known to detect chemical cues in water prior to ovipositing in pools of their choice (Clements., 1992). Females are noted to skim the water surface to detect these cues while the chemical receptors at the back of their leg primarily aid in this process. Studies conducting similar choice experiments with study species that use chemical cues to obtain information on ecological conditions have used water containing chemical cues of the ecological condition of interest (e.g. To test predator avoidance of tadpoles, study group used cues from caged tadpole-fed predators) in place of the actual ecological condition (e.g., tadpole fed-predators) (Angelon and Petranka., 2002; Mogali et al 2012., Afify and Galizia., 2015;). Results from formal testing of differences in oviposition when presented with pools with live predators and pools containing predatory-cues alone (i.e. water derived from pools with live predators) in several other species of mosquitoes and anurans indicate no differences in egg-laying (Mogali et al., 2012; see references in Ferrari et al., 2008). In this study, I used cue-water from tubs containing predators or competitor larvae or maintained as controls to test female oviposition responses. Females were provided with ovi-cups of 11cm x 4 cm. Since it is difficult to provide a range in whole organism density in such a small container, I used a previously standardized protocol to scale as per requirement. In addition, using cue-water was logistically feasible and minimized any random effects of anomalous behaviour of a predator or a batch of conspecific larvae during a trial.

In this experiment, I used two levels of larval predation and competition treatments along with control pools in the binary choices. The predator treatments consisted of groups of 2 or 4 predators and the competition treatments of either 20 larvae or 155 larvae, housed together in the cue-water tubs (Table 1). In total, I used 60 different predators whose body-size ranged from 0.9-1.2 cm for this study. The control cue-water did not contain either predators or larvae but was otherwise maintained in a similar way to the treatment cue-water. Fish food was used to maintain baseline nutrient content across all control and treatments. Cue-water was prepared fresh for each set of trials.

Table 1. Protocols used to prepare cue-water for the control and treatment conditions.

Day	Control	Competition	Predation
-1		Soak 1 ovi-strip in 1.5 L of water with 0.5 g of larval food in a separate tray	
0	Soak 0.2 g of fish food contained in porous bags in a tub with 1.5 L of water	Soak 0.2 g of fish food contained in porous bags in a tub with 1.5 L of water	Soak 0.2 g of fish food contained in porous bags in a tub with 1.5 L of water and place predators (either 2 or 4) in the tub
1	Leave the set-up undisturbed		
2	Remove food-bags from the tub	Remove food-bags from the tub and egg-strips from the tray. Transfer the required number of 1 st instar (either 20 or 155) larvae to tub	Remove food-bags from the tub

3, 4, 5	No further additions or changes are made. Water lost due to evaporation or when drawn for the experiment is replaced	Water lost due to evaporation or when drawn for the experiment is replaced. Larvae that pupate during the course of the experiment are replaced by larvae from the ancestral tray	No further additions or changes are made. Water lost due to evaporation or when drawn for the experiment is replaced. Predators are removed from the tub, fed 3-5 larvae every other day and placed back in the tub
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B] Maintaining Master Cage

Ovistrips containing about ~200 to 300 eggs were soaked in 1.5 liters of water with 0.5g of larval food. Ovistrips were removed after 72 hours to ensure that all adults were of nearly the same age-class. Two such trays were maintained for a period of 7-10 days until most larvae reached their pupal stage. 200 pupae from each tray were assigned to two separate master cages where adults emerged. Cages were supplied with ad libitum food source, moist cotton smeared with honey, which was replenished regularly. Cages were left otherwise undisturbed to ensure high-mating frequency in females. Subsequently, one of the cages was randomly assigned to be blood-fed for duration of six hours and the other remained undisturbed. To control for any effects due to aging, only one-week old and one-time blood fed adult-females were used in all the trials.

C] Experimental Setting

Two assay environments were created – solitary and social. Individual adult females with blood-fed distend abdomen were isolated using aspirators and placed in individual cages (0.3 m x 0.3m x 0.3 m) that were randomly pre-assigned to either solitary or social status. In the social cages, three females, which were aspirated from the master cage that was not subjected to blood-feeding, were placed along with the gravid female.

Each of the assay cages was supplied with ad libitum honey. On the third day from the introduction of the females into the assay cages, each cage was provided with two ovi-cups (4 cm x 11 cm). 100 ml of cue-water, control and treatment, which were drawn from the larger tubs, were transferred to the ovi-cups. The cups, each lined with ovistrips, were always placed at diagonally opposite ends of the cage and randomly assigned to one of the four possible combinations of positions. The same position was maintained within a cage throughout the trial. The ovistrips and cue-water were replenished on a daily-basis for a period of three days. Finally, the individuals from the trial were aspirated and stored in Eppendorf tubes. Master-cages were donated to the colony and the cue-water tubs were disassembled at the end of a block of trials.

Trials were carried out in blocks. Each block lasted for seven days and included preparation of cue-water for control and treatment conditions, maintenance of master cages and the running of 2 to 4 trials of different control-treatment binary choice experiments. The different control-treatment choice experiments used in this study were interspersed in time to avoid any confounding effects of time on oviposition responses. All experiments conducted were in accordance to the Animal Ethics Committee of IISc.

Analysis

The primary measure of oviposition behaviour was the number of eggs laid by an individual female. After every 24 hours, over three days, eggs from control and treatment pools were manually counted and recorded.

To understand how oviposition decisions were influenced by social status, I estimated 4 key response variables for each trial (a solitary female, or an individual female in a social setting with three other females): the propensity to oviposit, oviposition activity index (OAI), total fecundity and eggs deposited in control pools.

1. Propensity to oviposit: Here I recorded whether or not a female laid eggs during the trial. Females, after possibly assessing both pool type (control and treatment) and social status, can choose to either oviposit or entirely avoid the set of pools. Thus, propensity to oviposit was used as a measure of degree of choosiness at the larger spatial scale of the set of pools.

2. Total fecundity: The total number of eggs laid by a female during a trial, calculated as the sum of eggs deposited in control and treatment pools. Total fecundity can also be a measure of choosiness at the level of pool-network, if females can successfully manipulate total number of eggs laid during the trial after assessment of the two pools and social status.

3. Oviposition Activity Index (OAI)

$$\text{OAI} = \frac{\text{Treatment} - \text{Control}}{\text{Treatment} + \text{Control}}$$

This index is a measure of the degree of choosiness shown by the female at a smaller spatial scale, between the control and treatment pools. In this index, ranging from -1 to 1, 1 indicates maximum preference for treatment (all eggs deposited in treatment pool alone), -1 maximum preference for control and 0 no preference (equal distribution of eggs across both pools).

4. Eggs in control pool: The total number of eggs laid by a female over the duration of the trial in control pool alone. Control pool in the experimental set-up is an absolute risk free patch which is present across all treatment types and status. Measuring the eggs laid in these pools can provide information about the nature of pool evaluation i.e. if egg deposition in control pools is independent of adjacent pools.

To test the influence of social status on the oviposition responses of females to pools varying in risk, I ran separate linear mixed-effects models assuming normal errors with each of three response measures (OAI, total fecundity, eggs laid in control pool) as the response variable and with risk level (low/high) as the fixed effect, and block (week of trial), position of cups, and cage identity as random effects. For each response variable, separate linear mixed-effect models were run for predation and competition risk.

In addition, I ran separate generalized linear mixed models assuming binomial distributions with propensity to oviposit as the response variable and with risk level (low/high) as the fixed effect, and block (week of trial), position of cups, and cage identity as random effects for each type of risk (competition/predation).

To examine if being in a social setting promoted earlier egg-laying, I tracked the changes in egg-laying behaviour of a female over time. For this, the data obtained from each individual was divided into an "early" (egg-laying on Day 1 of the trial) and a "late" (Days 2 and 3) time period. For each trial, I calculated propensity to oviposit, total fecundity, oviposition activity index and eggs laid in control pools for early time periods.

For each analysis, cautious model reduction was performed. Only non-significant (p -value > 0.05) interaction terms were removed from the maximum model to aid in better understanding of relationships. Thus, inferences were based on final model inclusive of all main effects (significant or not), random effects, and interaction terms that were statistically significant. Additionally, to support my inferences about the strength of a relationship, effect sizes and bootstrapped confidence intervals were calculated. In comparing two means from the data, if confidence intervals of the two means do not overlap with the means of the other, then the means were considered to be statistically significant.

The R version 3.2.2 (R Core Team, 2015) was used for all analyses and lme4 package (Bates, Maechler, Bolker, and Walker., 2015) to run mixed-effects models.

Results

Previous work in the lab looked at larval growth and performance when larvae of different conspecifics densities were reared together (competition) and when larvae were preyed upon by varying densities of predators (predation) (Sharma et al., unpublished). Based on the results from this study, I chose larval and predator densities that would represent low and high risk of competition and predation respectively for offspring of ovipositing mosquitoes. The chosen low and high levels of competition and predation were 20 and 155 larvae, and 2 and 4 predators respectively. I hereby refer to the set of control and treatment pools together as pool-network (e.g. a set of pools with control and high predator pool would be referred to as high predator pool-network). In the following sections I describe the results for influence of social status on oviposition responses to larval competition and predation risk

Competition Risk

Social status did not influence the propensity to oviposit or total fecundity across the two levels of larval competition (Table 2, Table 3). Individuals were likely to oviposit 70% of the time when presented with a pool-network varying in competition risk, depositing a total of 41.8 ± 3.5 eggs in the two pools (Table 3, Figure 1, Figure 2). No differences in either measure were observed across competition levels within each status. Results from the mixed-effects model showed no clear differences in OAI in females across status and level of competition risk (Table 2, Table 3, Figure 3). Examination of the relative frequency of OAI in low competition pool-network, showed that while some females favour pools with low concentration of conspecific cues, most females from both statuses have individuals displaying a large variation in OAI (ranging from -1 to 1) (Figure 4). In contrast, individuals in social-setting displayed a strong preference to pools with high concentration of conspecific cue (60% of females with $OAI > 0.8$) than their counterparts (40% with $OAI > 0.8$) in high competition pool-network (Figure 4). In both levels of competition and status, associated with each of these peaks was a wide variation in total fecundity per female (Figure 5). While no differences in OAI were observed across levels of competition in solitary females, social females displayed a 4.5 times greater preference to high than low concentration of conspecific cues across high and low competition pool-network (Figure 3, Figure 4). Overall model results indicated that being in a social-setting did not influence the number of eggs laid in control pools (Table 2, Table 3). However, examination of means and confidence intervals indicated that, notwithstanding the substantial variation, females in social setting deposit nearly 50% fewer eggs than solitary females in control pools (Figure 6). In addition, while solitary females showed no differences in egg-laying in control pools across competition levels, social females deposited 50% fewer eggs in high than low competition pools. Comparison of means and confidence intervals showed that social females did not lay eggs earlier than did solitary females (Figure 7). Moreover, since propensity to begin egg-laying at an early time point was extremely low (< 0.25), the samples sizes were too sparse to carry out any further analysis.

Table 2: Statistical significance of variables included in the analyses of propensity to oviposit, total mean fecundity, oviposition activity index (OAI and mean number of eggs in control pool - in social versus solitary females (status) across levels of competition (type). Likelihood ratio tests (χ^2) are used to test the significance of fixed effects. Model reduction to obtain the minimal model was performed only if interaction between explanatory variables were non-significant. For variables included in the minimal model, likelihood ratio test statistic from comparison of final model with a model excluding the specified variable is reported.

Term	Propensity to Oviposit		Total Fecundity		Oviposition Activity Index		No. of eggs in control pool		df
	χ^2	<i>p</i> -value	χ^2	<i>p</i> -value	χ^2	<i>p</i> -value	χ^2	<i>p</i> -value	
Type	0.0528	0.8183	0.00143	0.96981	3.0754	0.2149	3.6468	0.1615	2
Status	0.2805	0.5964	3.0908	0.0787	0.3811	0.5370	1.6093	0.2046	1
Type:Status	0.9420	0.3318	0.41530	0.51928	1.8420	0.1747	1.0152	0.3137	1

Table 3: Parameter estimates for fixed effects from mixed effects models fitted to (a) propensity to oviposit, (b) total fecundity, (c) oviposition activity index (OAI) and (d) number of eggs in control pool as separate response variables and with status (solitary/social) and competition level (low/high) as the explanatory variables. Estimates are shown from final models, which included all main effects and statistically significant interactions alone.

Term	Propensity to Oviposit		Total Fecundity		Oviposition Activity Index		No. of Eggs in Control Pool	
	Estimate	Standard Error	Estimate	Standard Error	Estimate	Standard Error	Estimate	Standard Error
Intercept (Type: Low; Status: Solitary)	0.94109	0.36667	3.740290	0.0844	0.14593	0.12414	17.875	2.586
Type : High	0.09975	0.43250	-0.00391	0.1031	0.26295	0.15115	-5.531	2.927
Status : Social	0.24416	0.45634	-0.18341	0.1030	0.09181	0.15101	-3.831	3.075

Figure1: A comparison of propensity to oviposit by social and solitary individuals in response to varying levels of larval competition (Control vs Low conspecific density: Solitary (n=40), Control vs Low conspecific density: Social (n=27); Control vs High conspecific density: Solitary (n=26), Control vs High conspecific density: Social (n=34)). Error bars indicate 95% bootstrapped confidence intervals.

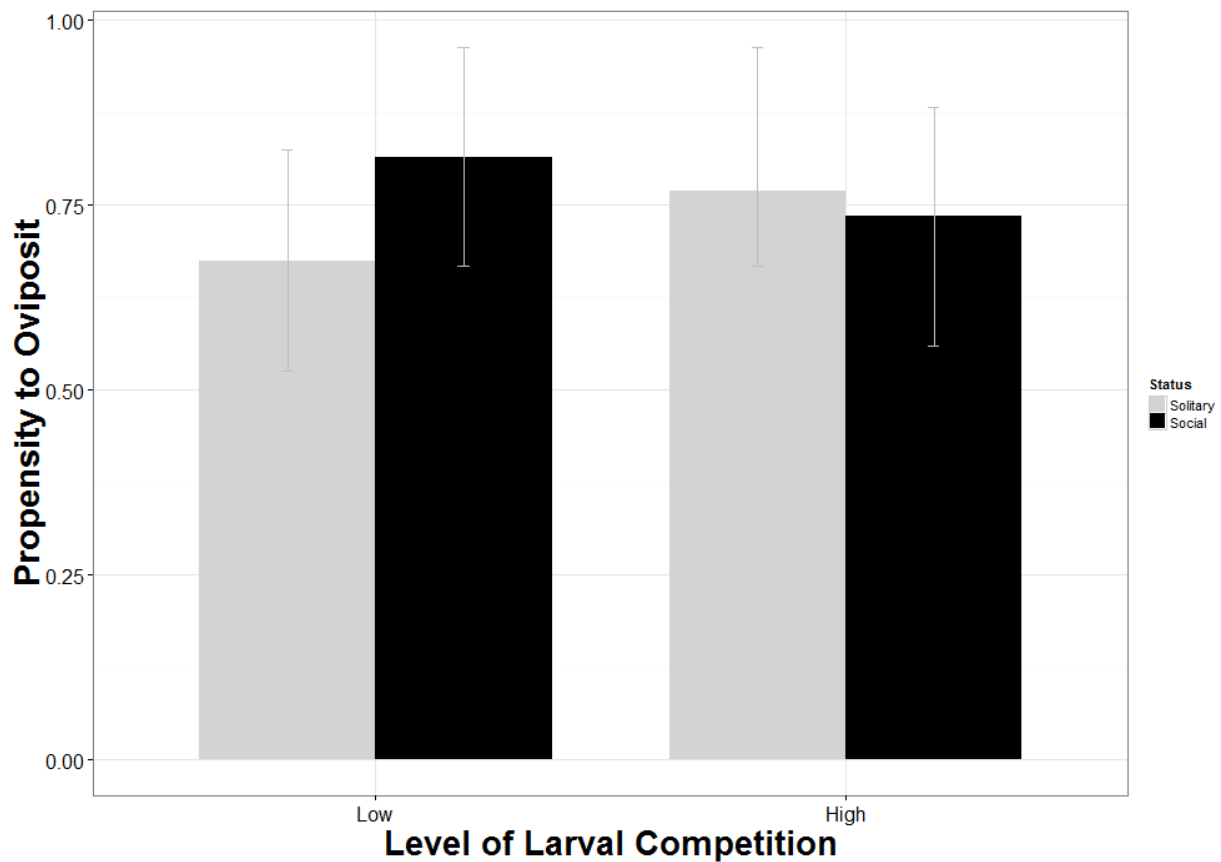


Figure 2: A comparison of total fecundity of social and solitary individuals in response to varying levels of larval competition (Control vs Low conspecific density: Solitary (n=27), Control vs Low conspecific density: Social (n=22); Control vs High conspecific density: Solitary (n = 20), Control vs High conspecific density: Social (n=25)). Error bars indicate 95% bootstrapped confidence intervals.

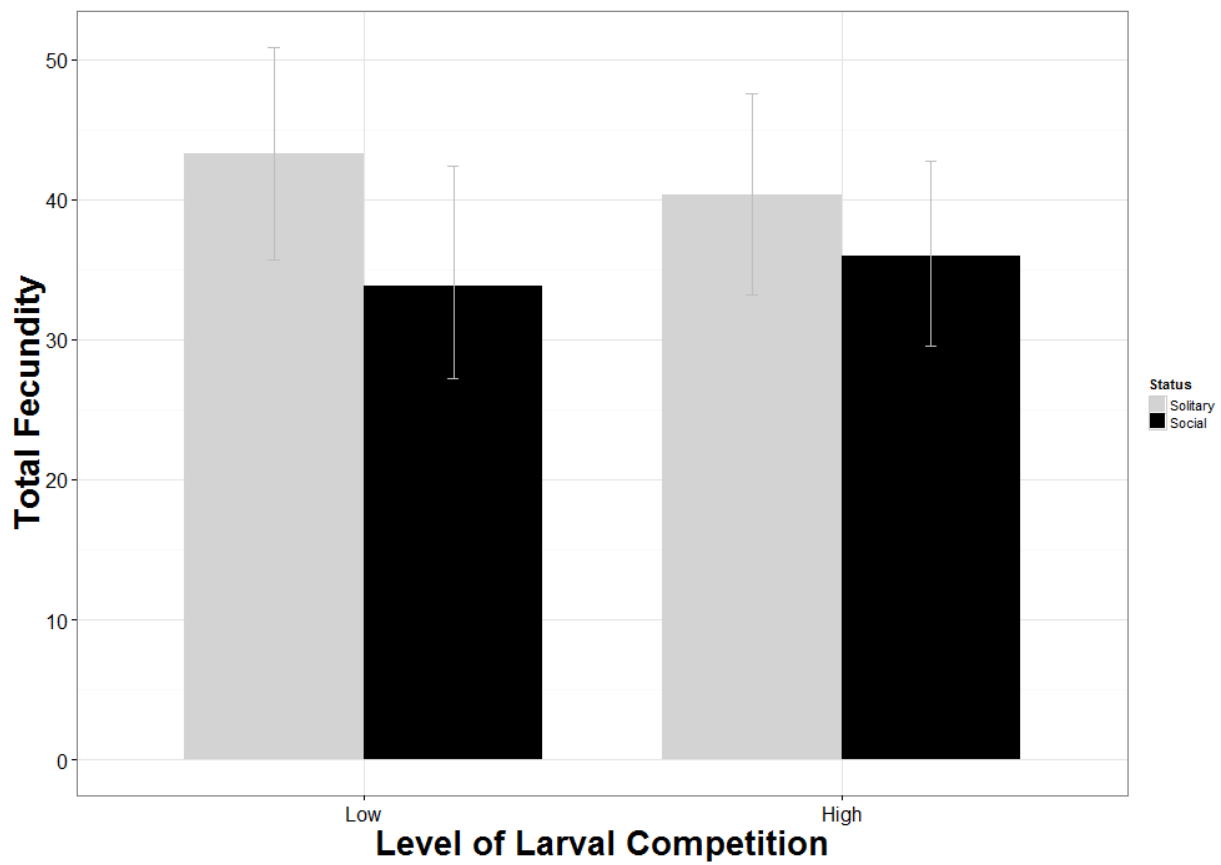


Figure 3: A comparison of oviposition activity index (OAI) of solitary and social individuals in response to varying levels of larval competition (Control vs Low conspecific density: Solitary (n=27), Control vs Low conspecific density: Social (n=22); Control vs High conspecific density: Solitary (n=20), Control vs High conspecific density: Social (n=25). OAI value of -1 indicates maximum preference to control pool, +1 as maximum preference to treatment pool, and 0 as equal preference to both pools. Error bars indicate 95% bootstrapped confidence intervals.

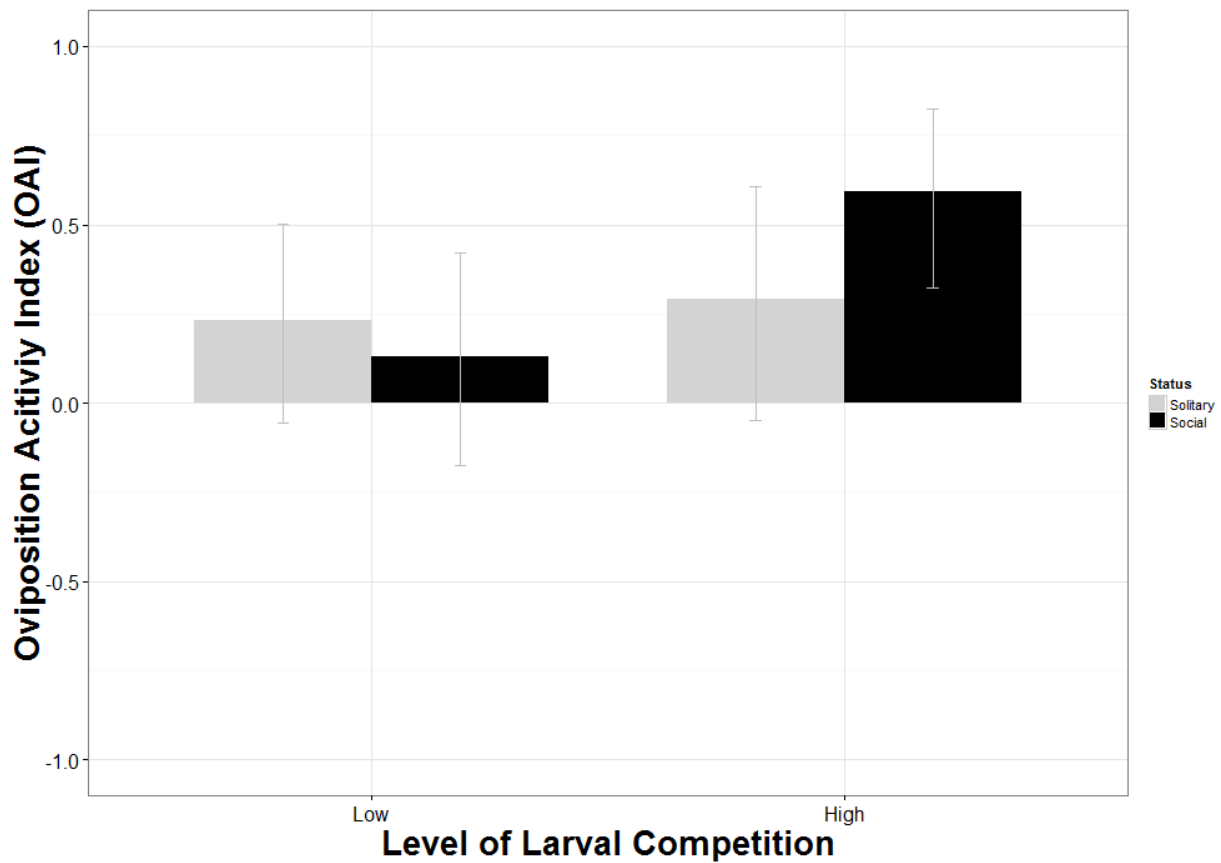


Figure 4: Frequency distribution of OAI in solitary and social individuals in response to varying levels of larval competition (Control vs Low conspecific density: Solitary (n=27), Control vs Low conspecific density: Social (n=22); Control vs High conspecific density: Solitary (n = 20), Control vs High conspecific density: Social (n=25)). OAI value of -1 indicates maximum preference to control pool, +1 as maximum preference to treatment pool, and 0 as equal preference to both pools.

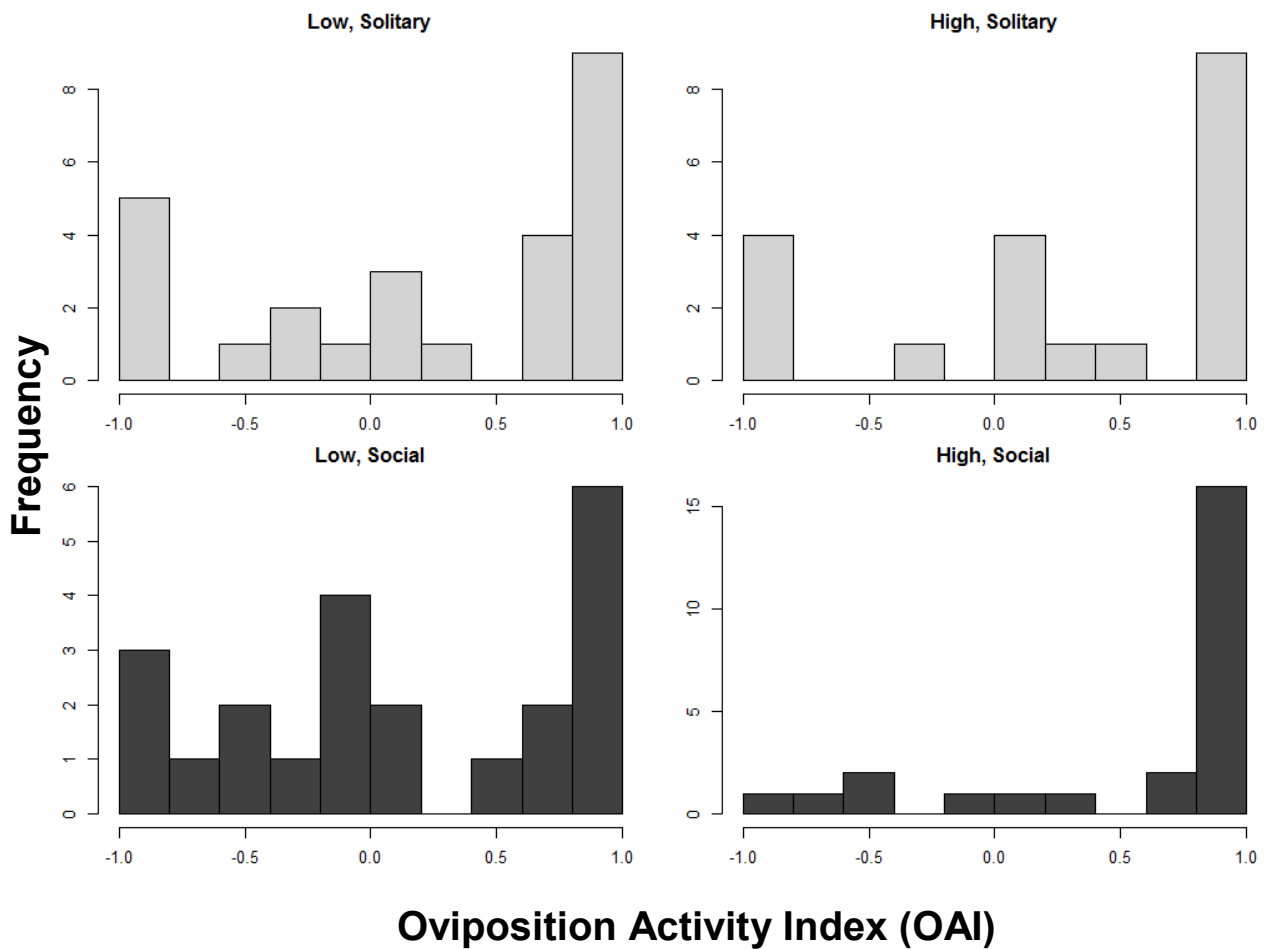


Figure 5: Scatter plots showing relationship between OAI (x-axis) and total fecundity of a female (y-axis) for solitary and social individuals in response to varying levels of larval competition (Control vs Low conspecific density: Solitary (n=27), Control vs Low conspecific density: Social (n=22); Control vs High conspecific density: Solitary (n=20), Control vs High conspecific density: Social (n=25)).

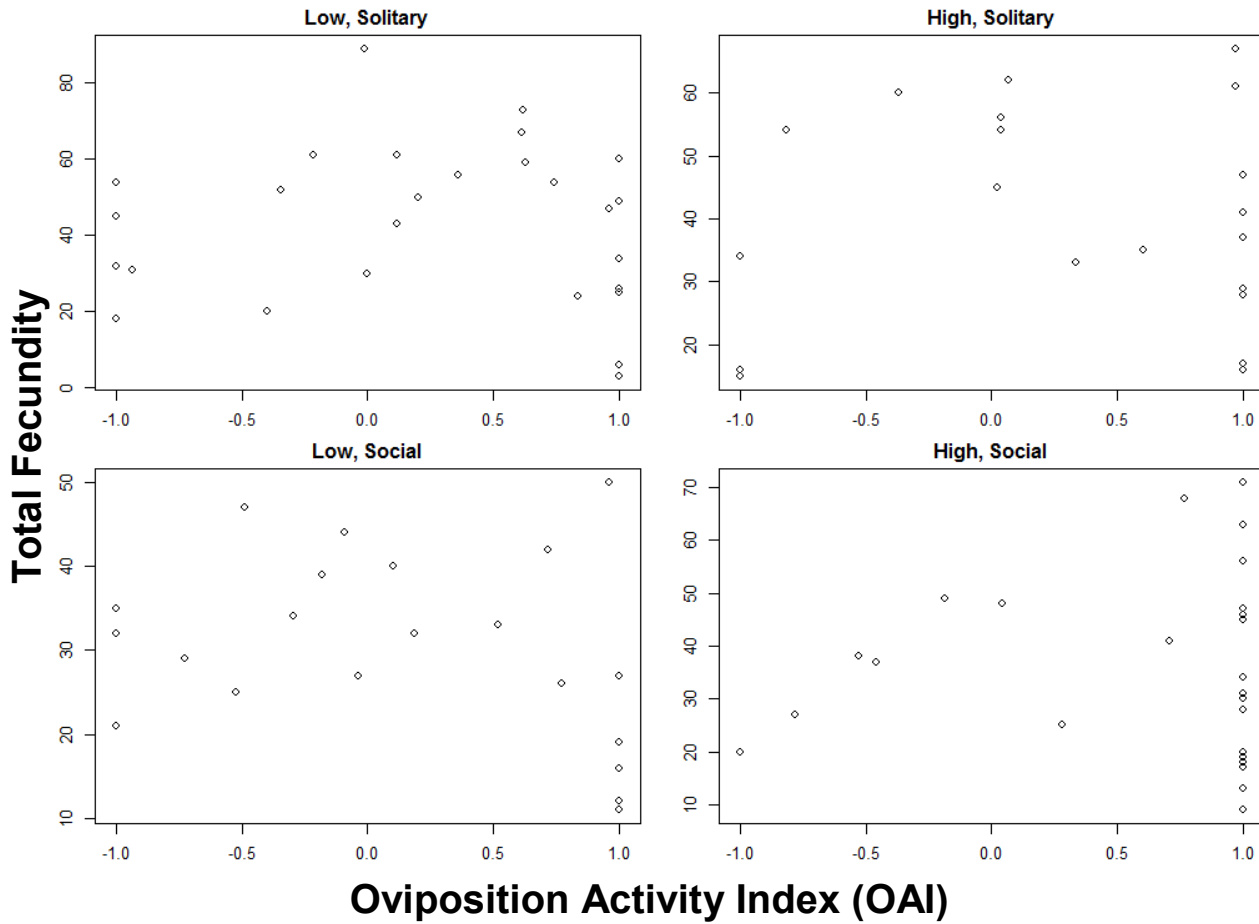


Figure 6: A comparison of number of eggs laid in control pools (alone) by social and solitary individuals in response to varying levels of larval competition (Control vs Low conspecific density: Solitary (n=27), Control vs Low conspecific density: Social (n=22); Control vs High conspecific density: Solitary (n=20), Control vs High conspecific density: Social (n=25)). Error bars indicate 95% bootstrapped confidence interval.

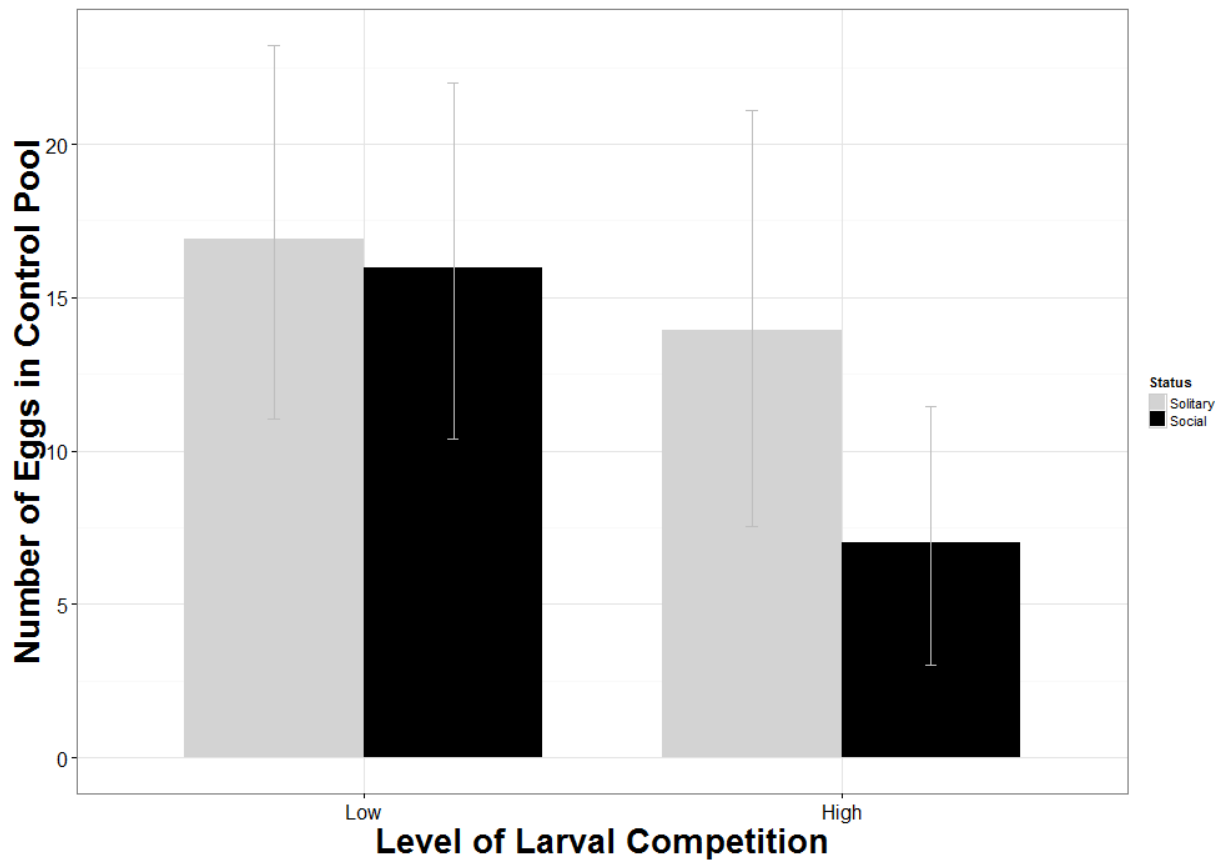
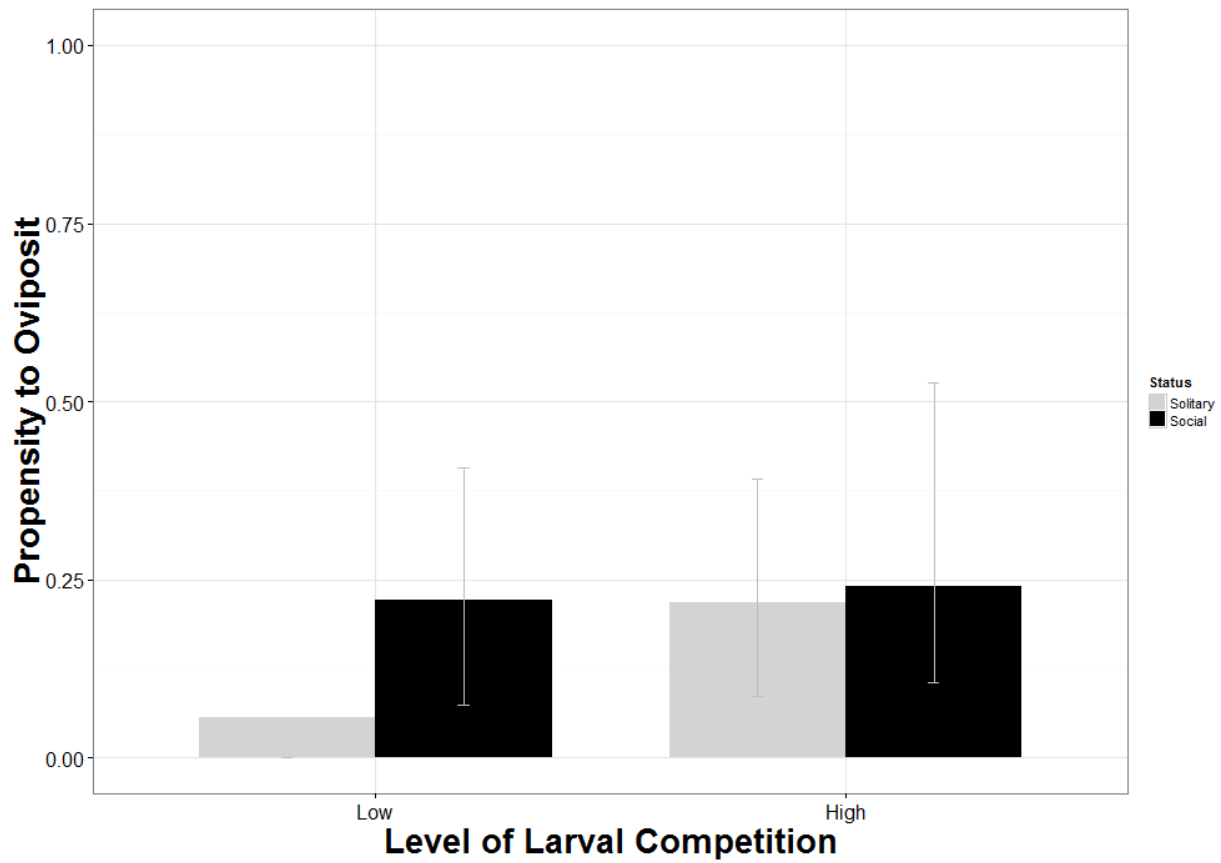


Figure 7: A comparison of propensity to oviposit by social and solitary individuals in response to varying levels of larval competition for the early time period (Control vs Low conspecific density: Solitary (n=35), Control vs Low conspecific density: Social (n=27); Control vs High conspecific density: Solitary (n=23), Control vs High conspecific density: Social (n=29)). Error bars indicate 95% bootstrapped confidence interval.



Predation Risk

Social status and the level of risk of larval predation influenced propensity to lay eggs in females (Table 4, Table 5). When compared with solitary females, while there was a 50% decline in propensity to oviposit in social females at low predator pool-networks, there was a 45% incline in propensity to oviposit among social females when faced with high predation pool-network (Table 5, Figure 8). In addition, when comparing low versus high predation pool-network, while there was a decline in propensity to oviposit in solitary females, social females showed an incline in propensity (Figure 8). Social females also displayed 1.5 times greater total mean fecundity (38.3 eggs) than their solitary counterparts when presented with high predation pool-network, but showed no differences in low predation pool-network (Table 4, Figure 9). Examining solitary status females, individuals deposited a total of 50% fewer eggs in high than in low predation pool-network levels, but no such differences were detected in social females. Results from OAI indicate no differences between social and solitary females for either predation level (Table 4, Table 5, and Figure 10). Examination of relative frequency distributions of OAI showed that while most females in both statuses chose pools with low concentration of predator cues over control pool, many others displayed a large variation in OAI (Figure 11). In contrast, when faced with high predation pool-network, while solitary females showed a bimodal distribution - only choosing to deposit all their eggs in control or treatment pools, social females presented a trimodal distribution where females chose either only control, treatment or both with equal probability (Figure 11). Relationship between OAI and total fecundity of individual females showed a large range in total fecundity associated with OAI across status and levels of predation (Figure 12). Model results also indicated that neither status nor level of predation risk affected the number of eggs deposited in control pools (Table 4, Table 5 and Figure 13). No differences in OAI or relationship between OAI and total fecundity were observed across predation levels within each status. No earlier egg-laying was reported in social females when compared with solitary females (Figure 14). Moreover, since propensity to begin egg-laying early was extremely low (< 0.25), the samples sizes were too sparse to carry out any further analysis.

Table 4: Statistical significance of variables included in the analyses of propensity to oviposit, total mean fecundity, oviposition activity index (OAI and mean number of eggs in control pool - in social versus solitary females (status) across levels of predation (type). Likelihood ratio tests (χ^2) are used to test the significance of fixed effects. Model reduction to obtain the minimal model was performed only if interaction between explanatory variables were non-significant. For variables included in the minimal model, likelihood ratio test statistic from comparison of final model with a model excluding the specified variable is reported.

Term	Propensity to Oviposit		Total Fecundity		Oviposition Activity Index		No. of eggs in control pool		df
	χ^2	<i>p</i> -value	χ^2	<i>p</i> -value	χ^2	<i>p</i> -value	χ^2	<i>p</i> -value	
Type	-	-	0.80973	0.36819	1.7149	0.4242	1.1857	0.5528	2
Status	-	-	0.62030	0.43093	0.0715	0.7892	1.1869	0.276	1
Type:Status	5.4477	0.01959	2.02861	0.15436	0.1440	1.0000	0.3532	0.5523	1

Table 5: Parameter estimates for fixed effects from mixed effects models fitted to (a) propensity to oviposit, (b) total fecundity, (c) oviposition activity index (OAI) and (d) number of eggs in control pool as separate response variables and with status (solitary/social) and predation level (low/high) as the explanatory variables. Estimates are shown from final models, which included all main effects and statistically significant interactions alone.

Term	Propensity to Oviposit		Total Fecundity		Oviposition Activity Index		No. of eggs in control pool	
	Estimate	Standard Error	Estimate	Standard Error	Estimate	Standard Error	Estimate	Standard Error
Intercept (Type: Low; Status:	1.7918	0.6236	3.5656	0.1418	0.27891	0.19129	2.3173	0.3491
Type : High	-1.5911	0.7687	0.1684	0.1808	-0.29353	0.23167	0.2755	0.4431
Status : Social	-1.2528	0.7843	0.1468	0.1805	-0.05293	0.23269	0.4318	0.4426
High x Social	2.4384	1.0628	-	-	-	-	-	-

Figure 8: A comparison of propensity to oviposit by social and solitary individuals in response to varying levels of larval predation (Control vs Low predator density: Solitary (n=21), Control vs Low predator density: Social (n=19); Control vs High predator density: Solitary (n=20), Control vs High predator density: Social (n=20)). Error bars indicate 95% bootstrapped confidence intervals.

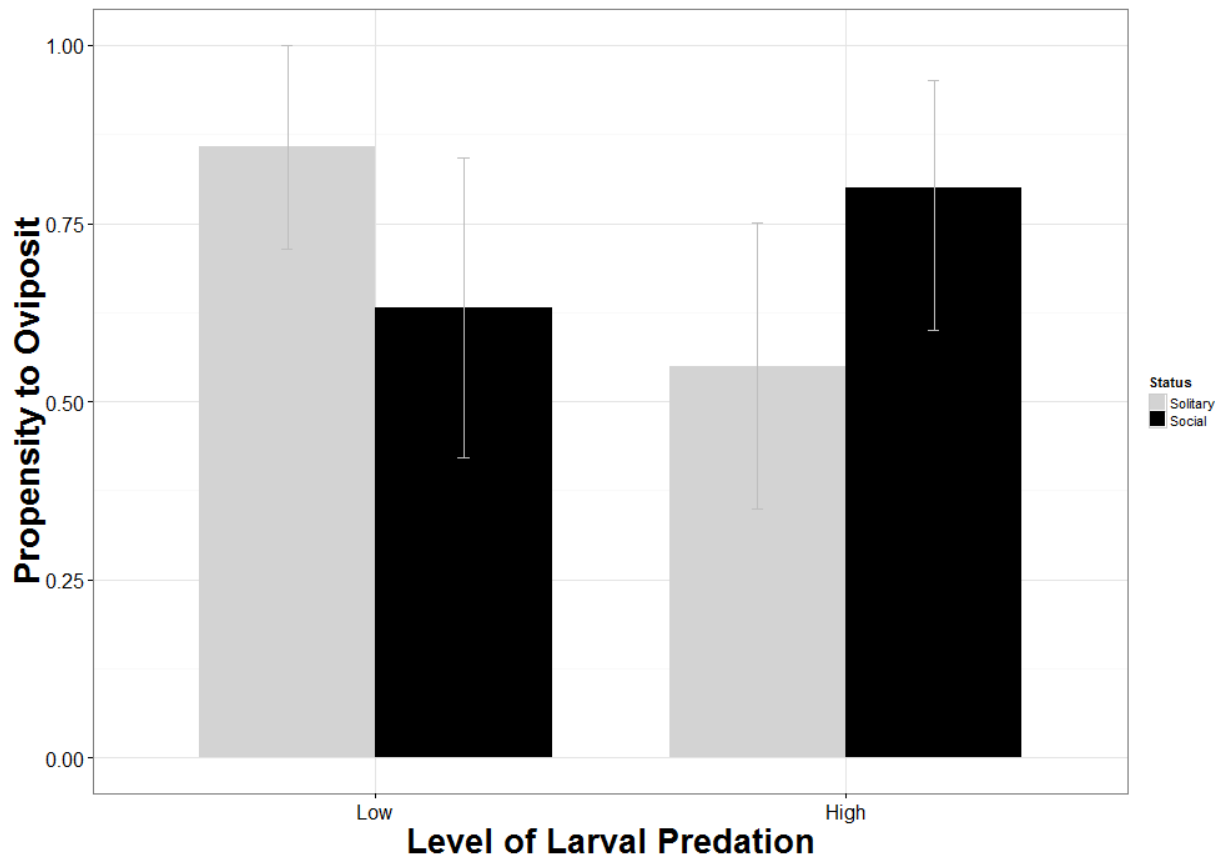


Figure 9: A comparison of total fecundity of social and solitary individuals in response to varying levels of larval predation (Control vs Low predator density: Solitary (n=18), Control vs Low predator density: Social (n=12); Control vs High predator density: Solitary (n=11), Control vs High predator density: Social (n=16)). Error bars indicate 95% bootstrapped confidence intervals.

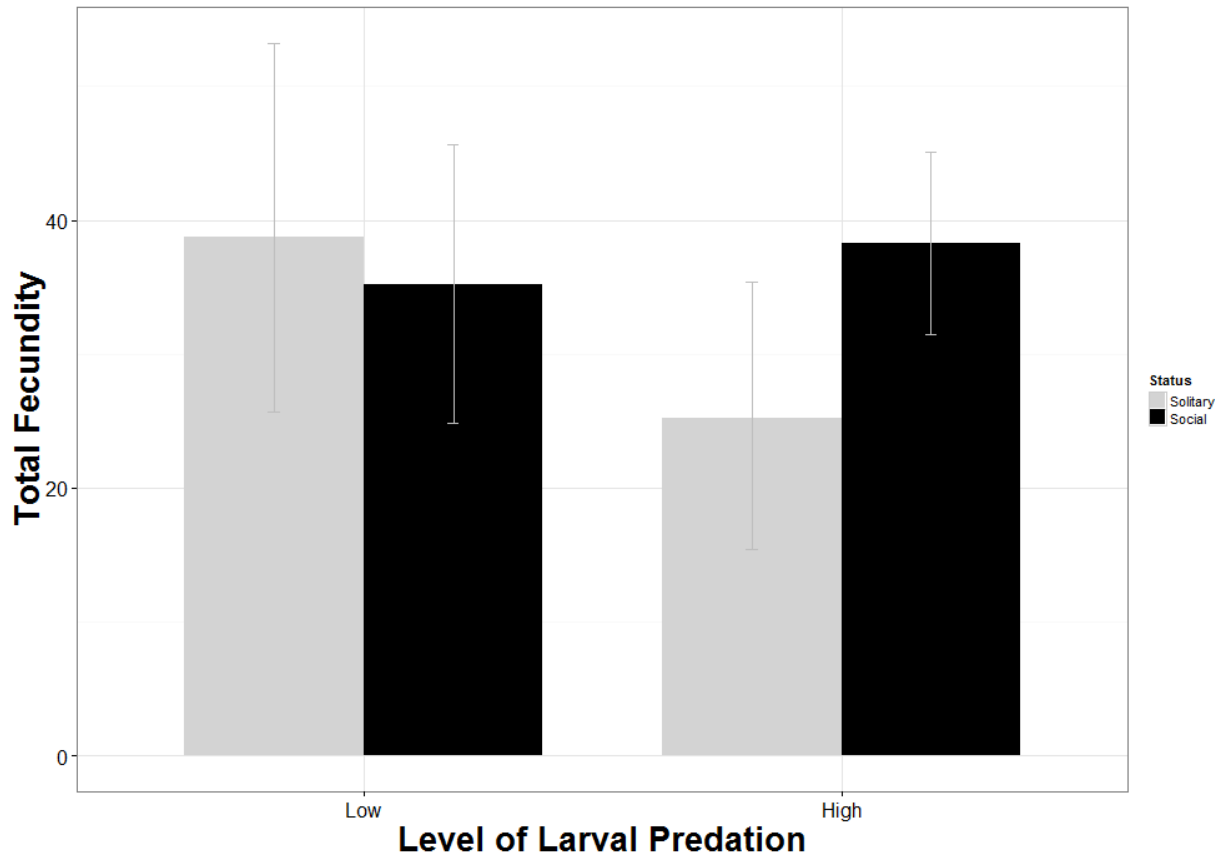


Figure 10: A comparison of oviposition activity index (OAI) of solitary individuals in response to varying levels of larval predation (Control vs Low predator density: Solitary (n=18), Control vs Low predator density: Social (n=12); Control vs High predator density: Solitary (n=11), Control vs High predator density: Social (n=16)). OAI value of -1 indicates maximum preference to control pool, +1 as maximum preference to treatment pool, and 0 as equal preference to both pools. Error bars indicate 95% bootstrapped confidence intervals.

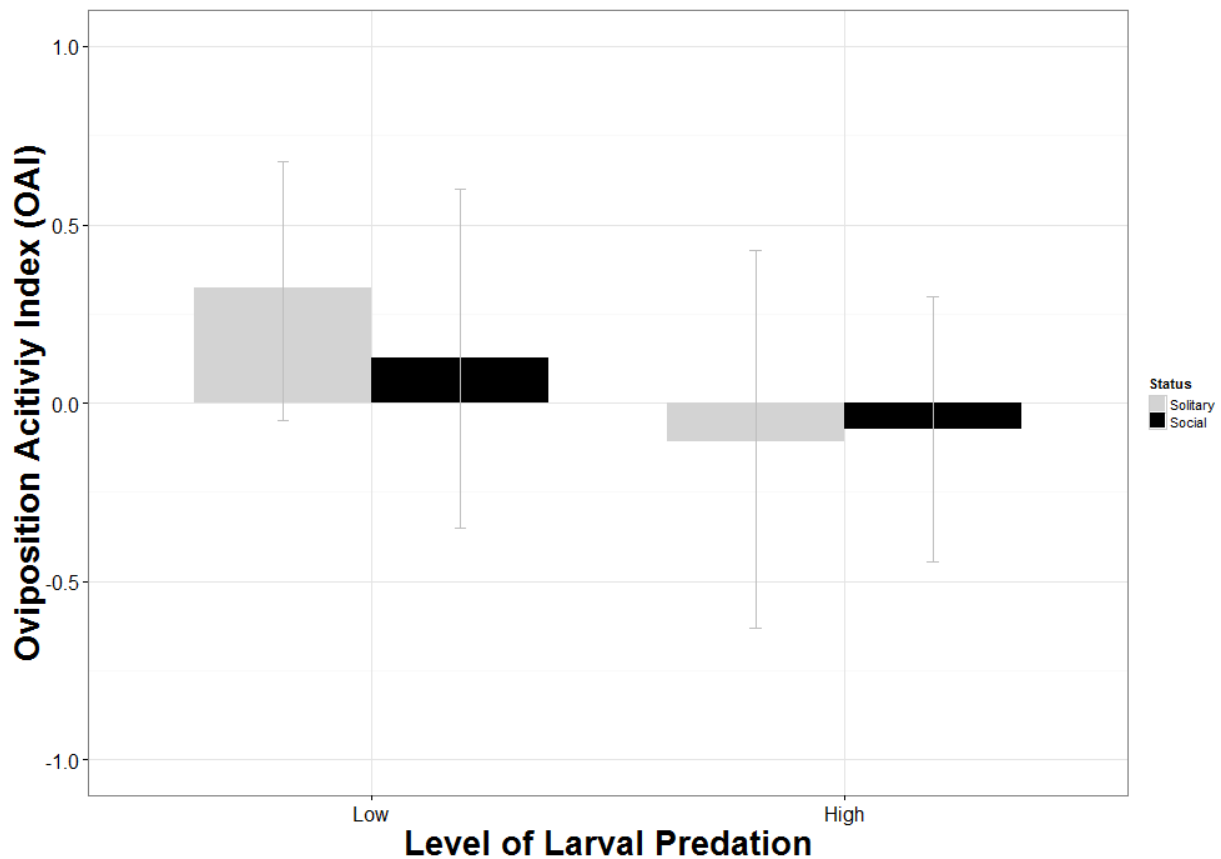


Figure 11: Frequency distribution of OAI of solitary and social individuals in response to varying levels of larval predation (Control vs Low predator density: Solitary (n=18), Control vs Low predator density: Social (n=12); Control vs High predator density: Solitary (n=11), Control vs High predator density: Social (n=16)). OAI value of -1 indicates preference to control pools, +1 towards treatment pool, and 0 as equal preference to both pools.

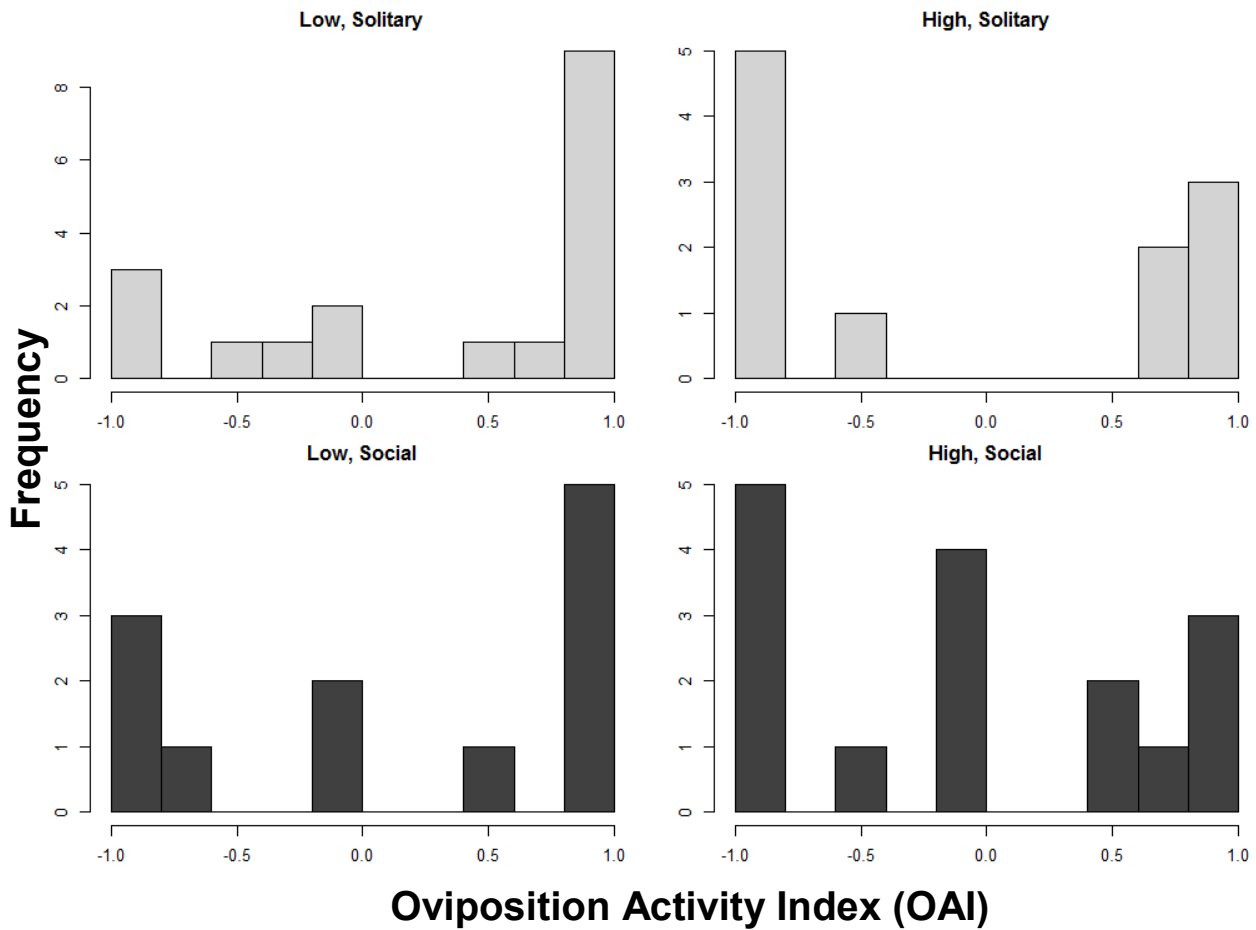


Figure 12: Scatter plots showing relationship between OAI (x-axis) and total fecundity of individual females (y-axis) for solitary and social individuals in response to varying levels of larval predation (Control vs Low predator density: Solitary (n=18), Control vs Low predator density: Social (n=12); Control vs High predator density: Solitary (n=11), Control vs High predator density: Social (n=16)).

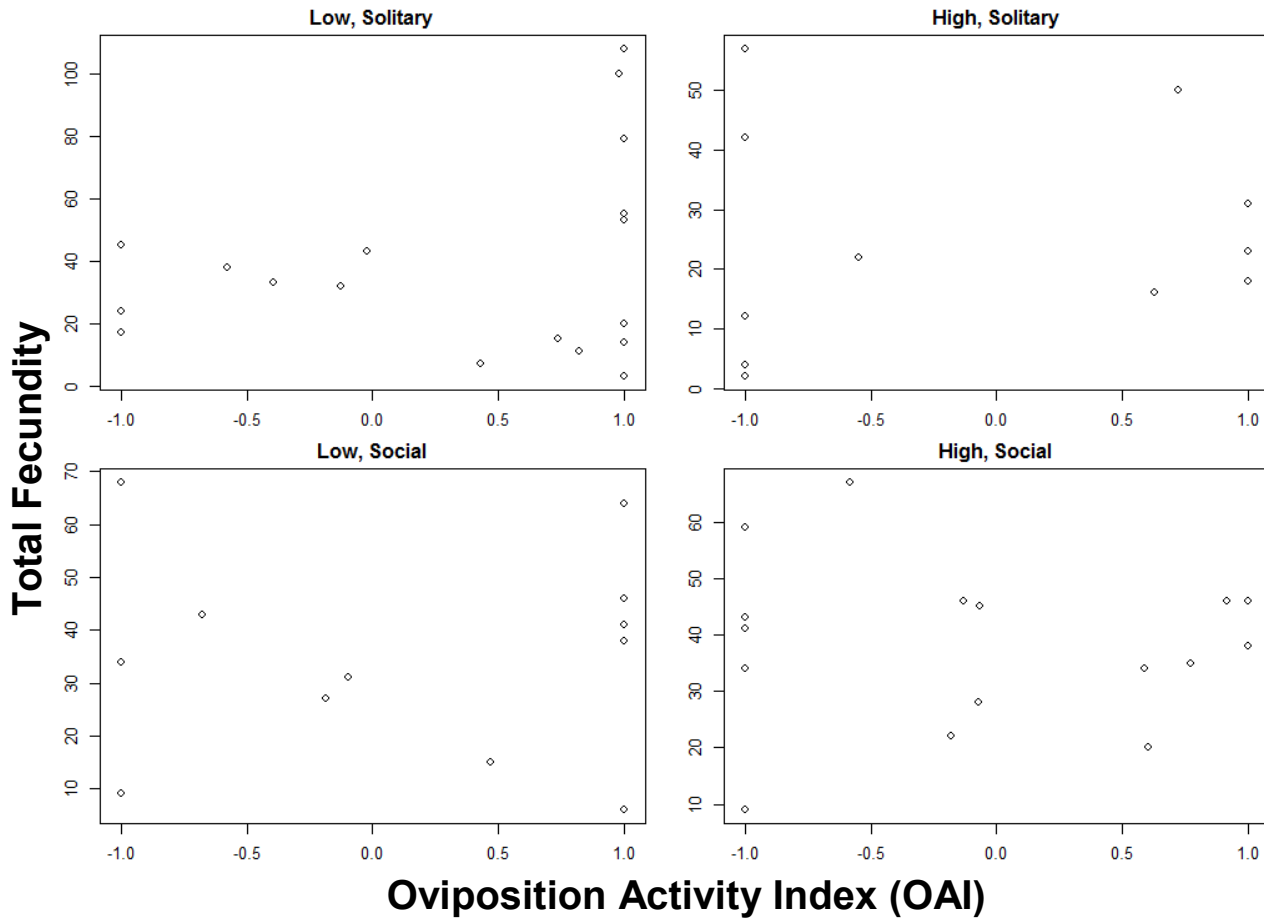


Figure 13: A comparison of number of eggs laid in control pools (alone) by social and solitary individuals in response to varying levels of larval predation (Control vs Low predator density: Solitary (n=18), Control vs Low predator density: Social (n=12); Control vs High predator density: Solitary (n=11), Control vs High predator density: Social (n=16)). Error bars indicate 95% bootstrapped confidence intervals.

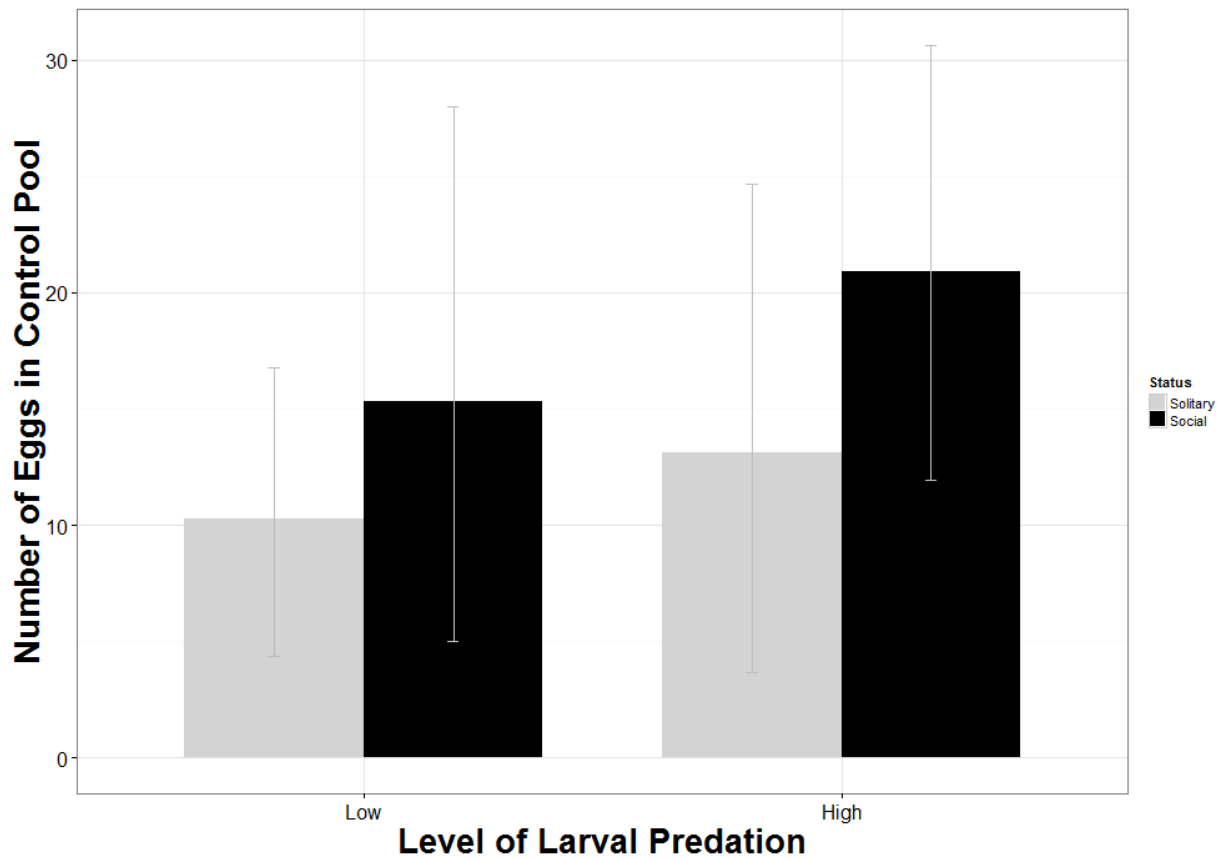
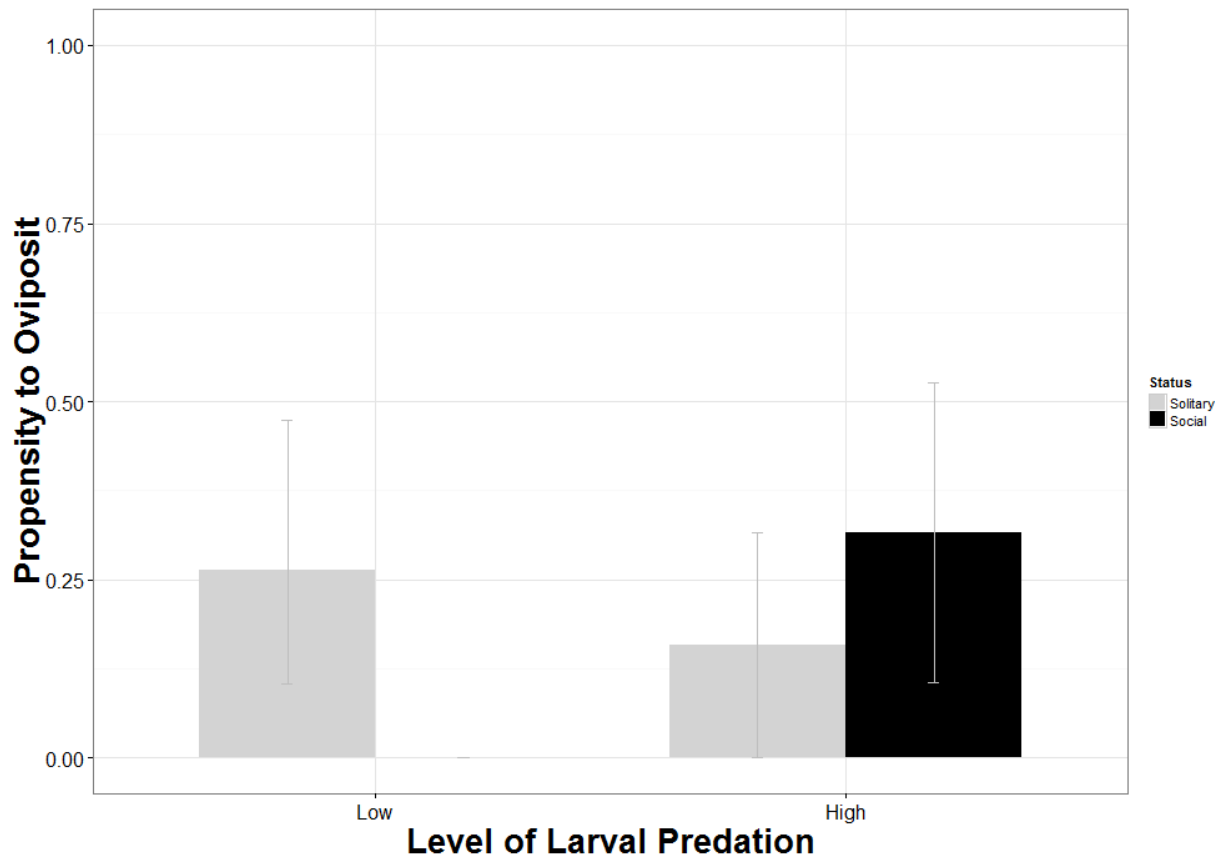


Figure 14: A comparison of propensity to oviposit by social and solitary individuals in response to varying levels of larval predation at early time period (Control vs Low predator density: Solitary (n=19), Control vs Low predator density: Social (n=13); Control vs High predator density: Solitary (n=19), Control vs High predator density: Social (n=19)).



Predation and Competition

Further analysis was performed, to understand how social females alone differed in their response to predation and competition risks. Using means and confidence intervals, I examined differences in oviposition measures across competition and predation - low larval risk (control and 20 larvae, control and 2 predators) and high larval risks (control and 155 larvae, control and 4 predators). Social females displayed no

differences in propensity to oviposit and total fecundity per female in competition versus predation for both levels of larval risks (Figure 15, Figure 16). While females also showed no differences in OAI in low larval risk, they exhibited nearly 5 times greater choosiness in picking pools with high concentration of conspecific over control, than pools with high concentration of predation cues over control (Figure 17). Social females also showed no differences in total eggs deposited in control pools of low predation and competition pool-network. However, females deposited nearly 3.6 times fewer eggs in control pools of high competition versus predation pool-networks (Figure 18).

Figure 15: A comparison of propensity to oviposit by social females alone in predation versus competition treatment types in response to varying levels of larval risk (Control vs Low conspecific density (n=40), Control vs Low predator density (n=19); Control vs High conspecific density (n=34), Control vs High predator density (n=20)). Error bars indicate 95% bootstrapped confidence intervals.

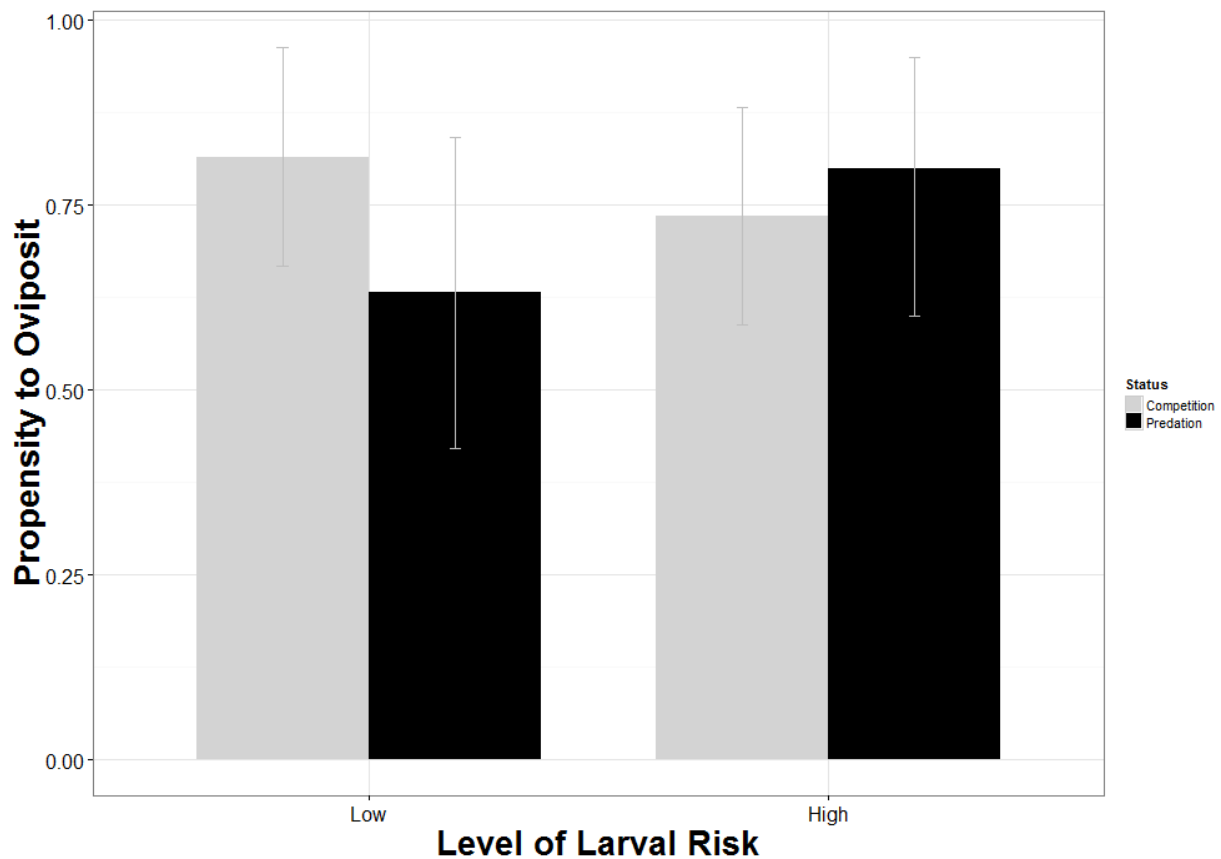


Figure 16: A comparison of total fecundity of social females alone in predation versus competition treatment types in response to varying levels of larval risk (Control vs Low conspecific density (n=27), Control vs Low predator density (n=12); Control vs High conspecific density (n=25), Control vs High predator density (n=16)). Error bars indicate 95% bootstrapped confidence intervals.

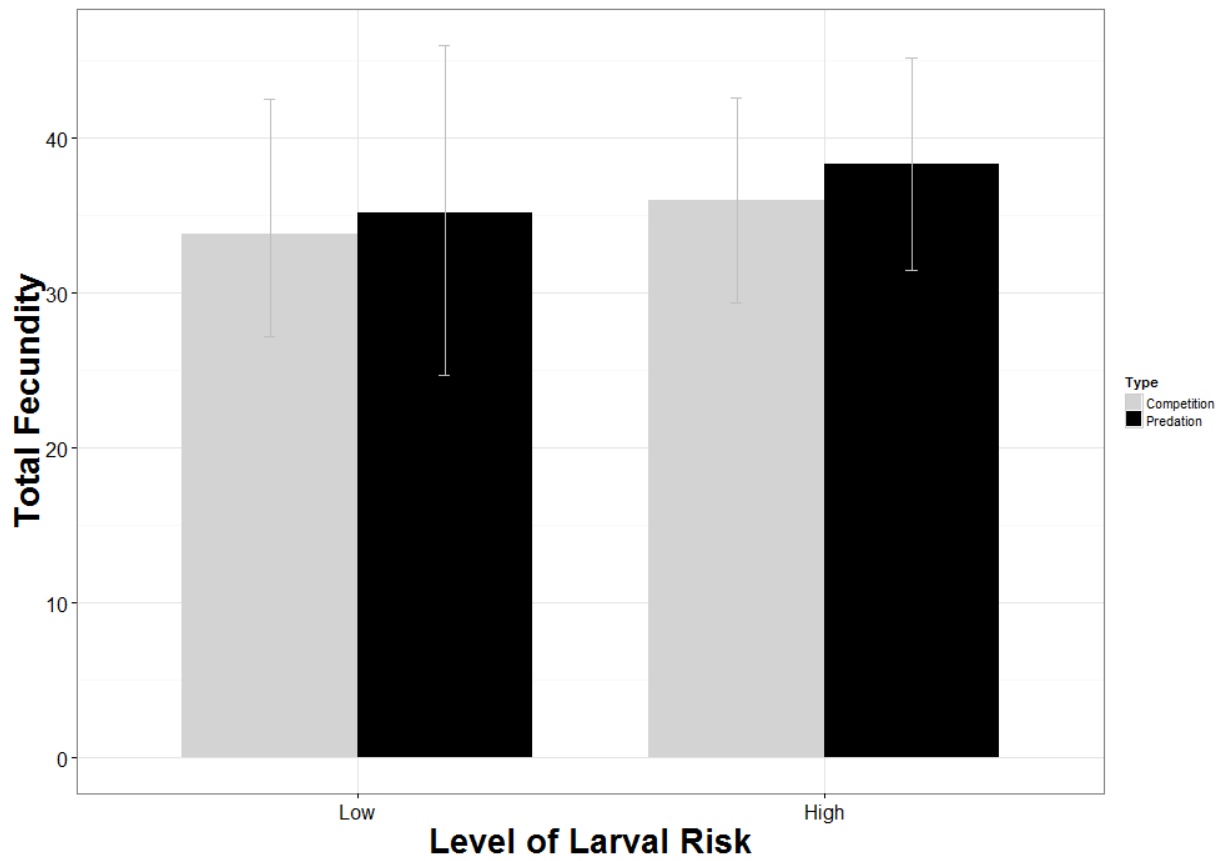


Figure 17: A comparison of oviposition activity index (OAI) of social females alone in predation versus competition treatment types in response to varying levels of larval risk (Control vs Low conspecific density (n=27), Control vs Low predator density (n=12); Control vs High conspecific density (n=25), Control vs High predator density (n=16)). OAI value of -1 indicates maximum preference to control pool, +1 as maximum preference to treatment pool, and 0 as equal preference to both pools. Error bars indicate 95% bootstrapped confidence intervals.

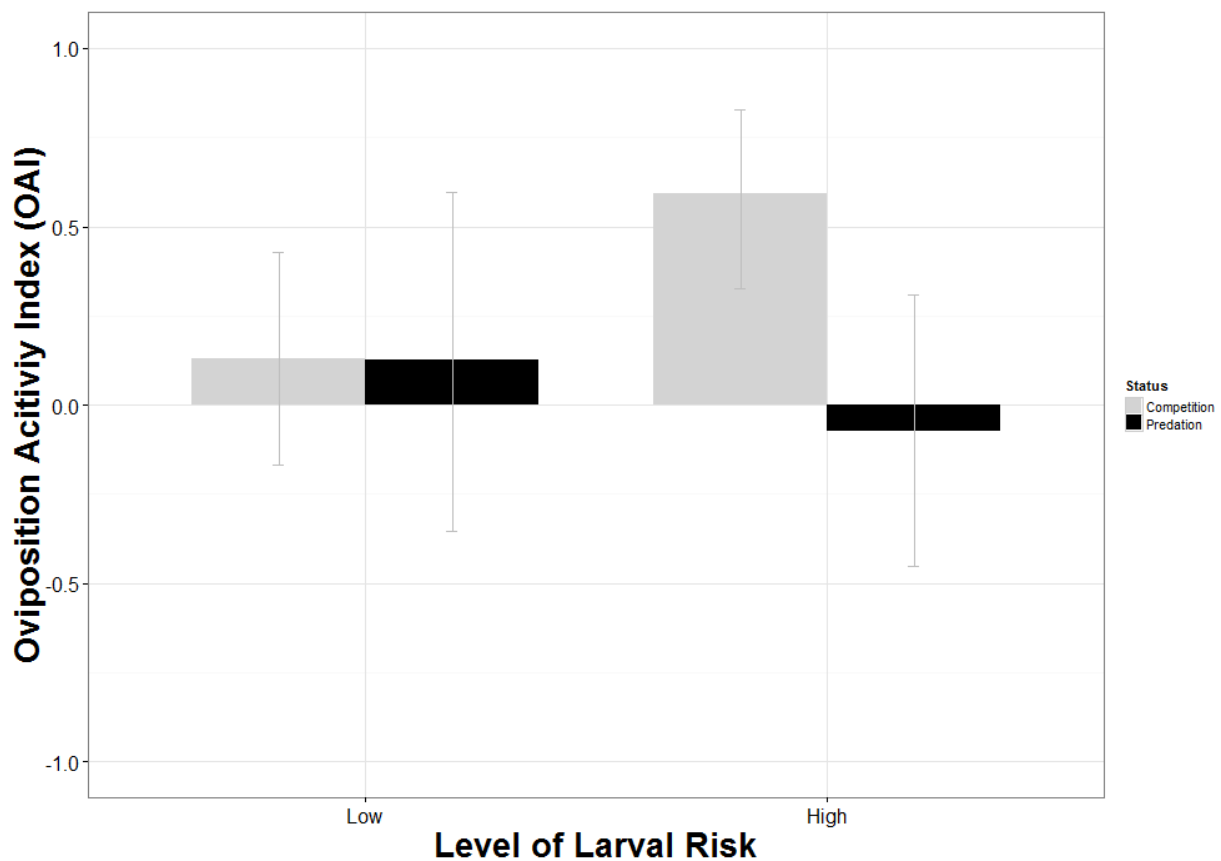
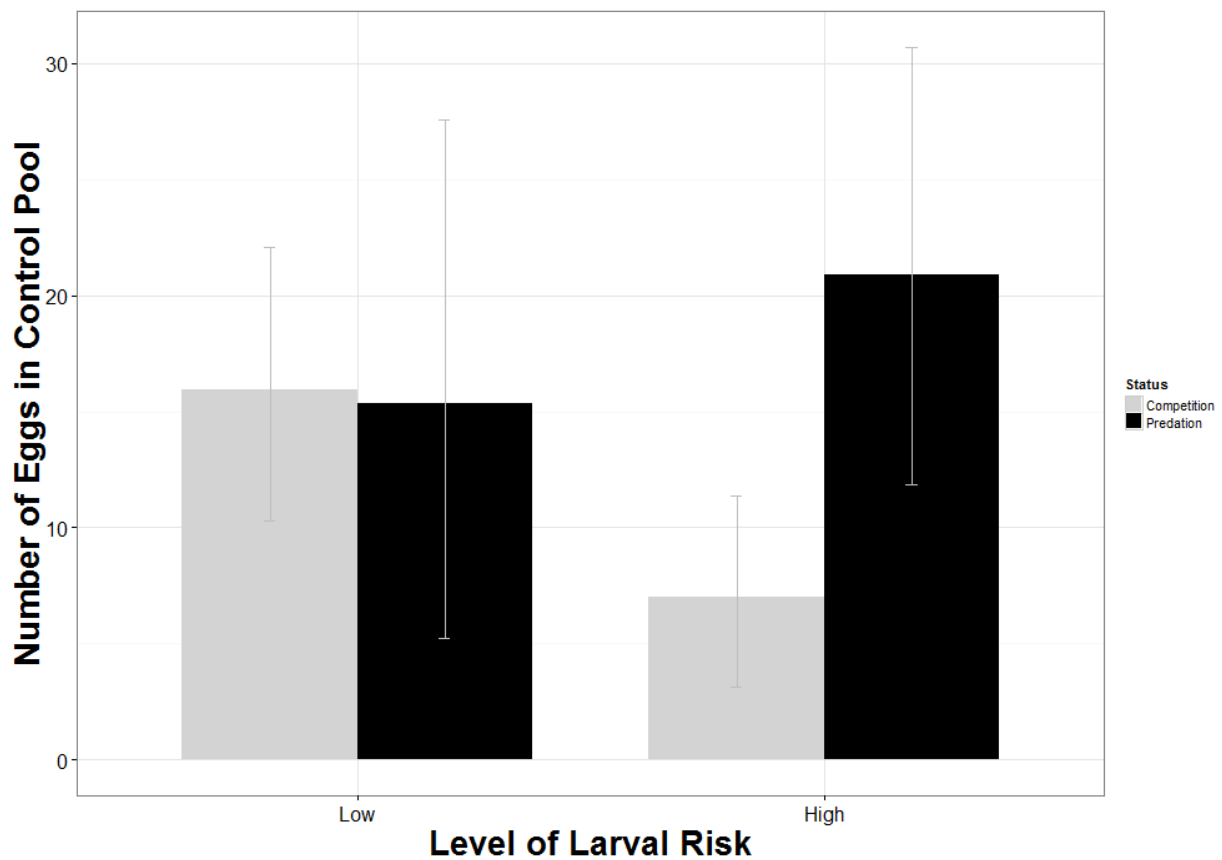


Figure 18: A comparison of number of eggs laid in control pools by social females alone in predation versus competition treatment types in response to varying levels of larval risk (Control vs Low conspecific density (n=27), Control vs Low predator density (n=12); Control vs High conspecific density (n=25), Control vs High predator density (n=16)). Error bars indicate 95% bootstrapped confidence intervals.



Discussion

Overview

In this study, I addressed if social cues can influence oviposition decisions in *A. aegypti* in the context of larval predation and competition risks. Here, solitary and social females were provided with two options - control and treatment (either low or high of either competition or predation). I hereby refer to the set of control and treatment pools together as pool-network (e.g. a set of pools with control and high predator pool would be referred to as high predator pool-network). I predicted that individuals in a social setting should show greater avoidance towards competition pool-network, whereas they should distribute eggs to a greater extent and display greater preference to predator pool-network. I provide evidence for influence of social-cues on oviposition behaviour but not always in the direction predicted. Social females showed no avoidance of competition pool-network nor did they decrease their egg-laying but they did display greater selectivity towards pools with high concentration of conspecific cues. I found that in high predation pool-networks, social females showed increased propensity to oviposit and increased fecundity but bet-hedged between pools more often than did solitary females. When predation and competition treatments were compared, my results showed that individuals were more likely to distribute eggs in low risk treatments and displayed greater choosiness in high risk predation and competition treatments. In addition, the findings from competition treatments indicate that females evaluate pool-network in its entirety prior to deciding whether to oviposit or not and that the choice to oviposit in a pool is strongly influenced by adjacent pool patches. Finally, neither type of risk elicited earlier egg-laying in social females as predicted.

How does social status influence oviposition response to pools varying in competition risk?

This study showed that, in general, social status had no discernible influence on oviposition decisions when presented with pools varying in larval competition risk. Social and solitary females followed similar egg-distribution patterns between pools with no and a low level of competition risk. Here, while a few females displayed slight

preference to treatment over control pools, the majority displayed a mixed strategy of egg-distribution - depositing eggs in varying ratios across pools. However, social females in the high competition pool-network appeared to follow a fixed strategy. Females here were highly choosy with an overwhelming majority of females depositing all their eggs in treatment pools (high concentration of larval cues) alone. These results appear to be contrary to the predictions made earlier. While studies do suggest that females avoid ovipositing in pools containing broods of other conspecific (Munga et al., 2006), several other studies also show that females may view the presence of conspecific larvae in a pool as an indicator of habitat stability and/or suitability (Wong et al., 2011; Zahiri et al., 1997). For instance, fishes use conspecific cues of previous occupants during oviposition site selection for their current brood. An experimental study showed that fishes may show no preference or even avoidance for sites devoid of conspecific cues. In contrast, fish were more likely and quicker to choose sites with conspecific cues (Byers 2002). Moreover, in the wild, it is reported that *A. aegypti* females oviposit just above the water-line and thus, eggs may hatch in batches only when there is a rise in water level in the pool (Clements., 1992). Thus, females may not view conspecific larvae currently occupying a pool as a threat because their progeny are unlikely to be in direct competition with the larvae currently residing in pools.

At low concentration of conspecific cues, where the contrast between treatment and control pools is less distinct, social females did not behave very differently than solitary ones. However, given that social females may anticipate egg-laying by other individuals, it was unexpected, based on the competition hypothesis, that social females displayed strong choice towards pools with high concentration of conspecific cues. One plausible explanation is that females may view the presence of other eggs or chemical cues from these eggs rather than from larvae as a measure of direct competition to their progeny. Since females do not encounter eggs from any other conspecifics yet, female may perhaps use this to their advantage and oviposit, without worrying about future competitors. However, studies on mosquito species - *Aedes triseriatus*, *Culex* spp. and sandfly showed that females show positive oviposition response to habitats with higher egg-densities. (Dia- Eldin and Ward., 1991; Edgerly et al., 1998). Authors explained that this may occur if eggs were to represent habitat stability or if aggregation of larvae

can prove beneficial (e.g. larval aggregates can prevent formation of scum in water thereby reducing predation and parasitism of larvae) (Pile., 1987; Edgerly et al., 1998). An alternative explanation is that high conspecific density is an indicator of pool capacity. Since the pool previously housed a large number of larvae and if nutrient recycling through debris, larval moults etc. results in a nutrient rich pool, treatment pools may signal higher habitat quality (Walker et al., 1991). This would mean that even if all females laid their eggs in the same pool, it may have no drastic influence on larval performance. Studies of larval performance, including survivorship, growth, and developmental time etc. would help address this hypothesis.

How does social status influence oviposition response towards pools varying in predation risk?

Social females responded differently to varying larval predation risk than did solitary females. As predicted, in a pool-network with high predation risk, social females showed a strong inclination to oviposit and did so with much greater numbers of eggs than solitary females. However, inconsistent with the predictions, I observed that social females did not make a shift towards depositing more eggs in predator pools. Instead, nearly a third of the females distributed their eggs similarly across both pools while the other two-thirds deposited eggs only in control or treatment pools like their solitary counterparts. In social conditions, asymmetric egg-distribution in favour of predator pools can be useful only when eggs in control pools are not at high risk of intraspecific competition from future broods and when offspring of other conspecifics can rescue an individual's eggs from competition. If individuals are uncertain about behavioural decisions of other individuals, or how intra-specific competition is mediated in predator or control pools with the addition of eggs in the future, or stochasticity in the environment, females may be better off bet-hedging and distributing eggs evenly across pools (Hopper., 1999; Khatchikian et al., 2009). In low predator pool-networks, both social and solitary females bet-hedged more often than in high predator pool-networks. Bet-hedging can be more favourable in low predator pool-networks as there are lesser differences in costs associated with larval performance. In high predator pool-networks, females showed three distinct pattern of distribution. While a third of the females were choosy in favour of predator pools as predicted, other females either chose control

pools or bet-hedged across pools. This large variation in female choice is consistent with previous results from a study that indicated that on average *A. aegypti* do show higher attraction towards predator pools when compared to 10 other related mosquito species, however while exhibiting great variation in their responses (Vonesh and Blaustein., 2010). In addition, my results showed that social females can completely avoid depositing in low predator pool-networks. This could possibly occur if they assessed that predation levels are not high enough to rescue larvae from future competition or if control pools cannot sustain high larval density. An alternate explanation is that females may be withholding eggs temporally. Reports from a related *Aedes* species suggest that, in the wild, females may withhold their eggs until they encounter pools or a season better suited for their larval performance (Edgerly et al., 1999). Thus, females may engage in temporal bet-hedging and scatter eggs across different pools, under different social settings, over time.

How differently do social females respond to predation versus competition risks?

In general, social females appeared to have similar propensity to lay eggs and similar fecundity under both predation and competition risks. Females appeared to respond similarly to the two types of risk, except in low predation pool-network where females exhibit greater avoidance to the pool-network. As discussed previously, females may choose to oviposit in pools with conspecific cues as it can reflect site-stability. Thus, signals of habitat stability and social facilitation through adult conspecifics appear to influence oviposition in social females. Similarly, social facilitation could lead to females favouring predator pools if their offspring are rescued from the negative effects of competition through predation and/or their mortality through predation is reduced via a dilution effect. Studies across a variety of taxa including anurans, gastropods, fish and mosquitoes have documented predator mediated competition and its largely beneficial influences on the offsprings (Persson., 1993; references in Wissinger and McGarfy., 1993; Chase et al., 2002; Mowles et al., 2011). The influence of social status on egg-distribution patterns differed between the two levels of risk in both predation and competition conditions. When contrasts between pools were less stark (low competition and low predation pool-networks), females were more likely to bet-hedge. For an individual, bet-hedging can be less costly in low risk conditions. Females appeared to

have more fixed strategies with increasing contrast between pools, however they exhibited differences in choosiness between the different competition and predation setups. For instance, social females exerted greater degree of choosiness in the pool-network that included a treatment with high concentration of conspecific cues than in any other type of risk, highlighting the importance of conspecific cuing in habitat selection. In contrast, while social females in high predation pool-networks also showed fixed strategies, there was no overall choosiness to particular pool in the network. Several studies from mosquitoes and other insect species show that females do not always make optimal choice with some providing evidence for selection of sub-optimal strategies. For instance, *W. smithii* mosquitoes oviposited more strongly in pools with higher conspecific larvae or in pools with greater larval food, but fewer eggs in neither or in the mixture of both. The author concludes that although preferences were sub-optimal, they were perhaps better than making random choices (Heard., 1994). Another field study on parasitoids showed that if female encounter rates with hosts were low, parasitoid females displayed superparasitism (i.e. ovipositing in hosts that were previously parasitised). Model results from the study showed that females who chose to superparasitise, sired only a few lesser offsprings than females who chose unparasitised hosts (optimal strategy). The study concluded that owing to the weak selection for the optimal strategy perhaps resulted in a mixed strategy in the population (Janssen A., 1989). Thus, along similar lines of reasoning and with evidence from larval performance, one could gain further insight into the selection for mixed strategies in social females in response to high predation pool-networks. In addition, one must note that a females current preference to a pool may not always reflect her future choices. Studies from parasitoid wasps show that although wasps may initially strongly favour ovipositing in low quality hosts, when presented with a mixture of high- and low- quality hosts, individuals rejected these low quality hosts over time (Van Alphen and Janssen., 1981). Additionally, my study also indicates that potential competition from offsprings of conspecific females for does not promote earlier egg-laying in social females. Thus, if females in social setting compete to oviposit closer to the water-line, simultaneous hatching of broods could result in high intra-specific competition. However, it could also be possible that I did not detect differences in latency to egg-laying owing to smaller sample sizes or due to limitations in the study-design.

How does social status influence oviposition response towards control pools under predation and competition risks?

Examining results from propensity to oviposit suggests that females not only avoid predation pools but also avoid control pools in certain pool-networks. Looking at total numbers of eggs laid in control pools show that, social females laid similar numbers of eggs in control pools in the low-competition, low-predation, and high-predation pool-networks, but laid fewer eggs in control pools in the high competition pool-network where OAI was skewed towards treatment pools. This indicates that females appeared to assess relative risks of adjacent pools prior to ovipositing in control pools. This is consistent with studies that show that parasitoid wasps were more likely to choose a particular larval host when presented with a host of lower quality than higher (Van Alphen and Janssen., 1991). While studies from mosquitoes, *Culiseta longiareolata*, in the wild showed that ovipositing females can quantify predation risks and lay near identical number of eggs in control pools, irrespective of conditions in the adjacent pool (Silberbush and Blaustein., 2011), social and solitary adults of *A. aegypti* do not appear to follow this fixed threshold model in this study.

Caveats of the study

This study design presents potential drawbacks that could perhaps influence the outcome of the study. First, in their ecological setting, females may encounter more than 3-4 other females, and it is possible that stronger differences may have been detected if higher conspecific densities had also been tested. With regard to the current study, to my knowledge, most empirical studies till date that expose focal adults to varying conspecific densities prior to measuring foraging or oviposition activity, use 1 – 6 conspecifics in the study design (Visser., 1995; Goubault et al., 2007). Studies that grouped females to test for influence of social facilitation also use group sizes ranging from 2 – 6. However, studies detect no detectable differences in egg-laying in higher groups (4 and 6) (Goff et al., 2010). Second, in the social-setting, it is possible that blood-fed females can detect that neighbouring females had not yet procured a blood meal and hence may not view them as potential competitors. However, evidence from the literature (Clements., 1992) and personal observations indicate that blood-fed

females take about 24 - 30 hours to digest their blood-meal. Thus, females are more likely to encounter only females with digested blood meals whose appearances are identical to non-blood fed individuals. There is not much information on if these mosquitoes can use odour to detect these differences. It is unlikely that mosquitoes would invest time to detect these differences and body-resources to develop mechanisms, especially when they are faced with more pressing challenges in the wild. Third, in this study I only assess how ovipositing modify their behaviour in presence of other individuals. It is unknown if these additional females are also testing the pools or involved in conspecific cuing. In the wild, neighbouring females may also be involved in these cuing behaviours, and that could affect oviposition decisions in females. Fourth, it has been documented in several species of mosquitoes that environment of larval rearing, aging and previous encounters can affect oviposition choice. For instance, adults of *A. albopictus* mosquito larvae reared in high larval conspecific density habitat, exhibited greater selectivity for habitats with conspecific cues and of more importance than nutrient content in water (Yoshika et al., 2012). Thus, in the wild when several factors are at play, it is difficult to assess if females continue to make similar choices as reported in this study. Finally, seasonality could play a major role in influencing egg-laying choices in females. Field studies with *Aedes triseriatus* has shown that females are more selective in the host pools that they choose in the beginning of the season than towards the end (Edgerly et al., 1999). Typically, at the end of the season females are more likely to be concerned with depositing all their eggs before winter sets-in and when eggs go into diapause. Also, since a female typically oviposits her eggs in multiple batches (around 5 for *A. aegypti*), the choices she makes later in her lifetime, may be dependent on the distribution of eggs earlier in the season.

Conclusion

A striking result of this study is the large amount of variation in oviposition patterns in both solitary and social individuals. First, females exhibited a great variation in choosing whether to oviposit or not given a pool-network. This implies that at a large spatial scale, some females have already rejected to oviposit. On a smaller spatial scale, these females chose not to make a choice between either of the pools presented to them. Of the females who did oviposit, once again there were large differences (ranging from ~10

to 100 eggs) in total eggs deposited per female even under identical pool-network and status. Furthermore, females did not exhibit strong unanimous patterns of egg-distribution across pools for a particular treatment, thus indicating perhaps females do not have a fixed response towards a treatment. Nevertheless, a second overall result in this study consists of some broad patterns in how social cues influence oviposition decisions. Social females exhibited great choosiness towards pools with high concentration of conspecific cues. Social females also favoured high predator pool-networks, and showed a surge in overall egg-laying but displayed a great variation in how they distributed eggs across these pools. Females appeared to adopt bet-hedging strategies, perhaps in response to the uncertainty in choices neighbouring females may make.

In the wild, females are faced with a mosaic of oviposition pools they must choose between. In addition, there are additional concerns such as the costs of travelling to procure food, hosts for blood meals and mates to account for. Under such conditions, using social-cues coupled with non-social cues, can lead to females making decisions that are cost effective or can help identify high quality pools while keeping costs at a minimum. Additionally, the variation in choosiness with no strong preferences or avoidance in any of the experiments perhaps indicates that females encounter much stochasticity both stemming from ecological conditions and from the decisions of other ovipositing females.

Future directions

This study has established that in *A. aegypti*, the presence of conspecific females can modify oviposition decisions across types of risks. To understand how this modification in oviposition affects larval performance (another fitness measure of the ovipositing female), one can measure survivorship, growth, developmental time and even offspring responses to different measures of oviposition behaviour. Next, since I find much variation in egg distribution patterns, one can also address what might maintain such behavioural variation. Finally, in its ecological setting, since adult females can receive social cues from several other types of individuals, one can measure influence of increasing conspecific female density, heterospecific females or even males.

References

- Abernathy, R. L., Teal, P. E., & Tumlinson, J. H. (1994). Age and crowding affects the amount of sex pheromone and the oviposition rates of virgin and mated females of *Helicoverpa zea* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America*, 87(3), 350-354.
- Afify, A., & Galizia, C. G. (2015). Chemosensory cues for mosquito oviposition site selection. *Journal of medical entomology*, 52(2), 120-130.
- Albeny-Simões, D., Murrell, E. G., Elliot, S. L., Andrade, M. R., Lima, E., Juliano, S. A., & Vilela, E. F. (2014). Attracted to the enemy: *Aedes aegypti* prefers oviposition sites with predator-killed conspecifics. *Oecologia*, 175(2), 481-492.
- Angelon, K. A., & Petranka, J. W. (2002). Chemicals of predatory mosquitofish (*Gambusia affinis*) influence selection of oviposition site by *Culex* mosquitoes. *Journal of chemical ecology*, 28(4), 797-806.
- Bentley, M. D., & Day, J. F. (1989). Chemical ecology and behavioral aspects of mosquito oviposition. *Annual review of entomology*, 34(1), 401-421.
- Bernardo, J. (1996). Maternal effects in animal ecology. *American Zoologist*, 36(2), 83-105.
- Betts, M. G., Hadley, A. S., Rodenhouse, N., & Nocera, J. J. (2008). Social information trumps vegetation structure in breeding-site selection by a migrant songbird. *Proceedings of the Royal Society of London B: Biological Sciences*, 275(1648), 2257-2263.
- Blaustein, L., & Kotler, B. P. (1993). Oviposition habitat selection by the mosquito, *Culiseta longiareolata*: effects of conspecifics, food and green toad tadpoles. *Ecological Entomology*, 18(2), 104-108.

Bonnie, K. E., & Earley, R. L. (2007). Expanding the scope for social information use. *Animal Behaviour*, 74(2), 171-181.

Byers, C (2007). The effect of conspecifics on selection of oviposition sites and territories. *Dissertation affiliated to University of Louisville*

Carter, J., Lyons, N. J., Cole, H. L., & Goldsmith, A. R. (2008). Subtle cues of predation risk: starlings respond to a predator's direction of eye-gaze. *Proceedings of the Royal Society of London B: Biological Sciences*, 275(1644), 1709-1715.

Chase, J. M., Abrams, P. A., Grover, J. P., Diehl, S., Chesson, P., Holt, R. D., & Case, T. J. (2002). The interaction between predation and competition: a review and synthesis. *Ecology Letters*, 5(2), 302-315.

Chess, K. F., Ringo, J. M., & Dowse, H. B. (1990). Oviposition by two species of *Drosophila* (Diptera: Drosophilidae): behavioral responses to resource distribution and competition. *Annals of the Entomological Society of America*, 83(4), 717-724.

Clements, A. (1992). Vol. 1: Development, nutrition and reproduction. *London [etc.]*: Chapman & Hall.

Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1-48

Edgerly, J. S., McFarland, M., Morgan, P., & Livdahl, T. (1998). A seasonal shift in egg-laying behaviour in response to cues of future competition in a treehole mosquito. *Journal of Animal Ecology*, 67(5), 805-818.

Elnaiem, Dia & Ward, R. D. (1991). Response of the sandfly *Lutzomyia longipalpis* to an oviposition pheromone associated with conspecific eggs. *Medical and veterinary*

entomology, 5(1), 87-91.

Ferrari, M. C., Messier, F., & Chivers, D. P. (2007). Variable predation risk and the dynamic nature of mosquito antipredator responses to chemical alarm cues. *Chemoecology*, 17(4), 223-229.

Gibbs, M., Lacey, L. A., Jones, M. J., & Moore, A. J. (2005). Egg size-number trade-off and a decline in oviposition site choice quality: female *Pararge aegeria* butterflies pay a cost of having males present at oviposition. *Journal of Insect Science*, 5(1), 39.

Gimnig, J. E., Ombok, M., Otieno, S., Kaufman, M. G., Vulule, J. M., & Walker, E. D. (2002). Density-dependent development of *Anopheles gambiae* (Diptera: Culicidae) larvae in artificial habitats. *Journal of Medical Entomology*, 39(1), 162-172.

Godfray, H. C. J. (1994). Chapter 3 : Oviposition in Parasitoids: behavioral and evolutionary ecology. *Princeton University Press*.

Goubault, M., Cortesero, A. M., Poinso, D., Wajnberg, E., & Boivin, G. (2007). Does host value influence female aggressiveness, contest outcome and fitness gain in parasitoids?. *Ethology*, 113(4), 334-343.

Goulson, D., Hawson, S. A., & Stout, J. C. (1998). Foraging bumblebees avoid flowers already visited by conspecifics or by other bumblebee species. *Animal Behaviour*, 55(1), 199-206.

Greene, C. M., & Stamps, J. A. (2001). Habitat selection at low population densities. *Ecology*, 82(8), 2091-2100.

Heard, S. B. (1994). Imperfect oviposition decisions by the pitcher plant mosquito (*Wyeomyia smithii*). *Evolutionary Ecology*, 8(5), 493-502.

Hirayama, H., & Kasuya, E. (2013). Effect of adult females' predation risk on oviposition site selection in a water strider. *Entomologia Experimentalis et Applicata*, 149(3), 250-255.

Hopper, K. R. (1999). Risk-spreading and bet-hedging in insect population biology 1. *Annual review of entomology*, 44(1), 535-560.

Irvin, N., & Hoddle, M. S. (2005). The competitive ability of three mymarid egg parasitoids (*Gonatocerus* spp.) for glassy-winged sharpshooter (*Homalodisca coagulata*) eggs. *Biological Control*, 34(2), 204-214.

Janssen, A. (1989). Optimal host selection by *Drosophila* parasitoids in the field. *Functional Ecology*, 469-479.

Khatchikian, C. E., Dennehy, J. J., Vitek, C. J., & Livdahl, T. P. (2010). Environmental effects on bet hedging in *Aedes* mosquito egg hatch. *Evolutionary Ecology*, 24(5), 1159-1169.

Le Goff, G. J., Mailleux, A. C., Detrain, C., Deneubourg, J. L., Clotuche, G., & Hance, T. (2010). Group effect on fertility, survival and silk production in the web spinner *Tetranychus urticae* (Acari: Tetranychidae) during colony foundation. *Behaviour*, 147(9), 1169-1184.

Levin, S. A., Carpenter, S. R., Godfray, H. C. J., Kinzig, A. P., Loreau, M., Losos, J. B., ... & Wilcove, D. S. (Eds.). (2009). *The Princeton guide to ecology*. Princeton University Press.

Liebezeit, J. R., & George, T. L. (2002). Nest predators, nest-site selection, and nesting success of the Dusky Flycatcher in a managed ponderosa pine forest. *The Condor*, 104(3), 507-517.

Miller, C. W., Fletcher Jr, R. J., & Gillespie, S. R. (2013). Conspecific and

heterospecific cues override resource quality to influence offspring production. *PLoS One*, 8(7), e70268.

Mogali, S. M., Saidapur, S. K., & Shanbhag, B. A. (2012). Tadpoles of the bronze frog (*Rana temporalis*) assess predation risk before evoking antipredator defense behavior. *Journal of ethology*, 30(3), 379-386.

Mowles, S. L., Rundle, S. D., & Cotton, P. A. (2011). Susceptibility to predation affects trait-mediated indirect interactions by reversing interspecific competition. *PloS one*, 6(8), e23068.

Munga, S., Minakawa, N., Zhou, G., Barrack, O. O. J., Githeko, A. K., & Yan, G. (2006). Effects of larval competitors and predators on oviposition site selection of *Anopheles gambiae sensu stricto*. *Journal of medical entomology*, 43(2), 221-224.

Persson, L. (1993). Predator-mediated competition in prey refuges: the importance of habitat dependent prey resources. *Oikos*, 12-22.

Pile, M. M. (1987). Pheromone-mediated behaviour of the mosquito *Culex quinquefasciatus* elicited by an oviposition attractant and its derivatives.

Prokopy, R. J., & Bush, G. L. (1973). Oviposition by grouped and isolated apple maggot flies. *Annals of the Entomological Society of America*, 66(6), 1197-1200.

Prokopy, R. J., & Duan, J. J. (1998). Socially facilitated egg-laying behavior in Mediterranean fruit flies. *Behavioral ecology and sociobiology*, 42(2), 117-122.

Refsnider, J. M., & Janzen, F. J. (2010). Putting eggs in one basket: ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annual Review of Ecology, Evolution and Systematics*, 41, 39-57.

Resetarits Jr, W. J., & Wilbur, H. M. (1989). Choice of oviposition site by *Hyla chrysoscelis*: role of predators and competitors. *Ecology*, 220-228.

Seppänen, J. T., & Forsman, J. T. (2007). Interspecific social learning: novel preference can be acquired from a competing species. *Current Biology*, 17(14), 1248-1252.

Silberbush, A., & Blaustein, L. (2011). Mosquito females quantify risk of predation to their progeny when selecting an oviposition site. *Functional Ecology*, 25(5), 1091-1095.

Silberbush, A., & Blaustein, L. (2011). Mosquito females quantify risk of predation to their progeny when selecting an oviposition site. *Functional Ecology*, 25(5), 1091-1095.

Templeton, J. J., & Giraldeau, L. A. (1995). Patch assessment in foraging flocks of European starlings: evidence for the use of public information. *Behavioral Ecology*, 6(1), 65-72.

Templeton, J. J., & Giraldeau, L. A. (1996). Vicarious sampling: the use of personal and public information by starlings foraging in a simple patchy environment. *Behavioral Ecology and Sociobiology*, 38(2), 105-114.

Thompson, J. N. (1988). Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia experimentalis et applicata*, 47(1), 3-14.

Van Alphen, J. J. M., & Janssen, A. R. M. (1981). Host selection by *Asobara tabida* Nees (Braconidae; Alysiinae) a larval parasitoid of fruit inhabiting *Drosophila* species. *Netherlands Journal of Zoology*, 32(2), 194-214.

Visser, M. E. (1995). The effect of competition on oviposition decisions of *Leptopilina heterotoma* (Hymenoptera: Eucoilidae). *Animal Behaviour*, 49(6), 1677-1687.

Vonesh, J. R., & Blaustein, L. (2010). Predator-induced shifts in mosquito oviposition site selection: a meta-analysis and implications for vector control. *Israel Journal of Ecology & Evolution*, 56(3-4), 263-279.

Walker, E. D., Lawson, D. L., Merritt, R. W., Morgan, W. T., & Klug, M. J. (1991). Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. *Ecology*, 1529-1546.

Wong, J., Stoddard, S. T., Astete, H., Morrison, A. C., & Scott, T. W. (2011). Oviposition site selection by the dengue vector *Aedes aegypti* and its implications for dengue control. *PLoS Negl Trop Dis*, 5(4), e1015.

Yoshioka, M., Couret, J., Kim, F., McMillan, J., Burkot, T. R., Dotson, E. M. & Vazquez-Prokopec, G. M. (2012). Diet and density dependent competition affect larval performance and oviposition site selection in the mosquito species *Aedes albopictus* (Diptera: Culicidae). *Parasit Vectors*, 5, 225-225.

Zahiri, N., Rau, M. E., & Lewis, D. J. (1997). Oviposition responses of *Aedes aegypti* and *Ae. atropalpus* (Diptera: Culicidae) females to waters from conspecific and heterospecific normal larvae and from larvae infected with *Plagiorchis elegans* (Trematoda: Plagiorchiidae). *Journal of medical entomology*, 34(5), 565-568.