Investigating the
Maternal effects of Predation on mosquito, *Aedes aegypti*.

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A dissertation submitted for the partial fulfillment of

BS-MS dual degree in Science

Under the guidance of

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Centre for Ecological Science

Indian Institute of Science

Indian Institute of Science Education and Research Mohali

June 2020

Certificate of Examination

This is to certify that the dissertation titled ***"Investigating the Maternal effects of Predation on mosquito, Aedes aegypti. "*** submitted by **Akshay p** (Reg. No. MS15162) for the partial fulfillment of BS-MS dual degree program of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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DECLARATION

The work presented in this dissertation has been carried out by me under the guidance of Dr. Kavita Isvaran at Indian Institute of Science Banglore and Dr. N.G Prasad at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

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In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Kavita Isvaran

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In my capacity as the co-supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. N.G Prasad

(Co-Supervisor)

ACKNOWLEDGEMENT

I express my deepest sense of gratitude and profound respect to my thesis supervisor Dr. Kavita Isvaran for taking me into her lab and mentoring me for the past one year. Her immense scientific, practical, and intuitive expertise guided me in completing my thesis work.

I would like to express my deepest appreciation and gratitude to my co- supervisor Dr. N.G. Prasad. I express my gratitude to Dr. Karthikeyan and Dr. Manvi Sharama for their guidance and understanding in designing the experiments and training me in the scientific field. It was a pleasure working with them.

I would like to thank Dr. Ravi for his insights and guidance in troubleshooting experiments. I am also thankful to lab members Kanika, Nitin, Gokul, Preeti, Pranav and Aravind. The group has been a source of knowledge and fun. Also, I would like to thank Kanchana for her help through out the experiment.

I would like to thank Abhijith and Ranjani, who was always there for me in my good and bad times. I thank my friends for their support during my five years in IISER.

I am forever grateful to my beloved parents and my brother for their love and continuous support. I owe everything to them. Also, I would like to thank my family members, cousins, for believing in me. All may not be mentioned, but none are forgotten.

AKSHAY P

ABSTRACT

Predator presence in the surroundings can shape the prey morphology, life history and behaviour. Prey employs various strategies to escape from predators and with apt phenotypic changes they can get away from predators. However, these predator escape strategies comes with a cost at the level of forgaing, mating or other activities. So predator presence can have negative impacts on prey physiological conditions. But very little is known about the transgenerational effects predation on prey. Maternal provisioning can reduce the risk of offspring being caught by predators by promoting growth rate and anti-predatory behaviours. Most of the studies on such maternal effects of predation are focused on the risk of predation experienced during adulthood. So in this study *Aedes aegypti* mosquitoes were used to test if the predation risk experienced during larval stage can affect i) mother's development, maternal investments in eggs and life history traits like longevity and body size (Wing length) and thereby (ii)influence offspring development, anti- predatory behaviour and other life history traits. Results revealed that *Aedes aegypti* responded to predation environment by developing faster and producing large eggs.The impact of predation risk in *Aedes aegypti* larvae carries over to next generations, through maternal effects.

Contents

List of Figures

List of Tables

Chapter 1

Introduction

Prey-predator relationships have long been studied by ecologists and evolutionary biologists. Both the prey and the predator employ various strategies to win their battle against the other. Selection acts on predators to evolve traits that would help them find and capture their prey efficiently, while on prey it acts to minimise the risk of being caught. If either of them temporarily wins the battle with the help of an apt adaptation, then it would change the selection pressure on the other one, leading to a counter adaptation and eventually to an evolutionary arms race between them (R.Dawkins & J. R. Krebs, 1979). Predators can have lethal and non-lethal effects on the prey (Chandrasegaran et al., 2017; Lima, 1998; Werner, 2012). Lethal effects are direct and can result in the death of the prey animal, but non-lethal effects are indirect and can influence prey morphology, behaviour and life history (Chandrasegaran et al., 2017; Crowl &Covich, 1990). For example, Physella virgata virgate snails in spring-fed Oklahoma streams normally grow to 3.5 mm size in around 3.5 months and start reproducing, but in the presence of predator crayfish Orconectes virilise, snails were observed to grow rapidly and reproduced very little till they become about 10 mm in size after 10 months (Crowl &Covich, 1990). Seeking refuge, aggregating, avoiding high risk areas and reducing high risk activities like mating and foraging, are common behavioural responses shown by prey organisms in the presence of predators (Chandrasegaran et al., 2017; Juliano &Reminger, 1992).

Responding to a perceived predator threat in the habitat, with suitable phenotypic changes, would increase the chances of prey survival by avoiding the direct effect of predation (lethality) (Chandrasegaran et al., 2017). Assessment of predator presence and threat level can be

done through physical cues, chemical cues, or through both from the habitat. Cues need not come directly from the predators, rather it can come from the conspecific that had been injured or killed by predators. Cues such as predator scent or metabolites released by predators are called kairomones while cues like injured prey components are called alarm cues or allomone (Chandrasegaran et al., 2017; Chivers &Smith, 1998; Grostal &Dicke, 1999). Upon sensing these cues, prey organisms might alter a few of its phenotypes (non-lethal effects) and with apt phenotypic changes, it may get away from predators. However, predator avoidance mechanisms have inevitable costs at the level of foraging, mating etc (Lima, 1998). So prey must vary the intensity of these responses in correspondence with the level of predation risk (threat sensitive predator avoidance hypothesis)(Seiter & Schausberger, 2015).

Since a wrong judgement about the predation threat in the habitat will have drastic effects on prey, it would be easy for the prey individual if someone in the population can forewarn about the environment. Mothers can sometimes do this through maternal effects. Maternal effects are defined as the influence of maternal phenotypes on offspring's phenotype, independent of the genetic contribution by the mother (Bernardo, 1996; Mousseau & Fox, 1998). Maternal experience of the environment can be translated into phenotypic variations in offspring through cytoplasmic factors in egg, like yolk amount, maternally transmitted mRNAs, lipids, hormones etc or through post zygotic influences via behavioural choices made by mothers (eg: choice of nesting sites) (Bernardo, 1996; Wolf & Wade, 2009). Females vary the amount and quality of resources invested in eggs depending on the environment (Bernardo, 1996; Qvarnström & Price, 2001). These altered resource investments in eggs can later influence various offspring traits like their growth rate, anti-predatory behaviour, longevity and can ultimately affect the chances of offspring survival and reproduction (Timothy A Mousseau & Fox, 1998; Tigreros et al., 2019). Sometimes through maternal effects, mothers can prepare their offspring to cope with the environment in a better way(Bernardo, 1996; T. A. Mousseau & Dingle, 1991; Seiter & Schausberger, 2015). These sort of anticipatory maternal effects can be interpreted as trans-generational phenotypic plasticity (Agrawal, 2002; Donohue, 2009). Studies on three-spined sticklebacks (Gasterosteus aculeatus) showed that mothers transfer information via eggs and can influence offspring development, growth and behaviour. Compared to control sticklebacks,

females that experienced predation threat produced larger eggs with more cortisol content in them, and consumed more oxygen immediately after fertilization. Also offspring from mothers who experienced predator threat, showed tighter shoaling, which is an anti-predatory behaviour in sticklebacks(Giesing et al., 2010).

Under an unfavourable environment, females can increase or decrease resources invested in eggs. Sometimes females increase the provisioning in eggs to produce high-quality offspring which would be better at handling the unfavourable environment, but such alterations in resources come at a cost in the number of offspring produced (Tigreros et al., 2019) . On the other hand, if the environmental condition is too severe so that it affects the female growth and physiological conditions badly, then these females may decrease the amount of resources invested per propagule (Tigreros et al., 2019). However, not all maternal alteration in resource allocation in eggs would result in better survival and performance of the offspring. Maternal effects are adaptive only if mothers anticipate the future offspring environment correctly and prepare offspring to perform better in that future (Agrawal, 2001; Timothy A Mousseau & Fox, 1998; Sheriff & Love, 2013). In an environment that is different from the one that the mother had anticipated, the altered offspring traits may have disadvantageous consequences. Field observations made by M J Sheriff and team on wild population of Snowshoe hare (*Lepus americanus*) and Lynx (*Lynx Canadensis*) demonstrate when maternal effects can be adaptive or maladaptive to offspring. This prey-predator system undergoes regular cyclic fluctuations in density. Predator density increases and then decreases with prey population density but with a delay of 1-2 years(Krebs et al., 1995; Sheriff & Love, 2013). Even though predator density is low after the decline phase, hare population density remains low for the next 2-5 years. The hares show maximum fecundity during the increase phase and minimum during the decline phase. However, hare reproduction rate stays very low till the late low phase (after decline phase), despite the number of predators being very low and vegetation being ample during this phase. Predation risk is maximum during the decline phase where this stress leads to maximum glucocorticoid (a stress hormone) level in hares and this maternal derived stress induces some phenotypic changes in offspring. Predator exposed females had offspring with reduced litter size, birth weight and size. Such maternally derived stress-induced phenotypic alterations were advantageous in decline

phase as maternal and offspring environments match. These individuals showed higher stress response and anti-predatory behaviours, which would increase the chances of survival. If a female mates at the end of decline phase where the number of predators were high and gives birth at the beginning of the low phase where the number of predators is low and ample amount of food resources are available, then the maternal and offspring environments are mismatched, so in such a case, an offspring with higher stress responses and higher anti-predatory response will have costs at the level of foraging and reproduction (Sheriff & Love, 2013).

In this project, I worked on /textitAedes aegypti mosquitoes also known as yellow fever mosquitoes. These widely distributed mosquitoes can spread agents of various diseases like dengue fever, chikungunya, zika fever, mayaro and yellow fever. These are container inhabiting mosquitoes and they lay eggs in stagnant water in unused pots, tanks, spare tire, tree holes, and other ditches. Since Aedes aegypti is a vector for many disease-causing agents and is in very close contact to our day to day life, it becomes necessary to control mosquito growth and breeding. Use of chemical larvicide and insecticide are very common mosquito controlling methods, but these chemical treatments can cause asthma and other serious health problems and can have side effects on the ecosystem itself. In order to control mosquitoes without using chemical sprays, people started growing natural predators of mosquito, like guppy fish and dragonfly nymphs, which would prey on mosquito larvae or pupae. In a predation environment, upon sensing physical, chemical or both cues, mosquito larvae respond by reducing the number of wriggle bursts and the number of wriggles per burst, and increasing time spent on resting (Chandrasegaran et al., 2017; Kesavaraju & Juliano, 2004). Many studies have been performed on the effects of predation on mosquitoes, however, the possibility of maternal effects of predation on mosquitoes has not been investigated. While using natural predators to control mosquitoes, it becomes important to know about the transgenerational effect of predation.

Most of the studies on maternal effects have looked at the effects of predation during reproducing period of individuals and mammals have been the model system for most of these studies (Bernardo, 1996; T. A. Mousseau & Dingle, 1991; Timothy A Mousseau & Fox, 1998). Since insects acquire most of their resources in their larval stage, predation in the larval stage are ex-

pected to have more intense effects on adult morphology, behaviour and life history (Qvarnström & Price, 2001). So in this study we tried to understand the maternal effects of predation during larval stage on Aedes aegypti mosquitoes. I examined if predation risk during larval stage has any effect on Aedes aegypti mosquitoes and if it can induce maternal effects. Specifically, I tested whether the risk of predation: (i) affects the mother's developmental, maternal provisioning (egg size), and adult traits (wing length & longevity) and (ii) offspring's developmental time, anti-predatory behaviour and adult traits (Wing length &longevity). Finally, I tested the effect of current vs maternal environment on offspring traits. Granite ghost (Bradinopyaga geminate) dragonfly were used as predators. Nymphs of Granite ghost consume mosquito larvae co-inhabiting the waterbody. Mosquitoes were reared under three different levels predation risk environments, namely Control, low predation and high predation. Control pools were predator free, low and high predation environments were hosted with 2 and 4 predators respectively. Predators were not allowed to roam around the pool freely, as they could eat up the entire experimental larvae in few days. So these predators were caged within the pools. Mosquito larvae were reared in these three environments in equal density. I made the following hypotheses about how life history and behaviour traits should be affected by non- lethal predation and by maternal effects of predation. First I assumed that the presence of caged predators and chemical cues released from them would induce stress in larvae cohabiting the environment, even though these larvae are not directly consumed by the predators. I predicted that individuals in predation environments will reduce their developmental time compared to control larvae, in order to morph into an adult faster and escape from the pool, as dragonfly nymph's aquatic life lasts much longer than mosquito's. I expected larvae in predation environments to perform anti-predatory behaviours in response to the predation cues in the environment. As the predation cues in water increase larvae are expected to increase the level of anti-predatory behaviour. Given that anti predatory behaviour trade off with foraging, individuals performing these escape behaviours would have less access to resources. I expected these stressed individuals with less access to resources to have compromised body size and longevity. In addition, predator-exposed females would anticipate future offspring environment to have predators, and so they could change the maternal provisioning to offspring by either increasing or decreasing investments in eggs. This

is because, if they invest more on eggs then they can have offspring with improved growth rate and anti-predatory behaviours. or alternately by distributing resources in more eggs but lesser in each, they can dilute the predation pressure on individual offspring. I also predicted that the eggs of predator exposed females will have less hatching propensity, because they might delay hatching until the concentration of predator cues in pool reduces. I expect that the maternal environments will have influence on offspring traits and the intensity of this influence would vary with the level of predation experienced by mothers.I would expect a negative effect on offspring body size and longevity of predator exposed mothers compared to offspring of predator naive mothers. Offspring of individuals from high predation environments were expected do well in predation environments than offspring of control individuals. Traits of offspring wouldn't be just a result of the environment experienced by mothers, rather the current environment wold also play a role in them. So influences from both the maternal experience and offspring experience were expected on offspring traits. Offspring in an environment same as that of mother were expected to perform well in that environment compared to offspring having different maternal environment.

Chapter 2

Materials and methods

2.1 Maternal (F0) Larval environments

All the *Aedes aegypti* mosquitoes used in this study were from a laboratory colony maintained at IISc, Bangalore and predator Bradinopyga geminata dragonfly nymphs were collected from crop fields at Malappuram, Kerala. New hatchlings from the eggs collected from the mosquito lab colony were reared until pupation in 18 large trays. Each tray had one of 3 different levels of predation environments, namely control (C), low predation (LP) and high predation (HP). Thus, each predation environment had, 6 replicates. Each tray contained 0.5 g of larval feed (mixture of dog biscuit and yeast in a ratio of 3:2 by weight) in 1.5L of tap water. To control for resource competition, each tray was kept with a similar larval density (70 larvae per tray) and water lost due to evaporation was refilled. Previous work showed that resource competition was low at this density (Manvi thesis ref). To mimic the risk of predation, we added 2 and 4 size matched predators (Bradinopyga geminate nymphs) in low predation and high predation environments respectively. In this experiment, the focus was on the non-consumptive effects of predation. Therefore, to ensure that predators did not cause direct mortality by preying on larvae, the predators were kept in individual transparent 50 ml containers within the tray pools, and water level in these containers were kept same as the level in tray pools. These predators were fed with eight 4th instar mosquito larvae every day and any uneaten live larvae were removed from container after 4 hours. Water from these containers were poured back into tray and refilled with tray water in every 12 hours. Thus, predators were unable to kill the experimental mosquito larvae but could still disperse physical and chemical cues. During pupation, each newly formed

pupae was collected and transferred to individual micro centrifuge tubes with a small hole on the top, until it eclosed. Then, females from all the three environments were used to determine the effect of their environment. All experiments were conducted in a room with LD 12:12 h photoperiod and an average 25C temperature. All experiments were conducted in a room with LD 12:12 h photoperiod and an average 25C temperature.

2.2 Master cage preparation

Master cages of each environment were created by keeping 100 females from respective environments in cloth cages with 50 unrelated males of same age and reared under control conditions. Each cage was provided with a Petri dish containing 2g of cotton with 20 ml of 10% sugar solution, as food. Food was replaced every alternate day. Cages were left undisturbed to ensure mating in females. After 2 days, mosquitoes were given blood for 4 hours and blood fed females were collected and transferred to individual ovi-vials for laying eggs. Individuals who did not have blood meal were again given blood after 24 hours and those who took blood were transferred to ovi-vials.

2.3 Ovi vials preparation

Ovi vials can be used as a mosquito oviposition site (S.Ioshino et al.,2018). To prepare ovi-vials, at the bottom of a glass vial (90mm high X 22 mm diameter), 0.4 g of cotton was inserted. Ovi-Cones are where mosquitoes lay eggs and are made from a whatman filter paper folded into cone, after cutting out a triangle wedge of 90 from a circle with 2cm radius. This cone was pushed down the glass vials until its tip touches the cotton. Cone and cotton were moistened with 4 ml of tap water, so that it could be an oviposition site for blood fed females. The top of the vial was closed using 0.5 g of cotton and 2 ml of 10% sugar solution was injected in it, as food for mosquito. Food was given every alternate day. Blood fed females were kept in ovi-vials for 6 days to lay eggs. These ovi vials represented inert environments without any cues of predators, so the eggs were only influenced by maternal provisioning.

2.4 Feamale fecundity and offspring provisioning

FFemales were removed from ovi vials after 6 days and were kept in individual vials to check for their longevity. Ovistrips (Ovi cones) were removed and dried. To estimate female fecundity, I collected and counted all the eggs that were laid on ovi- cones. A subset of these eggs were imaged using Leica MZ57 microscope for egg size measurements. Egg surface area was measured using ImageJ software .

Egg strips were soaked in individual 140 mm petri dishes with 140 ml of water and 46.7 mg larval food (0.5 g / 1.5 L). The newly emerged larvae per mother were counted for estimating hatchability. Hatchability of individual females was calculated using the following equation. Hatchability = number of eggs hatched / total number of eggs laid.

2.5 Offspring (F1) larval environments

Newly emerged larvae from the same maternal environment were pooled together. Next, to generate predator experienced and naïve offspring from each maternal environment, offspring from each maternal environment were randomly assigned to one of three levels of predation environments, namely control, low predation and high predation. Offspring environments were given 2 letter codes with first letter indicating predation level in maternal environment (C/L/H) and the second the predation level in offspring environment (C/L/H). For example, individuals in an environment free of predators and with mothers that had experienced high level of predation would be coded as HC. I had 9 (3X3) such combinations of predation levels with 2 replicates of each. The larval density and food in each tray was the same as in the case of the first generation (70 larvae in 1.5 L water with 0.5 g larval food). The number of predators in the different levels of predation were the same as in the case of the maternal environment. These predators were fed with eight 4th instar mosquito larvae every day and any uneaten live larvae were removed from container after 4 hours. Water from these containers were poured back into tray and refilled with tray water in every 12 hours. Water lost due to evaporation was refilled in trays. During pupation, each newly formed pupa was collected and transferred to individual micro centrifuge tubes with a small hole on the top.

2.6 Behavioural assay

In order to measure the level of antipredatory behaviours shown by second generation individuals in different predation environments, we test individuals, of different larval stages, from all the 9 combinations with cues from the three predation levels and measure their behavioural response. Since there is only a narrow window between any two consecutive larval stages we failed to do all these combinations, so the extreme cases were chosen for the behavioural assay. Individuals who were either in control or high predaton and if their mothers were also from either control or high predation environments, were chosen to measure the level of anti-predatory behaviour in either control or high predation cue water. That is, random samples of larvae from four combinations (CC,CH,HC,HH) were tested in either control or high predation cue water). Fourth instar larvae were chosen for the assay as they are big enough to observe easily. Once the larvae were 4th instar, they were held individually in 50 ml containers with 30 ml of cue water and their behaviour was video recorded for 12 minutes. Treatment containers were tested at a time. Twenty larvae from each of the four treatments were video recorded in control cue waters, and twenty each from the four different treatments in high predation cue water. Neither test subject larva nor cue water were reused in this assay. Oonce the recording was over, subject larvae were immediately transferred back into their respective environments. We assumed that this brief experience in the behavioural test environment would not affect life history traits much.

2.6.1 Cue water Preparation

Experimental larvae were held in water treated in one of the two ways. Control water was made by keeping 2 trays containing 70 conspecific larvae in 1.5 L water with 0.5 g of larval food. These larvae were held in these trays from their first instar stage to 4th (Same old as the experimental larvae) while high predation cue water trays had 4 sham predators (in transparent 50 ml containers) along with these. In high predation cue water trays, water from predator containers were poured back into tray and refilled with tray water every 12 hours. These predators were fed with eight 4th instar larvae every 24 hours and any live uneaten larvae were removed from the container after 4 hours. On the day of the experiment, predators and conspecific larvae were removed from these trays to get cue water. In both control and high

predation cue water, solid materials like larval faeces, and larval food accumulated during larval growth. Other than these, high predation cue water trays had faeces from dragonfly nymphs and uneaten bits of prey larvae. These materials remained in the cue water while recording behaviour of subject larvae.

2.6.2 Observation protocol

The first two minutes of the recorded video were not considered for observation since this time was given for the larvae to get acclimatised to the new treatment water. From each 12 min clip, three sample time intervals were taken for observation ie, first one minute after acclimatization period (2:00 -3:00 min), middle one minute (6:30-7:30min) and last one minute (11:00-12:00min). Since larval thrashing (wriggle movement) activity makes them more conspicuous to the predators and this activity is most likely to result in predation(Chandrasegaran et al., 2017) in these intervals wriggle movement was observed to estimate the level of anti-predatory behaviour shown by subject larvae. Number of wriggle bursts and the number of wriggles per burst were noted along with the time taken for each burst.

2.7 Developmental time

The time taken by an individual to become a pupa from the time of soaking eggs was used as developmental time of that individual. Any newly formed pupa was collected and time taken was noted. Larval pools were checked every four hours for newly morphed pupae. This protocol was followed in both the maternal and offspring generations. Separate sets of analysis were conducted for males and females since there is a considerable time delay between them.

2.8 Adult traits: Longevity and wing length

To check if adult longevity was impacted by larval environment (and maternal environment for the second generation), the time duration for which each individual stayed alive was noted. In the first generation, adults were monitored every day and in the second generation every 12 hours. Wings of dead individuals were dissected out and their images were taken using Leica

MZ57 microscope. Wing lengths were measured with the help of ImageJ software.

2.9 Analysis

To check how predation environment would affect maternal fecundity and resource provisioning, eggs were manually counted and following analysis were done.

2.9.1 Number of eggs

I collected egg strips from ovi vials and recorded the total number of eggs laid by females over the duration of the trial. Total number of eggs laid by females can give hints about the maternal strategy to cope with the predation environment.

2.9.2 Hatchability

The propensity of eggs to hatch was calculated using the following equation. Hatching propensity = No. of eggs hatched / Total no. of eggs laid. The value of hatching propensity ranges from 0 to 1. Hatching propensity value can be useful to understand the effects of predation on hatchability of eggs.

2.9.3 Egg size

I took images of eggs from each mosquito using Leica MZ57 microscope and measured their egg size using imageJ software. Egg size can be attributed to the maternal resource investements.

2.9.4 Developmental time

Developmental time was calculated as the time from the soaking of the ovistip, to when a pupa was detected. Pools were observed in every 4 hours for pupae. Newly formed Pupae were collected from pools for further experiments. This protocol has followed in both the maternal and offspring generation. I conducted separate sets of analyses for males and females, since females considerably differ in development time from males.

2.9.5 Behavioural assay

Wriggle count and wriggle burst: Number of wriggle bursts and number of wriggles per burst made by larvae in three different time intervals of the video were manually counted. These two measures can be used to understand the level of anti-predatory behavior shown by larvae

2.9.6 Wing legth

Wings of dead adult mosquitoes were dissected and imaged using microscope. Later wing lengths were measure using imageJ software. Wing length is good estimate of adult body size.

2.9.7 Longevity

Adults were stored in centrifuge tubes and observed in every 12 hours for dead individuals. Then longevity was calculated as time duration from soaking of eggs to the death.

Chapter 3

Results

3.1 Female fecundity and Offspring provisioning

Blood fed females responded to the predation environment experienced during larval stage by producing larger eggs. Mosquitoes in both low and High predation environments laid eggs with significantly larger surface area than the adults in control. However, the average surface area of eggs laid by individuals from Low predation environment (average egg surface area = 73957.47 square micrometer) were slightly larger than that of High predation (average egg surface area = 73599.17 square micrometer).

The average hatching propensity was below 0.5 in all the three environments. In control, eggs seemed to have higher propensity to hatch than the eggs in predator treatments. Eggs produced by individuals from Low predation environment had least tendency to hatch. But these observed differences were not statistically significant. Also no difference was observed in egg counts among the mosquitoes in three different levels of larval predation experiences.

3.1.1 Egg size

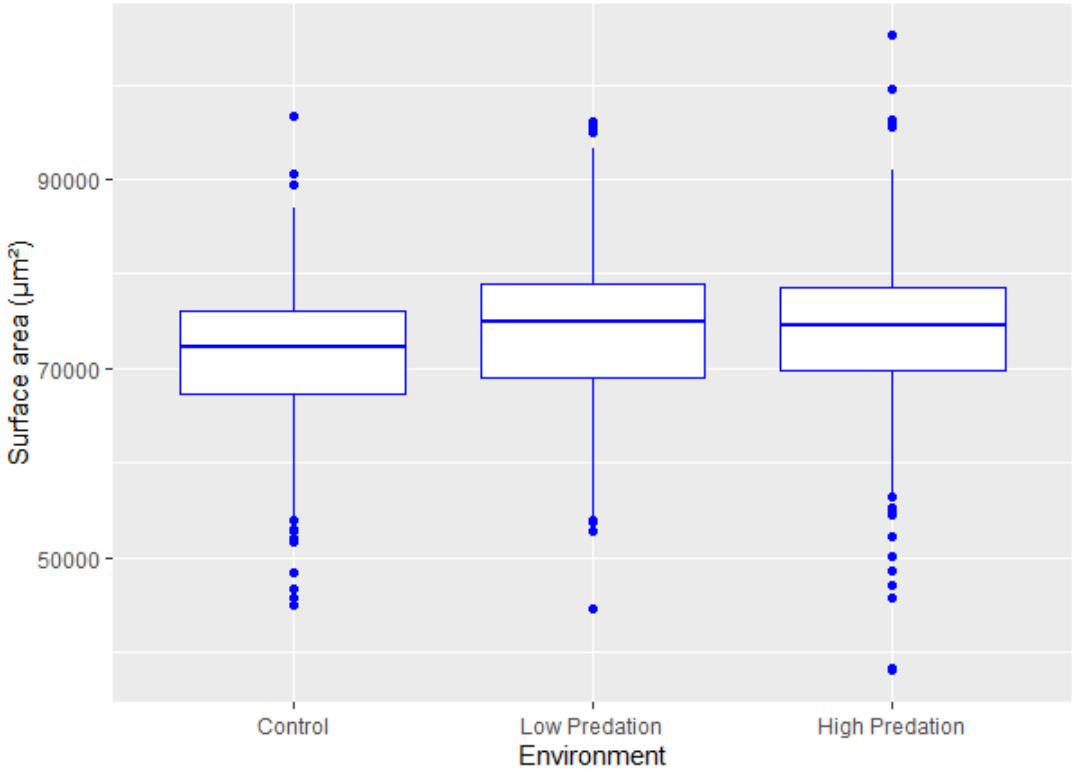

Figure 3.1: An egg size comparison of *Aedes aegypti* females, in response to the varying level of predation risk experienced during larval stage. Surface areas of eggs (from 31 control, 52 low predation and 44 high predation risk females) were measured to estimate the effect of predation risk experience on egg size.

Table 3.1: Coefficients of simple linear regression model for egg size as response.

	Estimate	Std. Error	t value	Pr(>| t |)
Intercept	71138.1	460.7	154.407	< 2e-16
HP Environment	2461.0	598.0	4.116	4.12e-05
LP Environment	2819.3	579.8	4.863	1.31e-06

3.1.2 Hatching Propensity

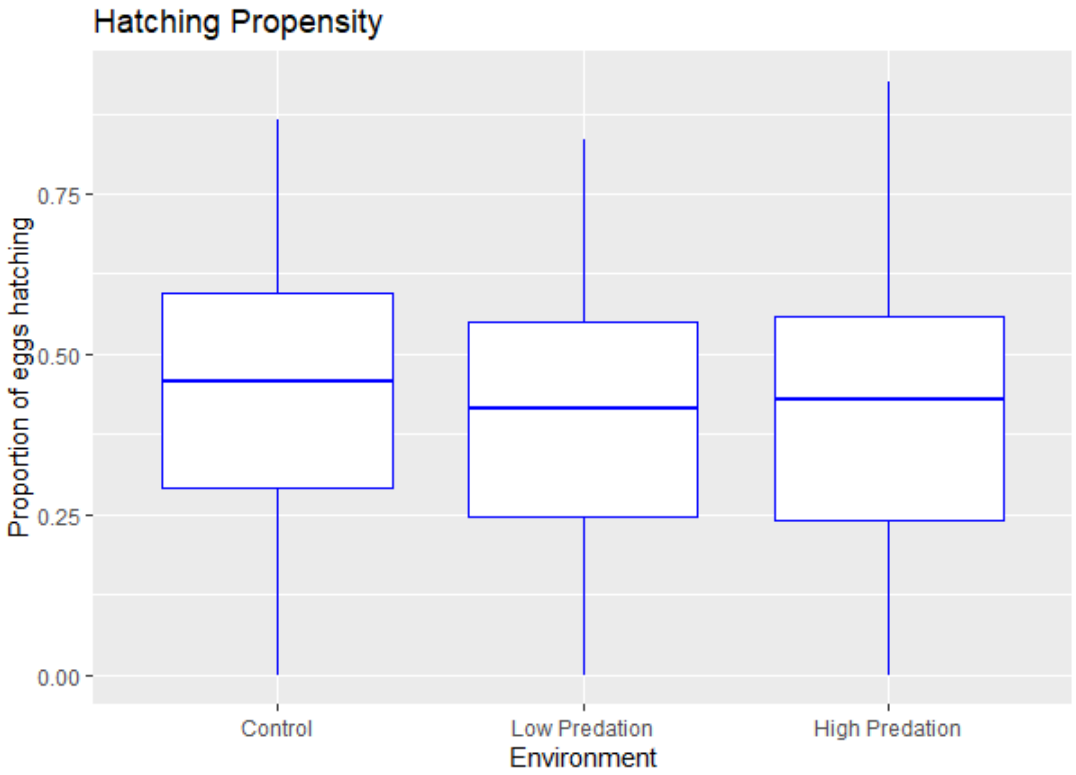

Figure 3.2: Hatching propensity of eggs as a function of predation risk level. Hatching propensity of eggs is estimated by calculating the ratio of number of eggs hatched to the total number of eggs laid.

Table 3.2: Coefficients of simple linear regression model for hatching propensity as response.

	Estimate	Std. Error	t value	Pr(>| t |)
Intercept	0.44430	0.03990	11.135	<2e-16
HP Environment	-0.04644	0.04993	-0.930	0.354
LP Environment	-0.01762	0.05175	-0.341	0.734

3.1.3 Egg count

Figure 3.3: A comparison of total eggs laid by individuals from control and predation risk treatments.

Table 3.3: Coefficients of simple linear regression model for egg count as response.

	Estimate	Std. Error	t value	Pr(>| t |)
Intercept	27.543	4.534	6.075	4.37e-09
HP Environment	8.501	6.250	1.360	0.1749
LP Environment	14.231	6.202	2.295	0.0225

3.2 Behavioural assay

To understand the level of anti-predatory behaviour shown by second generation individuals their larval movements were recorded. The total wriggle count and number of wriggles per burst made by mosquito larvae varied with the presence/absence of predation cues. When the maternal and offspring environments were same, mosquito larvae responded to predation cue

water by reducing the number of wriggle bursts made. On the other hand, when the maternal and growing environments were opposite, larvae tend to have more number of wriggle bursts in predation cue water than in control water. The highest average number of wriggle bursts (31.94) were observed when CC individuals were put in control water and the least average (22.78) for HH in high predation cue water. Larvae had less number of wriggles per burst in control than high predation cue water, when both the maternal and growing environments of offspring were same. But individuals having opposite maternal and growing environments made more wriggles per burst, if the trial cue water and growing environments matched. However maternal environment did not have significant effect on number of wriggle bursts. The main effects of maternal and growing environment on number of wriggles per burst were also not significant, but their interactive effect was significant.

3.2.1 Number of wriggle bursts

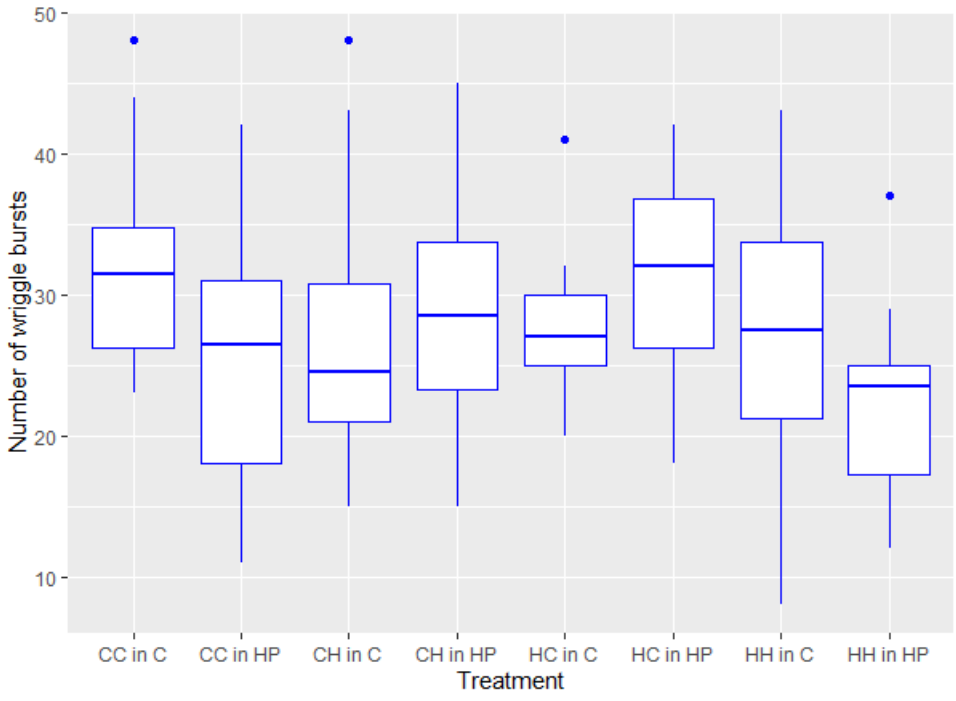

Figure 3.4: Total wriggle count produced by individuals from different combinations of maternal and growing environments, exposed to Control(C)/ High predation (HP) cue water. Control water contains conspecific cues and High predation cue water contain conspecific cues and high predation threat cues.

Table 3.4: Analysis of variation in number of wriggle bursts of mosquito larvae: Results from a linear regression model with number of wriggle bursts as response, and maternal and offspring environments as explanatory variables. Model coefficients, F test statistic and p values are shown.

Term	Df	Sums of squares	F value	p
Maternal environmet	1	45.56	0.7174	0.39845
Offspring environment	1	339.17	5.3401	0.02229
Maternal envt: Offspring envt	1	108.51	1.7171	0.1922
Residuals	140	8847.1		

3.2.2 Wriggles/ burst

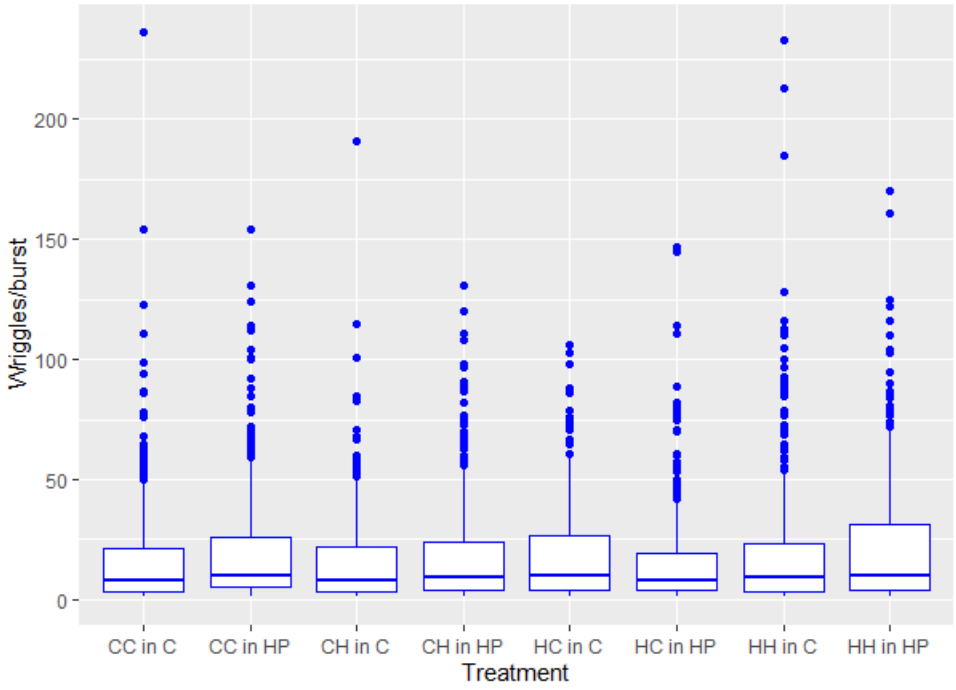

Figure 3.5: Number of wriggles per burst (wriggle burst length) observed in individual mosquito larvae from four different combinations of maternal and growing environments, exposed to Control(C)/ High predation (HP) cue water. Control water contains conspecific cues and High predation cue water contain conspecific cues and high predation threat cues.

Table 3.5: Analysis of variation in wriggles/bursts of mosquito larvae: Results from a linear regression model with wriggles/bursts as response, and maternal and offspring environments as explanatory variables. Model coefficients, F test statistic and p values are shown

Term	Df	Sums of squares	F value	p
Maternal environmet	1	709.32	1.3957	0.2375
Offspring environment	1	1362.42	2.6807	0.1017
Maternal envt: Offspring envt	1	5283.8	10.424	0.001255
Residuals	3570	1809603		

3.3 Developmental time

Mosquito individual varied in their time taken to mature in different larval environments. Since there is a time delay between male and female development time duration, males and females were separately analysed. In all the pools the developmental time duration of females were greater than males. However, both F0 males and females matured at shorter durations in predation threat environments. In F0 males and females, the average time development in three different environments decreased with the predation risk. The difference between control and low predation was very small but high predation individuals showed noticeable difference in their development time. Reduced development time enables the individuals to morph into adults and leave the pool earlier and thereby reducing the chances of predator attack on them. In F1 generation individuals having high predation maternal environment followed the same trend. However, offspring of control mothers in high predation environment had development time almost same as offspring in control environment. However, CL responded by reducing developmental time. Offspring from low predation maternal environments showed varying developmental time in sexes and treatments. Two way Anova results shows that there are statistically significant differences between sexes and between environments in F0 developmental time. In F1generation for males and females, two separate linear models were used with maternal and offspring environments as predictors. The main effect of maternal environment on development time wasn't significant in males and females. However maternal environment had significant interactive effect on developmental time.

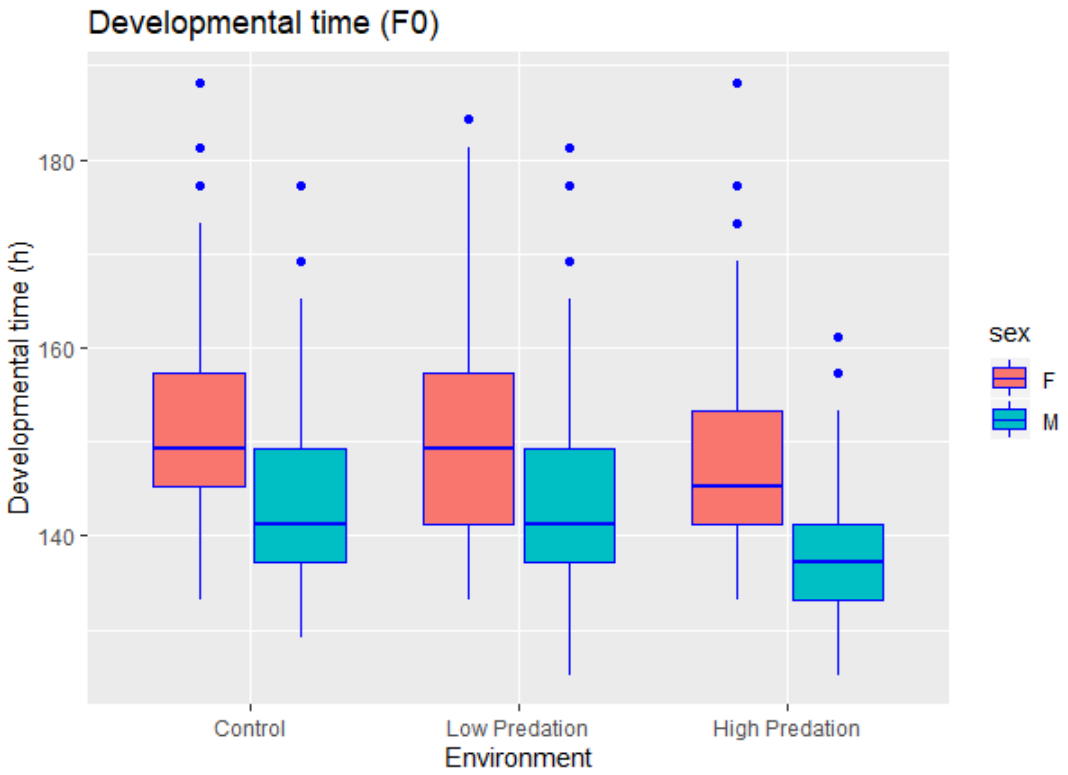

Figure 3.6: Developmental time (in hours) taken by F0 males and females under different larval environments:

Table 3.6: Analysis of variation in developmental time of F0 mosquito larvae: Results from a linear regression model with developmental time as response, and larval environment and sex as explanatory variables. Model coefficients, F test statistic and p values are shown.

Term	df	Sums of squares	F value	p
Sex	1	24117	286.154	2.2e-16
Environment	2	5448.8	32.325	2.19e-14
Sex:Environment	2	113.76	0.6745	0.5096
Residuals	1152	97145		

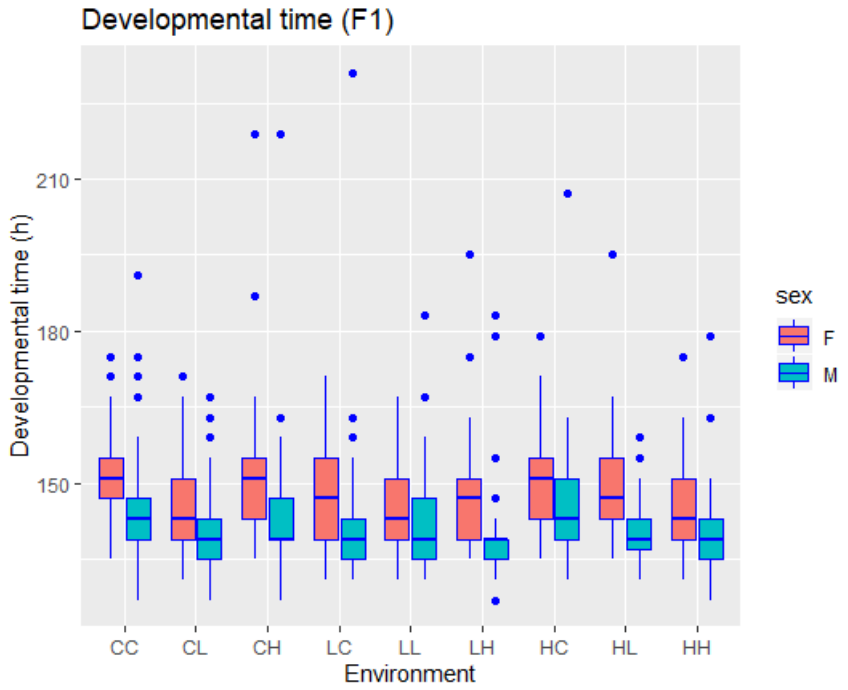

Figure 3.7: Developmental time (in hours) taken by F1 males and females under different larval environments:

Table 3.7: Analysis of variation in developmental time of F1-male mosquito larvae: Results from a linear regression model with developmental time as response, and maternal and offspring environments as explanatory variables. Model coefficients, F test statistic and p values are shown.

Term	df	Sums of squares	F value	p
Maternal environment	2	340.72	1.5893	0.20507
Offspring environment	2	933.15	4.3526	0.01335
Maternal envt: Offspring envt	4	923.19	2.1726	0.07092
Residuals	512	54389		

Table 3.8: Analysis of variation in developmental time of F1-female mosquito larvae: Results from a linear regression model with developmental time as response, and maternal and offspring environments as explanatory variables. Model coefficients, F test statistic and p values are shown.

Term	df	Sums of squares	F value	p
Maternal environment	2	214.76	1.1477	0.31817
Offspring environment	2	709.55	3.792	0.02319
Maternal envt:Offspring envt	4	1256.3	3.4204	0.008968
Residuals	508	46646		

3.4 Adult mosquito traits

3.4.1 Longevity

Longevity of F1 males were affected by the environment they were growing in. Maternal environment or offspring environment did not have any influence on longevity of females. Irrespective of maternal environment, level of predation risk experienced during larval stage had a negative effect on longevity of F1 males. Males from control environment had longer lifespan than individuals from predation environments. Longevity of males were higher than females in all the treatments.

3.4.1.1 Longevity (F0)

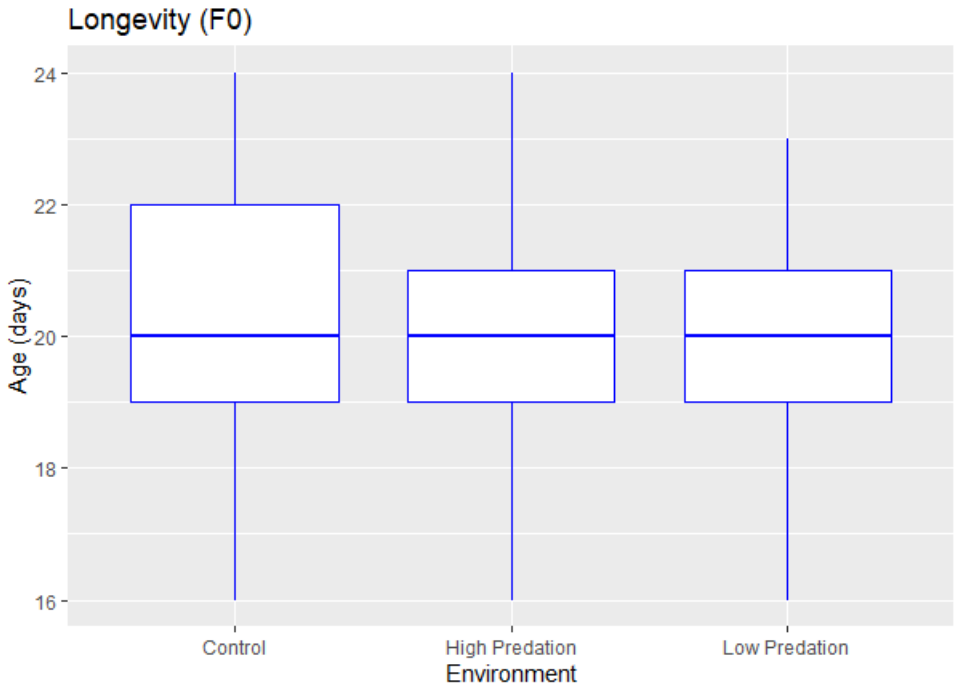

Figure 3.8: Number of days F0 females remained alive as a function of the level of predation threat experienced during larval stage.

Table 3.9: Coefficients of simple linear regression model for longevity as response.

	Estimate	Std. Error	t value	Pr(>| t |)
Intercept	20.2308	0.1933	104.658	<2e-16
HP Environment	-0.3238	0.2669	-1.213	0.226
LP Environment	-0.3085	0.2641	-1.168	0.244

3.4.1.2 Longevity (F1)

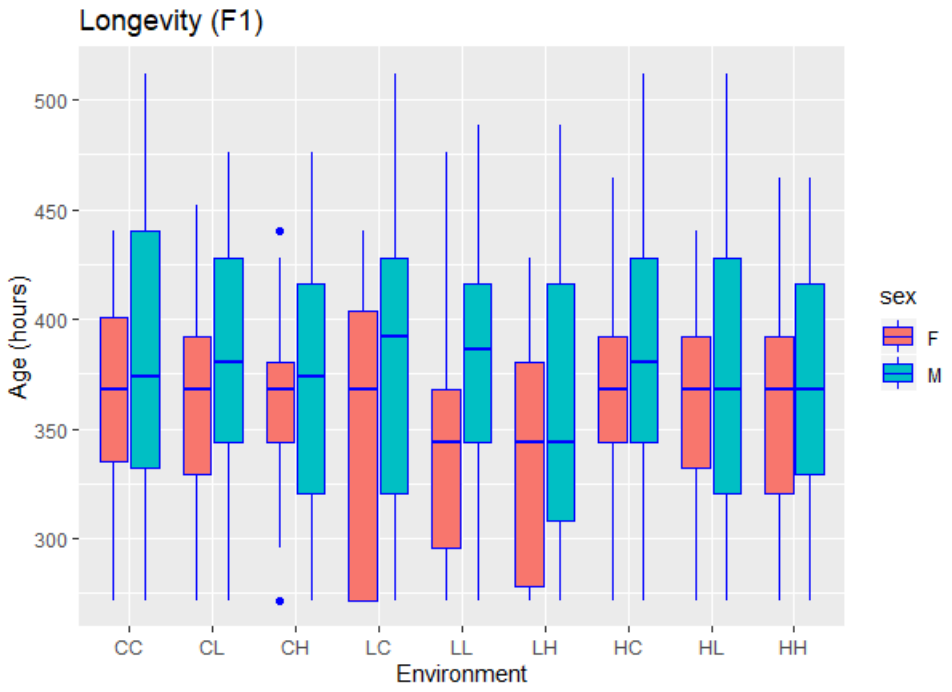

Figure 3.9: Number of days F1 males and females remained alive as a function of the level of predation threat experienced during larval stage.

Table 3.10: Analysis of variation in Longevity of F1-male mosquitoes: Results from a linear regression model with longevity as response, and maternal and offspring environments as explanatory variables. Model coefficients, F test statistic and p values are shown.

Term	df	Sums of squares	F value	p
Maternal environment	2	2438.6	O.3111	0.73279
Offspring environment	2	24895.7	3.176	0.04265
Maternal envt: Offspring envt	4	9759.4	0.6205	0.6481
Residuals	468	1840166		

Table 3.11: Analysis of variation in Longevity of F1-female mosquitoes: Results from a linear regression model with longevity as response, and maternal and offspring environments as explanatory variables. Model coefficients, F test statistic and p values are shown.

Term	df	Sums of squares	F value	p
Maternal environment	2	8089	1.5006	0.2241
Offspring environment	2	3776.1	0.7005	0.4969
Maternal envt: Offspring envt	4	1130.2	0.104	0.9811
Residuals	443	1203660		

3.4.2 Wing length

The impact predation risk on mosquito body size was evident on F0 female wing length. Control individuals had larger wings compared treatment individuals. Main effects of maternal environment and offspring environment were not significant on wing length variation, but their interactive effect was significant.

3.4.2.1 winglength (F0 females)

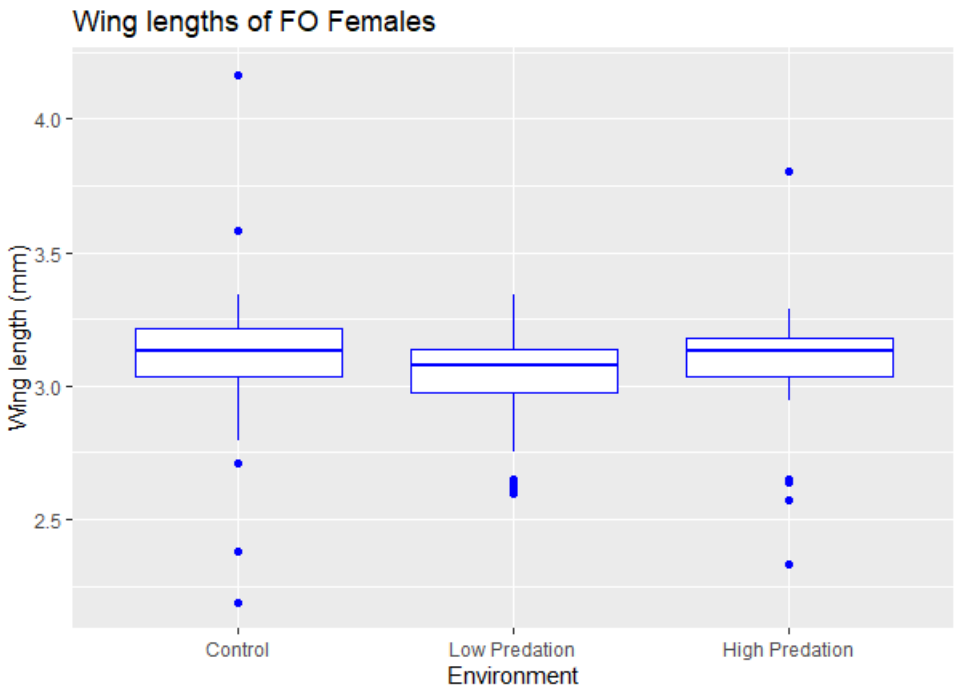

Figure 3.10: A comparison of wing lengths of F0-adults from different larval environments.

Table 3.12: Coefficients of simple linear regression model for F0-female wing length as response.

	Estimate	Std. Error	t value	Pr(>| t |)
Intercept	3.1115	0.02447	127.147	<2e-16
LP Environment	-0.07257	0.03304	-2.197	0.0292
HP Environment	-0.01931	0.03474	-0.5567	0.5789

3.4.2.2 wing length (F1 males and females)

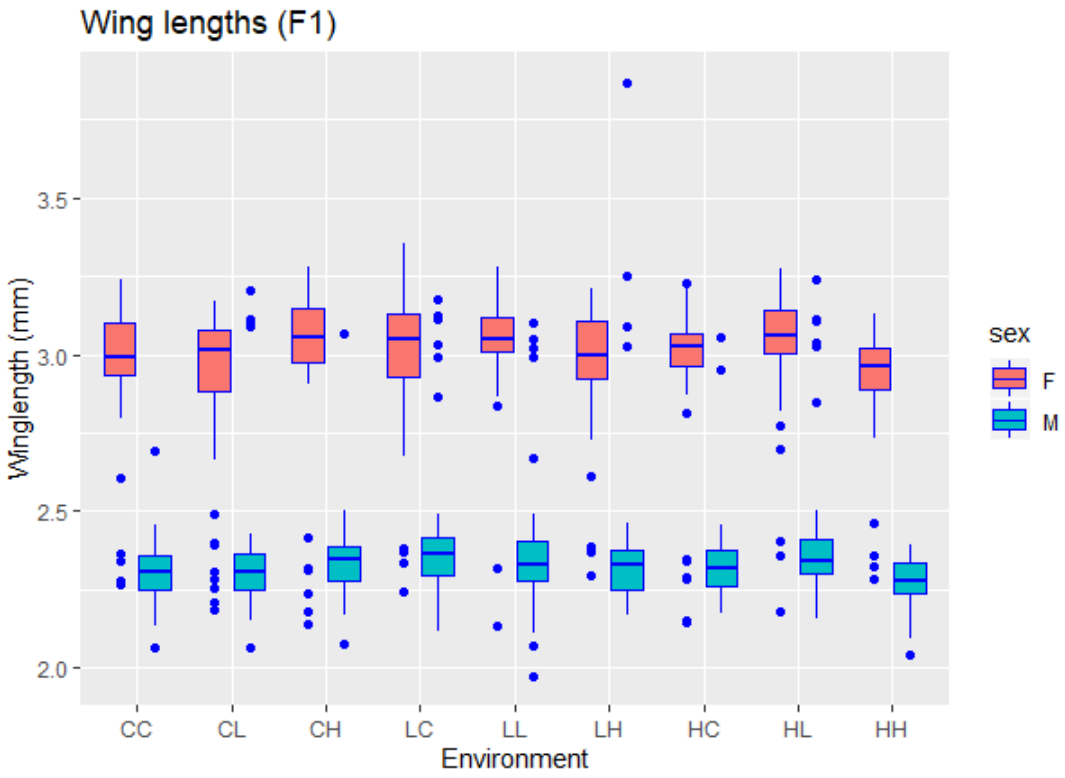

Figure 3.11: Wing lengths of F1 individuals as a function of larval environments.

Table 3.13: Analysis of variation in Wing lengths of F1-male mosquitoes: Results from a linear regression model with wing length as response, and maternal and offspring environments as explanatory variables. Model coefficients, F test statistic and p values are shown.

Term	df	Sums of squares	F value	p
Maternal environment	2	0.22339	2.6331	0.0729
Offspring environment	2	0.17399	2.0508	0.1298
Maternal envt: Offspring envt	4	0.55718	3.3485	0.01018
Residuals	473	19.677		

Chapter 4

Discussion

In this study I examined if the predation risk experienced during larval stage can affect i) mother's development, maternal investments in eggs and life history traits like longevity and body size (Wing length) and thereby (ii)influence offspring development, anti-predatory behaviour and other life history traits. To test this I made three different levels of predation risk environments namely Control(C), Low predation(LP) and High predation(HP) and the traits of mosquito larvae growing in these environments were observed. Offspring from each environment were further grown in these three environments to check the effect of current versus maternal environments on traits of interest. I predicted that individuals in predation environment would have reduced developmental time in order to morph into an adult faster and escape from the pool, as dragonfly nymph's aquatic life lasts much more than mosquito's. Constant exposure to predators can have negative impacts on prey physiology and thereby can compromise over body size and longevity. Also I predicted that these predators exposed females may invest in eggs such a way that the offspring will have better chances of survival in a predation environment and these offspring might perform increased level of anti-predatory responses. My results show that the predator exposed individuals had reduced developmental time compared to control individuals. Individuals matured in shorter time duration, as the level of predation risk increased in the growing environment. In F0 males and females, the average time development in three different environments decreased with the predation risk. The difference between control and low predation was very small but high predation individuals showed noticeable difference in their development time. Reduced development time enables the individuals to morph into adults

and leave the pool earlier and thereby reducing the chances of predator attack on them. This results are consistent with the Jonas Dahl Barbara L. Peckarsky's results on mayflies and Crowl and Covich's results on Physella virgata virgate snails. The main effect of maternal environment on development time wasn't significant in F1 males and females. However maternal environment had significant interactive effect on developmental time.

The average number of eggs laid in three environments were similar. Also no difference was observed in hatching propensities of eggs from different environments. However, Eggs laid by individuals grown in predation environments had significantly larger surface area compared to control eggs. This can potentially explain the comparatively reduced developmental time in offspring of predator exposed females. Larger eggs can be packed with more nutritional resources which can promote the faster growth and can improve predator escape behaviours of offspring (Tigreros et al.,2017). So producing larger eggs can have survival advantages in predation environments.

In a predation environment, upon sensing physical, chemical or both cues, mosquito larvae respond by reducing the number of wriggle bursts and the number of wriggles per burst, and increasing time spent on resting (Chandrasegaran et al., 2017; Kesavaraju amp; Juliano, 2004). However, in my results larvae responded to predation cues by reducing number of wriggle bursts only when the maternal and offspring environments were same. On the other hand, when growing environment and maternal environments were opposite they responded to predation cue water by wriggling more.However the main effect of maternal environment was not significant. The main effects of maternal and growing environment on number of wriggles per burst were also not significant, but their interactive effect was significant. From this it is clear that maternal effects of predation interact with the environmental context in which larvae develop and manifests on larval traits.

Mosquito longevity was not affected by the maternal environment. Growing environment had influence only on males. Presence of predators in the environment had negative impacts on male longevity. Wing lengths F0 individuals were negatively affected by predator presence and in F1 generation maternal environment interacted with offspring environment and influenced wing

length. In summary this study demonstrates that the impact of predation risk in Aedes aegypti larvae carries over different life stages and across generations, through maternal effects.

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