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Evaluation of a fitness-disrupting chemical for the control of *Aedes albopictus* mosquitoes

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ABSTRACT

Evaluation of a fitness-disrupting chemical for the control of *Aedes albopictus* mosquitoes

By: Alexandra Perez

Though *Aedes albopictus* mosquitoes have historically been considered secondary vectors of arboviruses such as dengue and chikungunya, the species has recently undergone a dramatic global expansion due to its ecological plasticity and strong competitive aptitude. Likewise, the pathogens vectored by *Ae. albopictus* have a high potential for geographic spread into areas where these vectors invade. Without vaccines or medical treatments for most arboviruses transmitted by *Aedes* mosquitoes, vector control is our most useful tool in disease prevention. Insect growth regulators such as pyriproxyfen (PPF) have been used to control mosquitoes at varying life stages due to its inhibition of morphogenesis, reproduction, and embryogenesis. Although PPF is effective and versatile in laboratory experiments, its use in mosquito control programs is limited by the high-cost, labor-intensive methods that are required to have a tangible, population-level impact. This study aimed to evaluate the use of PPF ingestion by female *Ae. albopictus* on inhibiting mosquito growth and reproduction at varying life stages. A dietary formulation of PPF combined with sugar solution was created, and sugar feeders were designed to allow mosquitoes to feed while reducing tarsal exposure to PPF. After access to PPF/sugar feeders containing 0, 1, 5, or 10 mg/mL PPF for 24 hours, female *Ae. albopictus* were reared in-laboratory and individualized for oviposition. Survival, fecundity, and fertility were determined for each female, and any transgenerational effects of parental ingestion of PPF were observed in the pupation and adult emergence rates of offspring. A combination of these experimental results and published literature were used to inform two stage-based matrix projections to determine the population-level effects of dietary PPF on *Ae. albopictus.* Overall, adult survival and fertility were not impacted by any of the tested PPF doses. Fecundity, pupation, and adult emergence were significantly reduced following ingestion of 5 mg/mL PPF (24%, 47%, and 43% reductions, respectively). Fecundity was reduced by 23% following treatment with 1 mg/mL PPF, and pupation was reduced by 22% following treatment with 10 mg/mL PPF. Treatment with 5 mg/mL PPF was selected for calculation of the matrix model parameters as this dose had the most consistent effect on reproductive output and outcome across all mosquito life stages. Population projections with no intervention grew exponentially over 100 days ($\lambda = 1.05$) while populations receiving treatment were projected to decay within that same time frame ($\lambda = 0.94$). The use of PPF/sugar feeding stations, in combination with current regimes, could provide a mosquito control strategy that acts through chemical and biological pathways to target mosquitoes at different life stages in a design that can be implemented easily at the household level.

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GLOSSARY OF TERMS

Abundance	Total number of individuals in a population
Arbovirus	Virus transmitted by an arthropod
Competitive aptitude	Capability of an invasive species to occupy a targeted ecological niche by exploiting the same resources as pre-existing species
Ecological plasticity	Ability to adapt to a large range of habitats
Emergence	Development of a pupa into an adult
Fecundity	Realized reproductive output, measured as the number of eggs laid by a single female
Fertility	Number of viable offspring produced by a single female, measured as the number of eggs to hatch per egg clutch
Life stage	Distinct stage of development in the life cycle of a mosquito (i.e., egg, larva, pupa, adult)
Oviposition	The act of laying eggs
Pupation	Development of a larva into a pupa
Reproductive outcome	The end result of a reproductive output
Reproductive output	Individual reproductive success, measured by fecundity and fertility per female
Stage duration	The number of days an individual spends within a stage of development in the life cycle
Sterilization	Inability to produce viable offspring
Transgenerational effect	Transmission of an adverse effect to offspring as the result of exposure in the parental generation
Vectorial capacity	Potential of a vector to transmit a pathogen

INTRODUCTION

Mosquito-borne pathogens are a major public health concern, with arboviruses dominating the global burden of disease [1-3]. Each pathogen is maintained by a cycle involving its specialized vectors and susceptible human and/or animal hosts [4]. Unambiguously named from the Greek word for “unpleasant” [5], *Aedes* mosquitoes are responsible for the transmission of arboviruses such as chikungunya (CHIKV) and dengue (DENV), along with many other, less notable infections throughout the world [1-6]. *Aedes aegypti*, a domestic, day-biting mosquito that preferentially blood feeds on humans [4], is usually considered the primary vector of these pathogens throughout Africa, Asia, and the Americas [7-8]. Though a number of *Aedes* species possess the ability to vector these same arboviruses [8], *Aedes albopictus* has been implicated as their most notorious secondary vector as viral isolations of CHIKV and DENV continue to be reported from field collections of the species on every major continent [8-13].

Ae. albopictus is widely considered to be a nuisance species known for its aggressive behavior and painful bites [13-14]. From the perspective of entomology, abundance of eggs and adults are used to determine the risk of exposure to mosquito-borne pathogens in a particular area [14-15]. However, since these nuisance behaviors are not significantly associated with increased mosquito productivity [14], *Ae. albopictus* has historically been considered a mere nuisance and unimportant vector of DENV, particularly in areas where the species co-exists with the primary vector, *Ae. aegypti* [13-14].

Changes in distribution and incidence of arboviral diseases are influenced by anthropogenic activities that inadvertently modify environmental conditions and the population dynamics of disease vectors [14-17]. Following the spread of *Ae. aegypti* and *Culex pipens*, *Ae. albopictus* has undergone a dramatic global expansion facilitated by human activities [13-16].

From its origins in the temperate and tropical regions of Asia, the species has spread to Europe, Africa, and the Americas over the last four decades [13]. This invasion represents the third instance of human activities enabling the global spread of a known mosquito vector [13].

These recent changes in the distribution of *Ae. albopictus*, in combination with its relevant biological traits, highlight its potential importance in the current and future emergence of pathogens. Likewise, the pathogens vectored by *Ae. albopictus* have a high potential for geographic expansion into areas where these vectors are present. As they continue to afflict increasingly larger portions of the world's population, it is crucial to consider the role these mosquitoes play in current and future emergences of arboviruses and the strategies used in disease mitigation and prevention.

Biology and Behavior of *Ae. albopictus*

The successful invasion of *Ae. albopictus* is rooted in its unique biological and behavioral traits in comparison with other mosquitoes of the *Aedes* genus. The species has been able to thrive in a wide range of habitats due to its ecological plasticity and strong competitive aptitude [13]. Though endemic to tropical and subtropical environmental conditions [13], the many subtypes of *Ae. albopictus* has a broad climactic threshold that has led to their establishment in more temperate regions [13,17-21]. Certain populations continue to breed in areas with mean temperatures as low as 10°C [13,22]. When temperatures dip below these thresholds, *Ae. albopictus* adapt by entering dormancy [22-25]. Their eggs are also able to withstand desiccation during extremely cold temperatures by undergoing diapause, or delayed hatching in response to adverse environmental conditions [13,18,20-22]. European strains of *Ae. albopictus* eggs have been shown to hatch following exposure to -10°C after diapausing in comparison to tropical strains with a lower temperature threshold of -2°C [23]. Ostensibly, the niche of invasive

populations has shifted beyond the species' native range so much that we now require a new set of expectations when trying to make predictions about *Ae. albopictus* population dynamics [20].

These traits have enabled *Ae. albopictus* to establish itself in areas with lower mean temperatures and lower annual rainfall than is required by other mosquito species, such as *Ae. aegypti* [13,17-25]. Recently, human mediation through climate change and urbanization has increased the competitive advantage of *Ae. albopictus* over resident populations of heterospecific mosquitoes, such as the observed decline of local *Ae. aegypti* populations in Brazil due to larval competition in shared aquatic habitats in the years following *Ae. albopictus* invasion [26-27]. Additionally, the competitive exclusion of *Ae. aegypti* by interference mating of *Ae. albopictus* (satyrization) has been documented in the United States [27]. Though reproductive interference occurs in many insect species, the mechanism of satyrization of *Ae. aegypti* by *Ae. albopictus,* in which interspecific mating prevents subsequent conspecific mating, appears to be unique to this particular interspecies dynamic [28]. Laboratory and field observations show that *Ae. albopictus* males are capable of satyrizing *Ae. aegypti* females, significantly decreasing their fitness and preventing the generation of hybrids [28]. Once established in new areas, factors such as satyrization appear to favor the ecological success of *Ae. albopictus* in competition with other resident mosquito species [13].

The *Ae. albopictus* niche includes a preference for sparse vegetative areas in rural, urban, and suburban habitats [29-30]. Taking advantage of the expanding urban landscape, juveniles are able use both natural and artificial water sources as aquatic larval habitats [29-30]. Females are aggressive daytime biters, able to complete a blood meal through multiple biting attempts [21,31-32]. They are also opportunistic feeders with a wide range of blood hosts (humans, domestic and wild animals, reptiles, birds, amphibians) [21,31-32]. Such diverse feeding

behavior is considered one of the main reasons *Ae. albopictus* is not a primary DENV vector to humans, compared to the anthropophilic species *Ae. aegypti* [13]. Recent evidence, however, suggest that *Ae. albopictus* is becoming increasingly endophilic with adults able to survive in indoor environmental conditions for long periods and larvae able to develop in indoor breeding sites [33-34]. Some populations found in urban areas have even developed anthropophilic behavior, such as seeking out humans as blood hosts [13,35].

Impact of *Ae. albopictus* on Health

The biology and behavior of *Ae. albopictus* lend the species high vector competence for the arboviruses it transmits. Due to its opportunistic feeding behavior, *Ae. albopictus* can act as a bridge vector, transmitting arboviruses between humans and wild or domestic animals [21,31-33]. Their aggressive biting allows females the opportunity to transmit pathogens to numerous new hosts within each gonotrophic cycle [21,31-32]. In laboratory experiments, *Ae. albopictus* has demonstrated the ability to transmit twenty-six arboviruses, though field surveys have only isolated CHIKV and the four DENV serotypes in wild populations [11,13]. The ecological plasticity, competitive aptitude, and high vector competence of *Ae. albopictus* merit attention in the public health sphere as the species continues to invade high-risk geographic areas.

In Europe, a non-endemic area where *Ae. aegypti* does not occur at all, *Ae. albopictus* is responsible for several outbreaks of CHIKV and possibly DENV [36]. Since its first appearance in Albania in 1979, *Ae. albopictus* has been observed in twenty European countries where it is now widely established as a nuisance species [36]. It is considered to be the invasive vector that poses the greatest threat to public health in Europe in terms of arboviral diseases [36]. It occupies most areas of Italy, the most heavily infested European country, and the east Mediterranean coastline of France [36]. *Ae. albopictus* was the primary vector responsible for the CHIKV

outbreaks in Italy in 2007 and 2017, in France in 2010, and the DENV outbreaks in France and Croatia in 2010, which constituted the first DENV cases to be reported in Europe since Greece in the 1920's [36].

Worldwide, *Ae. aegypti* is usually considered the primary vector of DENV in areas where the four serotypes are endemic [13,37]. When *Ae. albopictus* co-exists with *Ae. aegypti*, it is systematically viewed as a secondary vector [13,38]. However, *Ae. albopictus* appears to be a dominant candidate for historical outbreaks of DENV, including reports of an illness clinically compatible with DENV that were reported in the mid-nineteenth century in tropical Asia prior to the introduction of *Ae. aegypti* [37]. Though these strains of *Ae. albopictus* occur far from human habitation and feed on a wide variety of animals and birds [37], the species is suspected of being the primary vector in outbreaks of DENV in Reunión from 1977 to 1978, Hawaii from 2001 to 2002, in Reunión again in 2004, and Mauritius in 2009 [36-37]. *Ae. albopictus* was also implicated in the outbreaks of CHIKV in Reunión from 2005 to 2007 [36-37]. With the virtual absence of its usually vector, *Ae. aegypti*, field collections of mosquitoes in these areas revealed that CHIKV and DENV were only detected in *Ae. albopictus*. [38].

Following the major epidemic of CHIKV that occurred from 2005 to 2007 across the Indian Ocean Islands [36-39], analyses of full-length viral sequences have revealed the acquisition of a single adaptive mutation (E1-A226V) that provides selective advantage for replication and transmission of this viral strain following infection of *Ae. albopictus*, specifically [39-40]. The emergence of this new viral strain demonstrates that invasive *Ae. albopictus* can interfere with native host-vector-pathogen dynamics in a way that enhances viral transmission [39-40]. The ability of *Ae. albopictus* to adapt to new environments and transmit pathogens highlights the importance of the surveillance and control of this species.

Control Strategies for *Ae. albopictus*

In the absence of vaccines or effective drugs for the treatment of most mosquito-borne diseases, vector control is a critical step in disease prevention [41-42]. Conventional control for *Ae. albopictus* includes habitat and environmental control strategies, such as removal of aquatic larval habitats and the use of larvicides in potential breeding sites [43]. Chemical-based control through outdoor and peri-domestic space spraying with insecticides has been used to reduce adult mosquito density [43]. Some alternative methods utilize biological control methods through the release of laboratory-reared sterile insects or seek to reduce human-vector contact [43].

However, *Ae. albopictus* is notoriously difficult to control due to its elusive behavior, cryptic habitats, and reproductive biology [44-46]. The energy reserves of *Ae. albopictus* influence their preference for shaded resting areas such as bushes and shrubs that are protected from wind. *Aedes* mosquitoes are weak flyers with a flight range limited to about 200 m [45-46], and they prefer to fly relatively close to the ground [45-46]. *Ae. albopictus* has also been shown to avoid flying through open terrain and seek small, shallow water containers for oviposition [47]. Given the outdoor behavior and low dispersal ability of *Ae. albopictus*, insecticide-based vector control has focused on truck-mounted ultra-low volume (ULV) spraying, street-based thermal fogging, and residual barrier spraying [41]. Outdoor and peri-domestic space spraying has had limited effectiveness against this species as the chemical fails to reach their cryptic habitats [42,48-51]. Other vector control methods such as the use of larvicides and removal of breeding sites are also difficult to implement fully. Since *Ae. albopictus* uses skip oviposition to distribute eggs among multiple breeding sites within each gonotrophic cycle [49-50], it is often difficult to determine suitable sites for the placement of larvicides, traps, or conduct environmental management [50-51].

The potential range of *Ae. albopictus* does not necessarily represent the areas where infected mosquitoes exist or virus transmission is more likely to occur [51]. Because of their global distribution, these mosquitoes are widely considered a nuisance that adversely impacts daily life, even for those in low-risk geographic areas where *Ae. albopictus* play little to no role in pathogen transmission [13]. Peak feeding times occur during the early morning and late afternoon, and their aggressive behavior allows them to bite successively even after being swatted away [51]. Following their discovery off the coast of Queensland, Australia, a cost-benefit analysis was conducted to evaluate the economic threats posed by the establishment of *Ae. albopictus* in Brisbane [52]. Even though there was no reported presence of competent vectors of DENV, CHIKV, or ZIKV in Brisbane at the time, public demand for intervention was still projected to increase in response to the presence of a considerable biting nuisance [52]. The local population's willingness to pay for access to outdoor social and recreational areas free from *Ae. albopictus* was high enough for an eradication program to be more cost-beneficial than allowing for the mosquitoes' establishment [52]. Management strategies for nuisance mosquito species may use economic, ecological, and political factors to restrict mosquito densities to tolerable levels [53], which may not always coincide with actual human annoyance or serve as effective disease prevention strategies [53-56]. Intensive vector control programs in surrounding islands for over ten years has prevented *Ae. albopictus* from establishing in mainland Australia [57-58]. However, this accomplishment has been achieved because intervention programs were prepared with a thorough understanding of *Ae. albopictus* biology, acceptance of emerging vector control technologies, and recognition of the imminence of the species' invasion should they be regarded as anything less than a major public health problem [58].

The effects of *Ae. albopictus* on public health have been minimized since many consider the species to have a low capacity for transmitting arboviruses to humans and because it is a secondary vector in areas where it co-exists with *Ae. aegypti*, the primary vector of concern [13]. This has created a deficit in vector control and surveillance methods that are designed with the species-specific behaviors of *Ae. albopictus* in mind. Effective vector control depends on specific intervention strategies that target susceptible vector behaviors [59]. A greater understanding of the behaviors of *Ae. albopictus* that underpin its interactions with humans, pathogens, and the environment is needed in order to have tangible effects in reducing pathogen transmission [41]. Consequently, public health initiatives have shifted towards understanding certain biological and genetic mechanisms to mitigate the drawbacks associated with traditional chemical and environmental vector control methods [41,59-60].

Innovations in vector biology have fueled a renewed interest in sterile insect technique (SIT) for mosquito population control as such methods have low propensity for genetic resistance, can target vectors at varying life stages through different pathways, and offer an approach for controlling species when limited control options are available [61-63]. Rather than aiming to kill mosquitoes indiscriminately with neurotoxic substances, these methods rely on sterilization and prevention of vector behaviors that are associated with disease transmission [61-63]. Successful in agriculture, SIT involves rearing and releasing sterile male insects to block reproduction of wild-type fertile females. Sterility can be achieved through irradiation with gamma or x-rays, genetic engineering, or other laboratory processes [41,63]. The related incompatible insect technique (IIT) induces sterilization in males through vertical transmission of the endosymbiont bacteria *Wolbachia* which renders females unable to transmit pathogens [41,63]. These strategies eliminate the drawbacks posed by the elusory behaviors and cryptic

habitats of *Ae. albopictus* but are still highly dependent on mosquito behavior [41,64-65]. Both SIT and IIT rely on mating with wild females, but the mating competitiveness of laboratory-reared males will have a direct impact on these methods' effectiveness [41,64-65].

Insect Growth Regulators (IGRs)

Sterilization through the use of insect growth regulators (IGRs) is one possible solution to the challenge of genetic engineering or expensive irradiation equipment. Insect growth and reproduction are regulated by complex physiological processes involving hormones. Maintaining desired effects on targeted tissues depends on a precise schedule of synthesis, degradation, and titer changes that adjusts the quantity of hormone present in the hemolymph [62,66]. Juvenile hormones (JHs) are a group of structurally-related sesquiterpenoids involved in development and maturation [62,66-69]. In mosquitoes, JH-III titers are regulated by the balance between biosynthesis and secretion of the hormone by the *corpora allata*, as well as its degradation and uptake from the hemolymph by tissue uptake and excretion [67]. The amount of JH present controls the rate of molting and metamorphosis [62,67].

In females, JH continues to play a role in egg development into adulthood. A gonotrophic cycle proceeds with stages of previtellogenesis, ovarian resting stage, and vitellogenesis [62,67,70]. Females emerge with immature primary follicles which grow into mature previtellogenic follicles in the following 48 to 72 hours [62,67,70]. During this time directly following emergence, JH levels rise in response to the mosquito's first nectar meal [62,67,71]. JH targets the ovarian follicle to stimulate previtellogenic oocyte development, promotes competence in the ovary and fat body to respond to coordinating reproductive hormones (ovarian ecdysteroidogenic hormone (OEH) and 20-hydroxyecdysone (20E)), and induces mating and blood-feeding behaviors [62-63,66,70-72]. JH levels fall after a blood meal when OEH is

released and 20E levels rise during the ovarian resting stage [62,67,70]. JH remains low during the vitellogenic stage of egg development when the oocyte develops the yolk needed for the developing embryo [67,70]. Egg production in mosquitoes is cyclic, leaving the opportunity for subsequent processes of egg development. After the blood meal, tissues lose the competence to respond to 20E and OEH [62,67,70]. JH levels rise again to initiate previtellogenic growth of the next, secondary follicle and ensure competency is regained for target tissues in the presence of their respective hormones before the next blood meal [62,67,70].

Insect growth regulators include juvenile hormone analogs (Methoprene, pyriproxyfen) and chitin synthesis inhibitors (diflubenzuron, novaluron) [73]. JH mimics, such as pyriproxyfen (PPF), are synthetic juvenile hormones that take advantage of the anti-metamorphic effects of JH and inhibit adult emergence when exposed to mosquito larvae [62-63,66-72.74-75]. PPF has been administered in public health initiatives as a larvicide due to its low toxicity to mammals and high reactivity in small amounts [75]. Exposure methods currently being tested rely on release of laboratory-reared insects or contamination of larval breeding sites which pose challenges when the targeted vector is *Ae. albopictus* with its elusory behaviors and cryptic habitats [76].

Use of Pyriproxyfen (PPF) for Mosquito Control

According to a 2020 review on the use of PPF for the control of *Aedes* mosquitoes, 79.1% of studies focused on the efficacy of PPF granules, use of PPF in ultra-low volume (ULV), thermal fogging (TF) and fumigant technologies, insecticide resistance, and autodissemination or horizontal transfer [76]. PPF granules and dusts are solid forms of the chemical that can be applied to aquatic larval habitats as a larvicide. Depending on the concentration and time since application, PPF can inhibit adult emergence in juvenile mosquitoes

[76-79]. PPF as a larvicide has a high potential for control of *Aedes* mosquitoes, especially species such as *Ae. aegypti* that oviposit in small, temporary water containers in urban areas [76-79]. Conversely, full treatment coverage may be difficult to achieve to control a species like *Ae. albopictus* where a wide variety of oviposit sites would need to be reached [80]. Studies on PPF granules also face discrepancies in the duration that the active ingredient remains at a high enough concentration to be effective [76]. When used to target *Ae. albopictus,* quick deterioration would need to be counteracted with frequent reapplications of the larvicide, including to cryptic habitats [76]. Area-wide use of PPF as a larvicide would be labor-intensive and involve deploying and maintaining numerous sites [69,76].

Most studies evaluating the use of PPF in ULV, TF, and other space spraying techniques are performed using *Ae. aegypti*. Of the two studies involving *Ae. albopictus*, one utilized truck-mounted ULV area-wide applications of PPF [81] while the other used small-scale barrier treatments of PPF in urban and suburban residential yards [82]. While both studies found efficacy in laboratory experiments, neither found that PPF applied through space spraying was effective in reducing adult *Ae. albopictus* populations in the field [81-82].

Autodissemination is a process that utilizes female mosquitoes to transfer PPF or other IGRs to breeding sites or resting sites. During oviposition, residual PPF is transferred to the water contained in the aquatic larval habitat and results in juvenile mortality [68-69,83]. Adult females that subsequently oviposit at that site may also take up lethal concentrations of PPF which is then further disseminated [68-69,83]. Autodissemination with PPF can successfully increase the mortality rate of juveniles that were exposed [68-69,76,83]. However, the level of success achieved using PPF autodissemination varies greatly with method of exposure [76]. Most studies rely on PPF exposure through tarsal contact with surfaces contaminated with PPF

dusts or granules [76]. This method has had success in laboratory experiments [68-69,76,83-84], while other studies investigating ULV for autodissemination were not found to be effective [85].

Topical application of PPF to females during the adult stage is also shown to reduce egg production and changes blood-seeking behaviors [68-69,77,86-88]. Exposure affects egg development, egg production, and reduces the hatching of eggs [69,89]. As many methods currently involved in SIT and IIT may change mosquito mating and reproductive behavior, sterilization through PPF is a promising tool that could be used in these strategies. In some cases, exposure to PPF prevents females from producing any viable offspring during one or subsequent gonotrophic cycles [69,78,89-90]. Horizontal transfer of PPF between adult mosquitoes has also been demonstrated. Laboratory-reared male mosquitoes can transfer PPF dust to females during mating as a potential mechanism for indirect sterilization [91-92]. These studies demonstrated that PPF could be transferred by males to oviposition and resting sites (and subsequently transferred to females) and directly to females during mating. This approach could combine the benefits of SIT and enable higher coverage of mosquito breeding sites while addressing the barrier presented by the cryptic habitats preferred by *Ae. albopictus* that are difficult to locate during conventional larviciding [91-93].

While these methods are promising, they remain impractical in some contexts due to the chemical's high cost, slow response time, poor stability, and species specificity [43,66,70]. It has also been found that the direct effects of PPF on female fecundity and fertility change depending on the formulation of PPF, time of exposure, application method, and the species of mosquito being targeted [62,94-95]. PPF changes the egg-laying behavior of adult females depending on the time of exposure relative to the blood meal [88,93,96-97]. When females are treated with PPF within 24-hours of the blood meal, they are still able to oviposit and autodisseminate the

chemical to breeding sites [88,96]. However, when treated with PPF farther from the blood meal, females may not even attempt to lay eggs [88,96]. While autodissemination of PPF has been successful in laboratory studies, it is questionable if this method would be useful against mosquito species which use larger bodies of water as breeding sites [88]. However, exposure to PPF has been studied extensively for its sterilizing effects on *Aedes* mosquitoes [68-69,83-84,87-88,98] and can reduce the reproductive capacity of adults [69,87,99] depending on dosage and time of exposure in relation to the blood meal. Even so, mosquito control programs would need to be prepared to address the particulars of exposure method and targeted mosquito species when developing control plans involving autodissemination of PPF.

Evaluations of PPF performance have largely been performed with methods that do not vary in chemical formulation or exposure pathway [76,80,88]. Many studies rely on solid PPF in the form of dusts and granules on adult mosquitoes even though the majority of PPF commercial products are sold as emulsifiable concentrates that operate through residual surface contact [76-80,88]. Hustedt et al. (2020) identified several uncommon uses of PPF currently being studied, including: bed nets and mesh, release blocks, sugar baits, candles, topical treatments, ovitraps, and resin sticks [76]. While these may be novel uses for PPF, most continue to rely on tarsal contact for exposure [76,100]. For the control of mosquitoes such as *Ae. albopictus* that are not strong flyers, autodissemination strategies would depend on release of laboratory-exposed adult mosquitoes or the use of autodissemination stations [96]. If tarsal contact with PPF remains the primary method of exposure, these strategies would require a high degree of control and precision during administration, as PPF can have unintended negative effects on non-target populations with similar hormone systems and remain unchecked in aquatic ecosystems [86,101].

Dietary Formulations of PPF

Previous studies have used sugar feeders as a vehicle to target adult mosquitoes with insecticides and larvicides for mosquito control. Attractive-toxic sugar baits (ATSBs) operate by delivering insecticides and other toxic substances to adult mosquitoes through the ingestion of sugar solution containing a minimal effective dose of the active ingredient [78,100,102-104]. Though PPF is not considered to be toxic to adult mosquitoes, some studies combining PPF exposure and the lure of sugar baits have found success in mosquito control [103-107]. Adult mosquitoes are drawn away from natural sugar sources [102], and PPF exposure and subsequent autodissemination occur with contact with the sugar feeder. For *Ae. albopictus*, this exposure method would not necessitate locating cryptic habitats. Most of these ATSB designs continue to rely on tarsal contact with the insecticide to facilitate exposure [76]. Fulcher et al. (2014) investigated the use of an ATSB containing either 1% boric acid or eugenol that would also expose mosquitoes to PPF through surface contact [102]. These ATSBs mixed with PPF provided control of adult mosquitoes and additional control of larvae in the surrounding larval habitats [100,101-107]. Though the use of ATSBs and other sugar feeders is a novel method for delivering PPF to adult mosquitoes, this technique is subject to the same concerns as other strategies that rely on autodissemination through residual surface contact [100].

There is potential for a dietary formulation of insecticides combined with sugar feeders to reduce reproductive output in female mosquitoes. Schlein et al. (1990) used an ATSB resting above a larval habitat to disseminate the bacteria *Bacilluss phaericusto* to larvae[108]. The mosquitoes that had ingested the bacteria died and contaminated the larval habitats they fell into [108]. ATSBs mixed with PPF have been evaluated in a similar way for smaller insects, such as ants, for which PPF is toxic to adults [103-107]. Meola et al. (1996) studied the toxicity of PPF

ingestion to adult cat fleas and their eggs [109]. Instead of sugar, adult fleas ingested PPF combined with bovine blood through an artificial membrane system, resulting in both adult mortality and laying of non-viable eggs [109]. ATSBs containing insecticides have been evaluated for many species but may have limited range for contaminating larval habitats if the exposed adults die shortly after contact.

For mosquitoes where PPF is not considered toxic to adults, ingestion of dietary PPF offers the same benefits of chemical exposure through ATSBs with the additional possibility for autodissemination. Scott et al. (2017) evaluated the use of PPF ingested by adult *Ae. albopictus* for the control of larvae through fecal deposits [100]. When PPF is delivered through tarsal contact, it is autodisseminated by physical transfer of the larvicide from the mosquito's body to the breeding site [100]. When ingested, this study demonstrated that PPF can be disseminated into aquatic larval habitats through adult mosquito ingestion and excretion as it is transferred to surfaces through fecal deposits [100]. This study is also notable in that the dietary PPF formulation was dispensed in collagen sausages to prevent tarsal exposure, confirming that any transfer of PPF to surfaces occurred as a result of ingestion [100].

Anderson et al. (2016) studied the addition of dietary PPF to ATSBs containing sublethal doses of insecticides boric acid and eugenol. Rather than relying on its larvicidal effects, this study also determined the impact of dietary PPF on adult survival and reproductive potential in *Ae. aegypti* [110]. It concluded that PPF, both alone and in combination with other insecticides, did not consistently affect adult survival, fecundity, or fertility [110]. In fact, the study reported wildly different results for egg hatchability both between and within treatments [110]. However, this may be due to the fact that some treatments used a combination of PPF and sublethal doses of insecticides, which could have had an effect on the mosquitoes' feeding behavior and the

amount of sugar ingested [110]. The researchers also did not take measures to ensure PPF exposure occurred only occurred through ingestion rather than tarsal contact [110].

Though this paper was published after this project's experiments, Silva et al. (2021) has been one of the only studies to investigate both the effects of PPF on adult mosquito reproductive potential and autodissemination [111]. Females fed with ATSBs were found to excrete PPF, and their fecal deposits affected emergence inhibition of exposed larvae [111]. Females received a blood meal after PPF exposure and were allowed to oviposit. In contrast to the findings from Anderson et al. (2016), this study observed decreased fecundity and fertility of adult female *Ae. aegypti* that had ingested PPF through an ATSB [111]. However, similarly to the previous paper, this study did not go to any lengths to prevent tarsal contact with PPF [111].

Hypotheses

I hypothesize that there will be no statistically significant reduction in the survival of female *Ae. albopictus* following PPF ingestion compared to females fed a sugar solution without PPF. Of the survivors, I also hypothesize that *Ae. albopictus* females treated with dietary PPF will exhibit reduced reproductive output (decreased fecundity, decreased fertility) and adverse reproductive outcomes (decreased pupation and adult emergence among offspring) compared to untreated females, with the effects being dose-dependent. Further, I hypothesize that the effect of PPF on *Ae. albopictus* reproduction will translate into population-level effects due to the potential fitness reduction impaired by this dietary formulation.

Research Objectives

The objectives of this project are: 1) to formulate a sugar solution combined with PPF to be administered to adult *Ae. albopictus* using a feeding station that reduces PPF-tarsal contact; 2) to confirm survival of female *Ae. albopictus* treated with dietary PPF; 3) to evaluate the dose-

dependent effect of PPF ingestion on reproductive output of female *Ae. albopictus* and the reproductive outcome of their offspring; 4) to predict the potential population-level implication of fitness reduction due to dietary PFF administration.

METHODOLOGY

This project used two methods of data collection: laboratory-based experiments and population-level modeling (Figure 1). Laboratory experiments took place in Atlanta, GA, USA, at Emory University using colony-raised mosquitoes. Models were constructed, projected, and analyzed using R statistical software [112].

Mosquito Rearing

The *Ae. albopictus* colony used in this study originated from eggs obtained from the CDC, Centers for Disease Control and Prevention, Atlanta, GA, USA. The strain has been reared in-lab for over two decades, and it is known to be susceptible to all insecticides and chemical groups. Oviposition substrates containing eggs (Gainesville strain, MRA-804) were submerged in 1000 mL of deoxygenated water in plastic larval rearing trays (30×26×6 cm) and hatched inside an insect growth chamber (CARON Products, Marietta, OH, USA) set at 29°C and 80% relative humidity. To prevent larval performance from being affected by diet and density-dependent competition [107], this method was used to produce 500 mosquito larvae per tray. Larvae were fed daily with 10 mg/mL bovine liver powder solution (1 mL per fifty larvae) until pupation.

While in the pupal stage, individuals were isolated in clear polystyrene vials covered with netting to prevent adult males and females from mating upon emergence. A mouth aspirator was used to move adult males from vials into Bugdorm insect cages (30×30×30 cm, MegaView Science Co., Ltd., Taiwan); males were maintained in Bugdorms inside of the insect growth chamber and fed 10 mg/mL sugar solution *ad libitum*.

Test Chemical

The chemical tested in this project was pyriproxyfen (2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine, analytical grade, 100% purity, PESTANAL, Sigma-Aldrich, St. Louis, MO, USA). A 10% stock solution was created by dissolving PPF in hexane solvent in a 10:1 ratio (w/v).

Susceptibility of Pupae to PPF

The effect of PPF on colony pupae was determined in order to confirm the efficacy of the laboratory-made PPF stock solution. Batches of 10 pupae were added to 500-mL white plastic cups containing 99 mL of water and 1 mL of the above mentioned 10 mg/mL PPF/hexane stock solution. Controls consisted of 99 mL of tap water and 1 mL of hexane, and experimental controls consisted of 100 mL of water. Each treatment and control were replicated three times. All cups were capped with netting to prevent the escape of emerged adults (Figure 2).

Cups were monitored daily and continued until all pupae died or emerged successfully as adults. Cumulative totals of dead pupae and emerged adults were derived, and data were pooled for each treatment.

The percentage of emergence was calculated as the number of emerged adults divided by the initial number of pupae used multiplied by 100. The percentage of adult emergence inhibition was obtained according to the following formula:

$$\%\ emergence\ inhibition = 100 - \left(\frac{\%\ emergence\ from\ treated\ water}{\%\ emergence\ from\ untreated\ water} \times 100\right)$$

Preparation of PPF Sugar Feeding Stations

Specialized sugar feeding stations were created to ensure that mosquitoes ingested PPF sugar solution while preventing PPF exposure through tarsal contact (Figure 3A). The sugar

feeders consisted of a plastic 0.2-mL micropipette tip that protruded from the end of a black, 1.5-mL microcentrifuge tub with a hole drilled into the tip (Figure 3B,3C). Cotton soaked with sugar solution was inserted into the micropipette tip, and the microcentrifuge tube supported the tip in an upright position. Mosquitoes had access to the sugar-soaked cotton through the opening in the micropipette tip and were able to rest on the plastic while feeding (Figure 3D). With this design, tarsal contact occurred with the plastic rather than the sugar solution (Figure 3E).

The sugar solution was made by combining white cane sugar with water in a 10:1 ratio (w/v). This mixture was stirred, heated to 100°C, and cooled to room temperature. The 10 mg/mL PPF/hexane stock solution was left overnight in an Erlenmeyer flask under a fume hood to evaporate the hexane solvent. The PPF remaining in the flask was diluted with the 10 mg/mL sugar solution to provide the prescribed concentrations of PPF/sugar solution. Green food coloring was added to the formulation in order to visualize ingestion by the mosquitoes (Figure 3A).

The treatments used included 1, 5, and 10 mg/mL of PPF combined with 10 mg/mL sugar solution (1000, 5000, and 10,000 ppm, respectively) (Figure 1). When exposure to PPF dust is achieved through contact with contaminated surfaces, reduced reproductive capacity is observed in adult *Aedes* mosquitoes, depending on dosage and time of exposure in relation to the blood meal [68-69,83,87-88,99]. When female *Ae. aegypti* were exposed to surfaces contaminated with four different concentrations of PPF (0.75%, 0.075%, 0.0075%, and 0.00075%), the fecundity, fertility, pupation, and emergence of their respective clutches were reduced [113]. This occurred for each female, whether exposed before or after the blood meal, and reproductive disruption was significant for all, from the lowest to highest concentrations [113]. The sterilizing effects of

topical PPF have repeatedly been shown for female mosquitoes in a number of species [87-90,114].

However, there has been little evidence to suggest that comparably small concentrations have similar effects when exposure occurs through ingestion. Scott et al. (2017) created ATSBs containing PPF in concentrations of 0.5, 1, 5, and 10 ppb (PPF was in the form of a 10% emulsifiable concentrate) [100]. This resulted in adult emergence inhibitions of 42, 34, 46, and 57%, respectively, in larvae exposed to the fecal deposits of females following PPF ingestion, though effects on fertility and fecundity were not carried out [100]. Anderson et al. (2016) saw no significant effect on fecundity and fertility in adult female *Ae. aegypti* following ingestion of 0.5% PPF (PPF was in the form of a 10% emulsifiable concentrate) mixed into ATSBs, though this could have also been affected by the sublethal doses of boric acid and eugenol missed into some of the ATSBs [110]. Silva et al. (2021) was published after this project's experiments had concluded, but it is worth mentioning that this study prepared PPF/sugar solution by diluting solid PPF dust in hexane to create concentrations of 5, 50, 100, and 500 ppm [111]. These higher PPF concentrations, in comparison to the relatively lower concentrations ingested by mosquitoes in the previously mentioned papers, yielded a 41% reduction in the number of eggs laid and a 92% reduction in the number of eggs hatched by exposed females compared to controls, though there was no differential effect on fecundity or fertility between PPF doses [111]. The high concentrations of PPF (1, 5, and 10 mg/mL) in this experiment were chosen to reflect the larger doses of PPF that appear to be needed to reduce reproductive output through ingestion in comparison to the lower doses that are needed to have similar effects through topical application. Untreated controls received 10 mg/mL sugar solution delivered in specialized sugar feeders.

The final sugar feeders were created by inserting cotton soaked with 1 mL of PPF/sugar solution into the micropipette tips before they were secured into the microcentrifuge tubes. The sugar feeders were then secured to the bottom of mosquito cages using tape to prevent them from tipping over. In comparison with previous sugar feeder designs, the final version reduced tarsal contact with PPF and ensured that any observed reductions in reproductive output were the result of PPF ingestion (Figure 3).

Assessing Adult Female Survival

One-day old females were used in experiments for ingestion of dietary PPF. Females were held in 500-mL white plastic cups covered in netting during treatment. Each cup was prepared with a feeding station containing 1 mL of the prescribed sugar solution (Figure 4). Upon emergence, a mouth aspirator was used to move adult females from their individual polystyrene vials into cups (Figure 5). To ensure engorgement of each female, each cup contained 15 females, and experiments were replicated three times for a total of 45 females per treatment (Figure 4). Females were given access to PPF/sugar feeding stations for 24 hours following emergence. The number of dead females for each treatment was recorded 24 and 48 hours after PPF exposure was initiated.

Evaluation of Female Reproductive Output

After being allowed access to PPF/sugar feeding stations for 24 hours, all surviving females from each treatment were moved from cups to Bugdorms, each containing an equal number of males (Figure 5). For example, if all 45 females survived for one treatment, all 45 females were moved to a Bugdorm containing 45 males. One Bugdorm was used for each treatment.

Cotton saturated with 10 mg/mL sugar solution in 15-mL conical centrifuge tubes (Falcon, Corning, NY, USA) was provided to males and females *ad libitum*. Males and females were maintained in Bugdorms together for five days after access to PPF/sugar feeding stations ended (Figure 3). Females were fed with rabbit blood using artificial blood-feeding devices (Hemotek Membrane Feeding System, Hemotek Ltd, Blackburn, UK) at five days post emergence.

Adult females were individualized for oviposition in order to determine fecundity for each female replicate (Figure 1). Forty-eight hours after the blood meal, females were moved from Bugdorms into 50-mL conical centrifuge tubes (Falcon, Corning, NY, USA) covered with netting (Figure 5). The falcon tubes were filled with 15-mL of water and lined with strips of damp egg-laying substrate (Pellon, 931TD Fusible Midweight Interfacing, white) along the water margin (Figure 6). Strips of netting were placed at the bottom of each tube up to the water line to prevent mosquitoes from drowning. Cotton balls saturated with 10 mg/mL sugar solution were provided to the females *ad libitum* through the netting that covered each tube. Individualized females were kept inside of an insect growth chamber (CARON Products, Marietta, OH, USA) set at 29°C and 80% relative humidity for seven days. After females were removed from Bugdorms and individualized, male mosquitoes were euthanized in a freezer at -20°C.

After being allowed to lay eggs for seven days, individualized females were also euthanized in a freezer at -20°C. Egg substrates were removed from Falcon tubes, and the number of eggs laid by each female was recorded. Fecundity was calculated as the number of eggs laid with each female acting as a replicate. Humidity chambers were prepared by lining the bottoms of plastic Tupperware bins (33×20×13 cm) with damp cotton. After eggs were counted,

egg substrates were placed vertically in humidity chambers, and left in closed containers at room temperature/indoor conditions for 48 hours to dry.

Eggs from each dried substrate were transferred to a 60-mL white plastic cup and hatched inside of an insect growth chamber using 30 mL of deoxygenated water. Larvae were fed daily with 10 mg/mL of bovine liver powder solution (1 mL per fifty larvae). The number of larvae hatched in each cup was counted at 24, 48, and 72 hours after hatching. Fertility for each female was calculated as the number of larvae present at 72 hours after eggs were hatched. Individual hatching rates were also calculated as the number of eggs that hatched divided by the number of eggs that were laid by each female.

Evaluation of Transgenerational Effects of Dietary PPF

Larvae were transferred to new 60-mL white plastic cups at 72 hours post hatching. Cups were covered in netting and monitored daily until all larvae emerged successfully as adults or died in the juvenile stage as either larvae or pupae. Cumulative totals of offspring at each developmental stage (larvae, pupae, and emerged adults) were derived for each parental female and treatment. Pupation was calculated as the number of pupae that emerged out of the initial number of larvae per clutch, and adult emergence was calculated as the number of adults to successfully emerge out of the total number of pupae per clutch.

Statistical Analyses of Experimental Data

Analyses of data were carried out in R and RStudio [112]. Adult survival was treated as a binary variable (0=dead, 1=alive) and analyzed using a binomial generalized linear model (GLM) to test for the significance between treatment concentrations. The control group was used as the reference for the estimation of regression coefficients and odds ratios (OR). Individuals that did not survive in the time between PPF exposure and individualization (19 in total) were

excluded from all further analyses. Fecundity and fertility were analyzed as binary (0= no eggs/viable eggs; 1=presence of eggs/fertile eggs) and as counts of eggs/viable eggs per female. For the binary data on fertility and fecundity, a binomial GLM was used, whereas for the counts, a Poisson GLM. The overall fecundity/fertility data followed a Poisson distribution. Effect plots were generated from the best fit models to show the treatment effects on each parameter. Furthermore, the relative impact of PPF on each life history trait was evaluated by estimating the efficacy of each treatment, following the equation: EFF=OR for binomial models or EFF=(1-IRR) for count models. EFF ranges from 0 to 1, and represent the relative (proportional) reduction of a parameter due to PPF. Chi-square tests were used to quantify the statistical difference between PPF and controls, for the binary estimates of survival and ovipositing behavior.

Mathematical Model Development

Like all mosquitoes, the life cycle of *Ae. albopictus* is staged-based in nature (Figure 7) [115]. PPF exhibits both anti-metamorphic and sterilizing effects on mosquitoes, depending on the life stage in which exposure occurs [62,67,70]. Adult emergence is inhibited in mosquitoes exposed as juveniles, and mortality occurs at the larval or pupal stage [77-79]. Individuals that do manage to emerge as adults are not always stronger for it [116]. Biological parameters of the adult life stage that are impaired by juvenile exposure to PPF and other IGRs include delayed development, damage to the midgut, development of abnormal ovaries, and reduced longevity, wing length, body size, and overall fitness [88,116-118]. PPF exposure in the adult life stage of females reduces fecundity, fertility, and changes reproductive behavior [88,116-118]. Exposure to adult males can indirectly affect these conditions in females when PPF is transferred during mating [69].

A stage-based matrix projection was selected due to the differential effects of PPF on mosquitoes at varying life stages and the need to evaluate how these varying mortality and development rates for each life stage would change mosquito population dynamics under different treatment regimes. Based on the descriptions in Lefkovitch (1965), this matrix projection is a population vector that is multiplied iteratively by a transition matrix that details the probabilities of survival and development within and between each life stage across a discrete interval of time [119]. For each model output, a daily population vector is produced in each iteration from the multiplication of the transition matrix and the population vector from the previous time step (Figure 8). Since the population is divided into unequal groups or life stages, no assumptions are made about how the duration of a life stage may vary between individuals [119]. This method has been used to make predictions about many insect populations, including several species of mosquito vectors [120-126]. For this experiment, the stage-based transition matrix was beneficial for separating the growth and development probabilities and productivities by life stage to reflect the impact of PPF on mosquito development at each stage in turn.

The life stages used in each model include the four mosquito life stages: egg, larva, pupa, and adult. In addition to the three non-adult stages, the adult stage was further broken down into total adults and females as PPF affects all mosquitoes in the juvenile stage, regardless of sex, but only induces a sterilizing effect on adult females (Figure 9) [116].

Two models assessing the population-level effects of dietary PPF on mosquito development were parameterized using the results from our experimental data and published literature (Table 1). The first naïve model was constructed as a deterministic, stage-structured population model with the aim of projecting a local *Ae. albopictus* population uninhibited by external control strategies (Figure 9). Factors considered from our experimental data included

time elapsed between life stages and adult survival. The average time to pupation and adult emergence across eggs laid by untreated females were used as estimates of daily stage change rates when calculating growth and development probabilities for the transition matrix. Since the controls from this study had abnormally high mortality due to the nature of the methodology, egg mortality, fertility, pupation, and adult emergence were estimated using published literature in comparison with our results from mosquito colony rearing (Table 1).

Many field and laboratory experiments have demonstrated that mortality of *Ae. albopictus* eggs is highly dependent on air temperature, humidity, and the strain's desiccation tolerance [115,120,124-129]. Egg mortality was set to 33% and fertility was set to 67% based on previous studies that reported *Ae. albopictus* egg mortality and fertility at temperatures and relative humidity similar to the conditions used in our insect growth chamber (29°C, 80% relative humidity) [120,127,129]. These values are consistent with previous mosquito colonies reared in this lab using eggs from this strain (data not shown). Diet and density-dependent competition during the larval stage affect larval survival and time to pupation [130]. In our experimental conditions, 500 larvae were maintained per rearing tray with 1000 mL of water and fed with 1 mL of 10 mg/mL liver powder solution per 50 larvae daily. Pupation was set to 92% and adult emergence was set to 97% based on a study conducted with laboratory larvae that were reared under similar diet and density conditions [130]. Females in this study were individualized for egg laying and were not given the choice of multiple oviposition sites. Daily fertility per female was set to 40 eggs, comparable to the ovipositing behavior of *Ae. albopictus* in other laboratory experiments who chose ovipositing sites containing few or no preexisting larvae [130]. A 1:1 sex ratio between males and females was assumed [115]. Longevity of adults was

set to 22 days with a gonotrophic cycle of 7 days, in line with both experimental conditions and published literature [115,123].

The naïve model was contrasted with the alternate model to explore the population-level effects of PPF treatment (Table 2). Experimental data was used to modify the parameters of the naïve model to reflect the transgenerational effects of PPF ingestion on stage-specific survival and development. Treatment with 5 mg/mL PPF was selected for the alternate model because this dose had the most consistent effects on development and survival across all life stages. Parameters for fertility and adult survival remained consistent between the naïve and alternate models because there was no statistically significant difference in either of these variables between the treatment and control groups (Tables 2). Fecundity, pupation, and adult emergence were significantly reduced; 1-IRR values obtained from Poisson GLMs were used to calculate percent reductions and modify each parameter accordingly (Table 2).

Model Assumptions and Initial Conditions

In this population projection, matrix multiplication was used to predict the number of individuals in a particular life stage at the next time step. Typically, individuals can either survive and grow to the next life stage, survive and remain in the same life stage, or die [115]. Mortality in the immature stages may be due to factors such as infertility (for eggs that do not hatch), environmental conditions, and predation [131]. In our naïve and alternate models, juveniles that failed to develop to the next life stage were treated as mortalities, including adults that died during emergence. Eggs that failed to hatch were considered infertile, a cause of egg mortality. Failing to develop was the only factor considered to be responsible for mortality. Other factors such as predation, parasitism, and environmental conditions such as temperature were excluded from these calculations [131]. In the alternate model, it was assumed that failure

to develop was caused by the transgenerational effects of dietary PPF exposure on individual fitness, rather than factors such as environmental stochasticity or intraspecific competition.

Similarly, development to the next life stage was consistent with overall survival for all individuals within that stage group. Developmental time for each life stage (the number of days an individual remained within a size class) was used to determine the daily probability an individual would develop to the next life stage or remain in the same life stage over the course of a time step (Figure 8). The use of daily stage-change rates allowed for the model to create daily population projections, since mosquitoes grow and develop on a daily time scale.

After determining matrix values for both the naïve and alternate models, the projection cycles were simulated for a total of 100 days. This time scale was chosen to reflect how local mosquito populations would proliferate from the beginning of mosquito proliferation (mosquito season) to mid-summer (the time of year expected to have peak *Ae. albopictus* densities) [120]. The alternate model projected the population's response if intervention with 5 mg/mL PPF/sugar feeding stations were to be introduced at the beginning of mosquito season and maintained through the following 100 days. Simulations were initiated with a population of 50 females to match experimental conditions.

Model Statistics

For the sensitivity analysis, perturbation was used to examine how changing the parameter values in the alternate model would change its output. Sensitivity of the alternate model was measured based on changes in fecundity, pupation, and adult emergence, the three parameters that were significantly reduced by 5 mg/mL dietary PPF. Table 2 shows how the parameters were modified to create the alternate model; six scenarios were created by simulating a 10% increase or decrease for each parameter. Validation of the alternate model was also done

by comparing outputs of the total female abundance across all 100 elapsed days for all models (naïve, alternate, and all six sensitivity models).

RESULTS

Ae. albopictus females were treated with PPF formulated in a dietary sugar solution. Sugar feeders used to deliver treatments were designed to allow for minimal to no tarsal contamination on mosquitoes (Figure 3). Overall, 160 *Ae. albopictus* females were used in these experiments, of which 115 were exposed to either of the three PPF doses tested (1 mg/mL, 5 mg/mL, 10 mg/mL). A total of 2710 eggs were produced by surviving females over the duration of the fitness and fertility studies. In total, 430 eggs hatched, 337 larvae pupated, and 321 pupae emerged as adults (Table 3).

Pupal Susceptibility to PPF

Table 4 shows the inhibitory effects of PPF on adult emergence to pupae. For all pupae tested, 90% adult emergence was achieved for untreated controls compared to 56.67% and 30% adult emergence for pupae exposed to hexane and 10 mg/mL PPF/hexane, respectively. Adult emergence was inhibited by 66.67% in pupae treated with PPF and 37.04% in pupae treated with hexane compared to pupae reared in water alone. In comparison to hexane, adult emergence was inhibited by 47.06% in pupae treated with PPF.

Adult Female Survival

Overall, dietary PPF did not significantly affect the survival of adult female *Ae. albopictus*. Between 24 and 48 hours post ingestion, 84.4% to 95.6% of treated females survived compared to 97.78% of untreated survivors (Chisq = 0.62222; df=1, P=0.4302). Figure 10 shows the survival of *Ae. albopictus* 24 to 48 hours post PPF exposure, compared to the control group. At 48 hours post exposure, PPF had no statistically significant effect on mosquito survival (Table 5).

Sterilizing Effects of Dietary PPF

Dietary PPF did not appear to affect the ovipositing behavior of female *Ae. albopictus,* which ranged between 55.56% and 84.44% across treatments (Figure 11). Overall, PPF treatment (PPF exposure: yes/no) was not associated with the chance of a female laying eggs (Chisq = 5. 24759; df=1; p=0.6188). The odds of a female laying eggs were not significantly reduced at any of the prescribed concentrations of PPF compared to untreated females (Table 5).

The fecundity of females either exposed to PPF or to a sugar solution is shown in Figure 12. The figure suggests a reduced fecundity for the treatments 1 mg/mL and 5 mg/mL. A GLM identified a significant reduction in fecundity at those PPF doses (Table 6). The numbers of eggs laid by females fed a diet of 1 mg/mL PPF was reduced 22% compared to untreated females, and a diet of 5 mg/mL PPF reduced fecundity by 24% (Table 6). At 10 mg/mL, no statistically significant reduction in fecundity was detected. A GLM was used to project the estimated fecundity by each treatment, confirming the statistically significant reduction at intermediate PPF doses (Figure 13).

Fertility (measured as number of eggs that hatched per female) appeared to be similar across treatment, with outlier observations found in all PPF concentrations (Figure 12). A GLM found no association between fertility and PPF exposure (Table 6). A GLM-based prediction of fertility showed no significance across treatments (Figure 13). Such lack of significance was confirmed when PPF exposure was considered binary (exposure yes/no; Chisq=24.467; df=18, P=0.1403).

Transgenerational Effects of Dietary PPF

Overall, when the offspring of PPF-exposed females were reared, a statistically significant reduction in pupation rate and adult emergence was detected. Exposure to PPF in

parental females led to a statistically significant reduction in pupation when females were exposed to 5 mg/mL or 10 mg/mL of PPF (Table 7). A GLM-based prediction of pupation confirming such findings is shown in Figure 13. Ingestion of 5 mg/mL or 10 mg/mL PPF reduced pupation 47% or 28% respectively (Table 7). Adult emergence was further reduced for larvae from females exposed at 5 mg/mL by 43% (Table 7). For the other diets, no statistically significant effect of PPF exposure on parental females was detected (Figure 13).

Population Projections

In the naïve model, a population of *Ae. albopictus* that is initiated with 50 untreated females grew exponentially over the course of 100 days ($\lambda = 1.05$). When treatment with 5 mg/mL dietary PPF was introduced to the initial 50 females in the population, abundance decayed within the same time span (Figure 14). After an initial boom in eggs laid and larvae hatched, reduction in pupation rate and adult emergence rate due to the transgenerational effects of PPF prevented continued proliferation of pupae and adults ($\lambda = 0.93$).

Sensitivity analyses in the alternate model produced similar results to the original. When fecundity, pupation rate, or adult emergence rate were increased or reduced by 10% in the transition matrix, populations showed nearly identical booms initially in egg and larvae abundance that were not met with similar abundance for pupae and adults (Figure 15). Increased parameters resulted in an increased eigenvalue of 0.94; reduced parameters maintained the eigenvalue of the original alternate model ($\lambda = 0.93$).

The total number of adult female *Ae. albopictus* that the models predicted would emerge over the 100 days varied between projections (Table 8). A population receiving no treatment grew to have 18,157 adult females, while the population treated with 5 mg/mL PPF produced a total of 111 females in the time allowed. Female abundances from sensitivity analyses were

either 119 for all with a parameter increased by 10% or 105 for all with a parameter decreased by 10%, regardless of which parameter was being modified.

DISCUSSION

The impact of PPF exposure on key mosquito fitness and development traits such as fecundity, and pupation and adult emergence of their offspring were reported. Moreover, the effect appeared to vary across PPF doses, with 5 mg/mL exerting the strongest effects across life-history traits.

Previous research has illustrated the ways sublethal doses of insecticides may affect mosquito populations, acting as repellents or changing their mating and blood feeding behaviors. If a vector control method relies on adult mosquitoes to mate and autodisseminate PPF to larval rearing habitats, the dietary PPF formulation should not inhibit their survival. In this study, PPF ingestion was not associated with increased mortality of exposed females. At 48 hours post exposure, PPF ingestion did not cause a statistically significant reduction in the odds of survival at any of the tested concentrations. Silva et al. (2021) investigated reduction in the reproductive potential of adult female *Ae. aegypti* following PPF ingestion, but did not report survival of adults following treatment [111]. Similarly, Scott et al. (2017) focused on the larvicidal effects of PPF that had been autodisseminated by adult females following ingestion without reporting any effects on adult survival [100]. In Anderson et al. (2016), ATSBs containing 5% PPF alone were found to have insignificant effects on adult mortality. However, most of the ATSBs tested in this study contained PPF and sublethal doses of boric acid and eugenol with some differential effects on adult mortality [110]. Anderson et al. (2016) and Scott et al. (2017) both used pyriproxyfen that was originally formulated in a commercial product (10%, NyGuard, MGK, Minneapolis, MN). According to this company, the exact percent concentration of PPF is withheld as a trade secret, and they do not identify the solvents and emulsifiers used to produce the emulsifiable

concentrate [132]. While it is unknown if these ingredients contribute to mosquito fitness, it is unlikely such information can be obtained without experiments using the required controls.

Measures of reproductive output varied greatly with PPF dose and mosquito life stage. The proportion of ovipositing females did not vary across treatments, but fecundity was reduced in those who ingested 1 or 5 mg/mL PPF. Comparatively, Silva et al. (2021) saw a 41% decrease in average number of eggs obtained from females fed with ATSBs containing PPF [111]. Though this study's treatment levels ranged from 5 to 500 ppb PPF, there were no significant differences noted between concentrations. We saw no overall effect of PPF in reducing fecundity, but results varied greatly between concentrations. Fertility was impacted neither by PPF concentration nor overall PPF effect. This is at odds with Silva et al. (2021) where a 92% reduction in fertility was observed as the overall effect of PPF at comparably smaller concentrations, though they did not observe any differences in fertility between concentrations [111]. By contrast, Anderson et al. (2016) reported values for the number of larvae obtained from females fed PPF that varied wildly between replicates [110]. These conflicting results would suggest that PPF may have a dose-dependent effect, where PPF concentration is not directly related to inhibition of viable egg production in female mosquitoes. It is also possible that other factors have contributed to these differential effects. Both Anderson et al. (2016) and Silva et al. (2021) observed that mosquitoes were reluctant to fully feed on their PPF/sugar formulations, though neither of these studies tested to confirm engorgement [110-111]. It is also possible that tarsal contact with PPF was a factor in reducing reproductive output, particularly in Silva et al. (2021) where no measures were taken to prevent tarsal contact with the PPF/sugar solution. In this study, it is possible that the relatively lower effects of PPF on fecundity and fertility occurred due to the sugar feeder design that ensured low or no tarsal contamination. Many

studies have already confirmed that topical PPF reduces fecundity and fertility in female mosquitoes of different species [68-69,83-84,87-88,98-99], so tarsal contact with the PPF/sugar solution in Silva et al. (2021) and Anderson et al. (2016) may have increased the overall reductions observed in reproduction, that were reported as a consequence of PPF ingestion.

Each of these studies also varied in the schedule that was used to rear mosquitoes, deliver PPF treatment, and allow for oviposition. Since the sterilizing effects of PPF differ depending on when exposure occurred relative to the blood meal [88], it is possible that the impacts of dietary PPF occur in a different time window than topically-applied PPF. Silva et al. (2021) reported that PPF remained present in fecal deposits of mosquitoes up to 96 hours after feeding from ATSBs containing PPF [111]. Results from these experiments could also vary due to external factors that impact whether a mosquito will feed within the time it has access to dietary PPF (as opposed to topical application).

Both pupation and adult emergence from the offspring of females fed with PPF were significantly reduced depending on the prescribed dose. Pupation decreased significantly with both 1 and 5 mg/mL PPF, and emergence was significantly inhibited only after ingestion of 5 mg/mL PPF. None of the aforementioned studies reported reproductive outcome beyond egg laying and egg hatchability. These effects could likely be due to a dose-dependent effect of PPF on reproduction. It is also possible for PPF or its concentrations to target a mosquito differently at its life stages. Yadav et al. (2019) investigated the fecundity, fertility, pupation, and adult emergence in *Ae. aegypti* females treated with topical PPF and reported that the number of eggs, larvae, pupae, and adults that developed per clutch of exposed females declined significantly in all tested PPF concentration [113]. However, since PPF was applied topically to the mosquitoes, it is difficult to conclude whether the transgenerational effects of PPF occurred as a result of

female sterilization or juvenile exposure (since PPF could have been autodisseminated to the breeding sites). A few other studies have reported that, of the adult insects that manage to emerge following juvenile exposure to PPF, they exhibit reduced fitness, such as abnormal wing development [133-134]. In mosquitoes that successfully emerge as adults in spite of PPF exposure at the larval or pupal stage, fitness and longevity are decreased [116]. It is possible that the transgenerational effects of PPF will affect mosquitoes in their life stages differently than the effects that have been studied for larval exposure.

While the sterilizing effects of dietary PPF were studied on the individual level in laboratory experiments, the mathematical models generated projections for PPF ingestion at the population level. Ultimately, mosquito control is delivered to mosquito populations, and these strategies need to be evaluated for those effects. Both models constructed in this study projected *Ae. albopictus* populations over 100 days to mimic the effects of mosquito control strategies that are implemented at the beginning of mosquito season and are maintained to the time of year associated with peak mosquito abundance. In the alternate model, survival and mortality factors were limited to being caused by the effects of PPF on inhibiting mosquito development. In either model, failure to develop to the next life stage within the chosen stage change duration was synonymous with death. Assuming that no other external or intrinsic factors impacted mosquito growth, development, and reproduction, PPF ingestion was sufficient in decreasing mosquito abundance in comparison with the naïve model that showed exponential growth. This could be a promising addition to existing mosquito control strategies.

Despite the fact that traditional reliance on insecticide spraying has successfully controlled mosquito populations in the past, indoor residual and space-spraying in residential areas can be resource-limited and hampered by poor public perception [135]. For these reasons,

greater emphasis has been placed on community-based approaches to provide sustainability to vector control programs [136]. The rationale posits that sustainable mosquito control must be accomplished by those who live in the houses where the problems occur [135-136]. However, commercial insecticides have a high propensity for improper and frequent use by consumers, a problem that is accelerated by a lack of public knowledge and exacerbates the current problems faced by inefficient vector control strategies [137]. Unsystematic use of aerosolized insecticides at the household level reduces treatment coverage, limits product efficacy, and selects for insecticide resistance in the same manner as outdoor spraying [137-142]. Conversely, the extensive health education and community outreach required for community ownership of prevention programs are slow and costly [136].

A method implementing dietary PPF would not rely on large-scale applications of insecticides that may indiscriminately kill both targeted and non-targeted species. It would also provide a control strategy that operates through chemical and biological pathways, targeting mosquitoes differently depending on life stage. For mosquito species with narrow flight ranges, the use of PPF sugar feeders as autodissemination stations could be used for mosquito control at the household level. The sugar feeding stations developed for these experiments were created to minimize tarsal exposure to PPF in mosquitoes, but the design also doubles as a preventative measure against environmental contamination. Sugar feeders providing a minimal effective dose of PPF could be an option for a commercially-available mosquito control tool that is easy to implement and mitigates many of the problems faced by current options that are on the market.

Novel mosquito repellents that are commercially-available are regularly being found from unexpected sources. In one study, Victoria Secret Bombshell fragrance was found to strongly repel *Ae. aegypti* and *Ae. albopictus* as well as commercial repellents with effects

lasting for more than 120 minutes [142]. However, prevention of mosquito/human contact (and subsequent prevention of disease transmission) could be as simple as offering the mosquitoes a different meal that one's own blood. In spite of some assertions, vectorial capacity of mosquitoes is linked to their reproductive biology as females require a blood meal in order to facilitate egg development. However, the molecular pathways that regulate mosquito host-seeking behavior can be manipulated for mosquito control. Dittmer et al. (2019) found that sugar feeding decreased the attraction of *Ae. albopictus* females to human hosts [143]. Sugar feeding alone was found to induce a response in several genes in the female fat body that play a role in vitellogenesis, resembling the transcriptional response that typically occurs after a blood meal [143]. With the addition of PPF to commercially-available sugar feeders, this method in mosquito control may have the additive effect of decreasing human-vector contact in addition to its use to reduce population size.

In spite of this, the varying efficacies of PPF across laboratory studies illustrate the broad spectrum of factors that need to be considered when determining how to implement PPF into current vector control strategies. In order for PPF to have its intended effects on target populations, it must be administered in a way that considers the way that PPF and external factors interact with mosquitoes through varying life stages. In this study, female mosquitoes were exposed to dietary PPF beginning on the day they emerged, but *Ae. albopictus* may not be prepared to feed until one day after emergence [144]. Sugar feeder designs were also limited in their design. High concentrations of PPF were chosen for treatments, and only small quantities of PPF/sugar solution could be produced for experiments. Minimal amounts of cotton had to be used to ensure that the equally small amounts of sugar solution did not evaporate in the insect growth chamber before mosquitoes were able to feed. The quantity of sugar solution in each

sugar feeder was small, and engorgement could not properly be determined because the mosquitoes did not feed enough to cause distended abdomens or a visible color change. It is possible that some of the mosquitoes tested in each treatment did not properly feed on their respective sugar solutions and led to skewed reproductive outputs.

CONCLUSIONS

The use of PPF for mosquito control is a promising, multipurpose addition to the fight against the global burden of arboviral diseases. PPF exhibits anti-metamorphic effects against juveniles and reduced reproductive output following adult exposure. However, the large-scale integration of PPF into control strategies is hampered by the fact that the molecular mechanisms and pathways that PPF takes to regular mosquito development and reproduction are still in the process of being discovered. PPF is known to be bioactive in very small amounts when used as a larvicide or applied topically to adult female mosquitoes in laboratory settings, but the results from this experiment and similar studies indicate that higher doses of dietary PPF may be required to produce similar reductions in fecundity, fertility, and subsequent transgenerational effects. However, the population projections created with the provided parameters suggest that implementation of dietary PPF through autodissemination stations or release of laboratory-reared mosquitoes could have intended effects for mosquito population control. This is especially promising for the use of PPF in sterile insect technique (SIT) and incompatible insect technique (IIT) where the currently available methods of sterilization may change mosquito mating and reproductive behavior. For mosquito control strategies that depend on mating between laboratory-reared mosquitoes and wild populations, sterilization through PPF exposure is a possible option. Male mosquitoes have been shown to transfer PPF to females. Though the process is unknown, it possibly occurs through mating or autodissemination by males to ovipositing sites and shared resting sites. As this process could have unintended consequences on non-target species, future studies should investigate whether ingestion of PPF by male mosquitoes could be transferred venereally to females during mating and lead to sterilization. Laboratory studies are starting to evaluate novel methods as mosquito control strategies move

away from the traditional insecticide-based spraying they have long relied on. However, as these laboratory experiments move forward, it will be equally important to consider how these small-scale studies will translate to having entomological and epidemiological effects when applied to large scale interventions.

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TABLES

Table 1: List of population parameters used in models

Parameter	Description	Naïve Model	Alternate Model (% Reduction)	Sources of input
EE	Daily probability an egg will remain unhatched	0.33	0.33 (0)	Yang et al. (2020) Juliano et al. (2002) Löwenberg et al. (2004)
EL	Daily probability an egg will hatch	0.67	0.67 (0)	Yang et al. (2020) Juliano et al. (2002) Löwenberg et al. (2004)
LL	Daily probability a larva will remain in the larval stage	0.013	0.013 (0)	Yoshioka et al. (2012)
LP*	Daily probability a larva will pupate	0.15	0.078 (46.65)	Yoshioka et al. (2012)
PP	Daily probability a pupa will remain in the pupal stage	0.014	0.014 (0)	Yoshioka et al. (2012)
PA*	Daily probability a pupa will emerge as an adult	0.44	0.25 (43.33)	Yoshioka et al. (2012)
AA	Daily probability an adult will survive	0.81	0.81 (0)	Erikson et al. (2010) Löwenberg et al. (2004)
AF	Daily probability a female will survive	0.09	0.09 (0)	Erikson et al. (2010) Löwenberg et al. (2004)
FA	Daily probability a female will oviposit	0.45	0.45 (0)	Erikson et al. (2010)
FF	Daily probability a female will survive to the next gonotrophic cycle	0.05	0.05 (0)	Erikson et al. (2010)
F*	No. of eggs laid per oviposition	40	30.59 (23.54)	Erikson et al. (2010) Löwenberg et al. (2004)

Table 2: Parameter calculations and modifications for population models and sensitivity analysis

	Population Projections			Sensitivity Analysis	
Parameter	**Naïve Model**	**Reduction (%)**	**Alternate Model**	**10% Increase**	**10% Reduction**
FE*	40	23.53659	**30.585364**	**33.6439004**	**27.5268276**
EE	0.33	0	0.33	0.33	0.33
EL	0.67	0	0.67	0.67	0.67
LL	0.012698413	0	0.01269841	0.01269841	0.01269841
LP*	0.146031746	46.64502	**0.07791521**	**0.08570673**	**0.07012369**
PP	0.013636364	0	0.01363636	0.01363636	0.01363636
PA*	0.440909091	43.33333	**0.2498485**	**0.27483335**	**0.22486365**
AA	0.81	0	0.81	0.81	0.81
AF	0.09	0	0.09	0.09	0.09
FA	0.45	0	0.45	0.45	0.45
FF	0.05	0	0.05	0.05	0.05

*Indicate variables that were significantly reduced for treatment with 5 mg/mL dietary PPF in experimental trials. Cells highlighted in yellow indicate values that were reduced from the naïve model to reflect treatment conditions in the alternate model. Cells highlighted in green indicate values that were changed (increased or decrease by 10%) to test the sensitivity of the alternate model.

Table 3 : Survivorship data and stage duration for offspring of female *Ae. albopictus* treated with dietary PPF

Treatment	Total (n)	Died (n)	Survival (%)	Stage Duration (mean days ± std)
Untreated				
Eggs	820	702	14.39	1.102 ± 0.304
Larvae	118	19	83.90	3.847 ± 0.791
Pupae	99	2	97.98	1.909 ± 0.322
Adults	97	-	-	-
1 mg/mL PPF				
Eggs	548	461	15.88	1.239 ± 0.479
Larvae	87	19	78.16	4.023 ± 0.934
Pupae	68	7	89.71	2.118 ± 0.322
Adults	61	-	-	-
5 mg/mL PPF				
Eggs	627	509	18.82	1.361 ± 0.607
Larvae	118	37	68.64	3.782 ± 0.931
Pupae	81	7	91.36	1.988 ± 0.561
Adults	74	-	-	-
10 mg/mL PPF				
Eggs	715	608	14.97	1.213 ± 0.494
Larvae	107	18	83.18	3.796 ± 0.851
Pupae	89	0	100	2.079 ± 0.527
Adults	89	-	-	-

Table 4: Inhibitory effects of PPF on adult emergence of *Ae. albopictus* pupae			
Treatment	**Adult Emergence[1] (%)**	**Adult Emergence Inhibition[2] (%)**	**Adult Emergence Inhibition[3] (%)**
Untreated control	90	-	-
Hexane control	56.67	37.04	-
PPF	30	66.67	47.06

[1] Percentage of adult emergence is the number of emerged adults divided by the initial number of pupae used multiplied by 100.

[2] Cells highlighted in blue indicate percentage of adult emergence in comparison to untreated controls. Percentage of adult emergence inhibition is 1-(% adult emergence from treated water / % adult emergence from untreated water)*100.

[3] Cells highlighted in orange indicate percentage of adult emergence in comparison to hexane controls. Percentage of adult emergence inhibition is 1-(% adult emergence from treated water / % adult emergence from untreated water)*100.

Table 5: GLM analyses for survival and ovipositing behavior of female *Ae. albopictus* mosquitoes tested across four dosages of dietary PPF					
Treatment	**β**	**95% CI (β)**	**P-Value (β)**	**OR**	**95% CI (OR)**
Survival 24 hours after treatment					
Intercept	21.57	-166, 1452	0.996	2.32E+09	1.22E-72, Inf
1 mg/mL PPF	-7.821E-11	-462, 424	1.000	1.00	3.21E-201, 1.14E+184
5 mg/mL PPF	-18.50	NA, 509	0.997	9.26E-09	NA, 1.03E+221
10 mg/mL PPF	-18.50	NA, 509	0.997	9.26E-09	NA, 1.03E+221
Survival 48 hours after treatment					
Intercept	3.78	2.26, 6.659	0.00018 ***	44.0	9.62, 779.83
1 mg/mL PPF	-1.46	-4.45, 0.504	0.20082	0.233	0.0116, 1.65
5 mg/mL PPF	15.78	-116.03, NA	0.99232	7.14E+06	4.07E-51, NA
10 mg/mL PPF	-1.76	-4.73, 0.124	0.11612	1.73E-01	0.00881, 1.13
Presence of oviposition					
Intercept	1.068	0.414, 1.80	0.0022 **	2.909	1.513, 6.04
1 mg/mL PPF	-0.334	-1.318, 0.64	0.5004	0.716	0.268, 1.90
5 mg/mL PPF	0.960	-0.157, 2.20	0.1038	2.612	0.855, 9.02
10 mg/mL PPF	0.420	-0.635, 1.53	0.4409	1.522	0.530, 4.61

Table 6: GLM analyses for fecundity and fertility of *Ae. albopictus* females tested across four dosages of dietary PPF						
Treatment	**β**	**95% CI (β)**	**P-Value (β)**	**IRR**	**1-IRR**	**95% CI (IRR)**
Fecundity						
Intercept	2.9481	2.879, 3.0158	< 2E-16 ***	19.070	-18.07	17.794, 20.405
1 mg/mL PPF	-0.2527	-0.361, -0.1450	4.6e-06 ***	0.777	0.223	0.697, 0.865
5 mg/mL PPF	-0.2684	-0.373, -0.1646	4.2e-07 ***	0.765	0.235	0.689, 0.848
10 mg/mL PPF	-0.0134	-0.114, 0.0868	0.79	0.987	0.013	0.892, 1.091
Fertility						
Intercept	1.2964	1.110, 1.4723	<2E-16 ***	3.656	-2.656	3.033, 4.36
1 mg/mL PPF	-0.0494	-0.329, 0.2266	0.73	0.952	0.048	0.719, 1.25
5 mg/mL PPF	-0.1633	-0.419, 0.0928	0.21	0.849	0.151	0.657, 1.10
10 mg/mL PPF	-0.0576	-0.321, 0.2044	0.67	0.944	0.056	0.726, 1.23

Table 7: GLM analyses for pupation and adult emergence of the offspring of *Ae. albopictus* females tested across four dosages of dietary PPF						
Treatment	**β**	**95% CI (β)**	**P-Value (β)**	**IRR**	**1-IRR**	**95% CI (IRR)**
Pupation						
Intercept	1.7619	1.558, 1.9526	< 2E-16 ***	5.824	-4.824	4.750, 7.047
1 mg/mL PPF	0.0743	-0.237, 0.3795	0.636	1.077	-0.077	0.789, 1.462
5 mg/mL PPF	-0.6282	-0.918 ,-0.3406	1.9E-05 ***	0.534	0.466	0.399, 0.711
10 mg/mL PPF	-0.3312	-0.616, -0.0473	0.022 *	0.718	0.282	0.540, 0.954
Adult Emergence						
Intercept	1.6666	1.453, 1.8663	< 2E-16 ***	5.294	-4.294	4.274, 6.464
1 mg/mL PPF	0.0464	-0.283, 0.3688	0.77973	1.047	-0.047	0.754, 1.446
5 mg/mL PPF	-0.5680	-0.876, -0.2624	0.00028 ***	0.567	0.433	0.416, 0.769
10 mg/mL PPF	-0.2113	-0.504, 0.0814	0.15633	0.810	0.19	0.604, 1.085

Table 8: Total abundance of adult female *Ae. albopictus* under for population projections and sensitivity analyses from changes to selected parameters							
Population Projections		**Sensitivity Analysis**					
Naive	**Alternate**	**10% Increase**			**10% Reduction**		
		FE	**LP**	**PA**	**FE**	**LP**	**PA**
18,157.32	111.7166	119.3770	119.3770	119.3770	104.9591	104.9591	104.9591

FIGURES

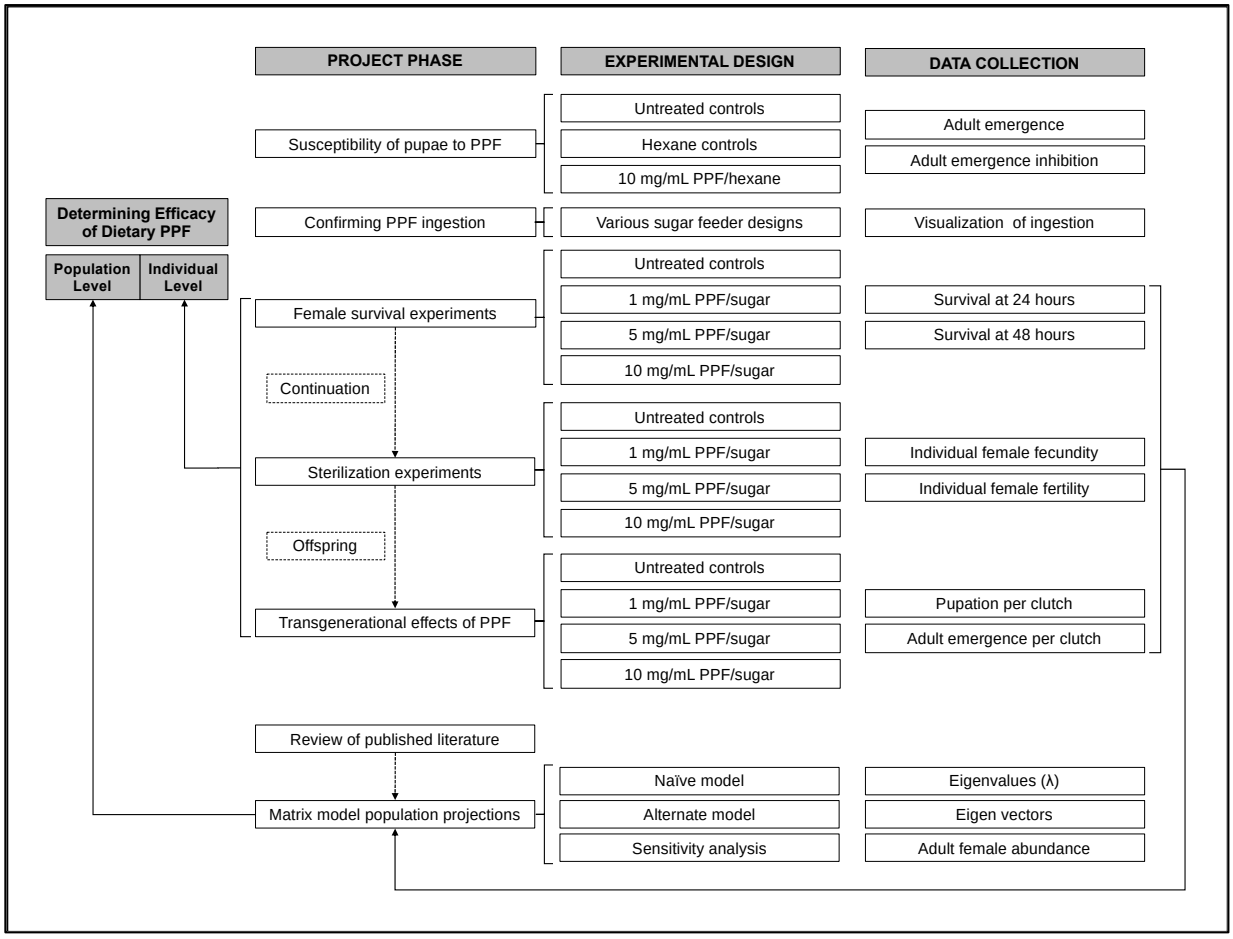

Figure 1: Overall design and protocol for evaluating the use of dietary PPF on *Ae. albopictus* control based on reduced reproductive capacity.

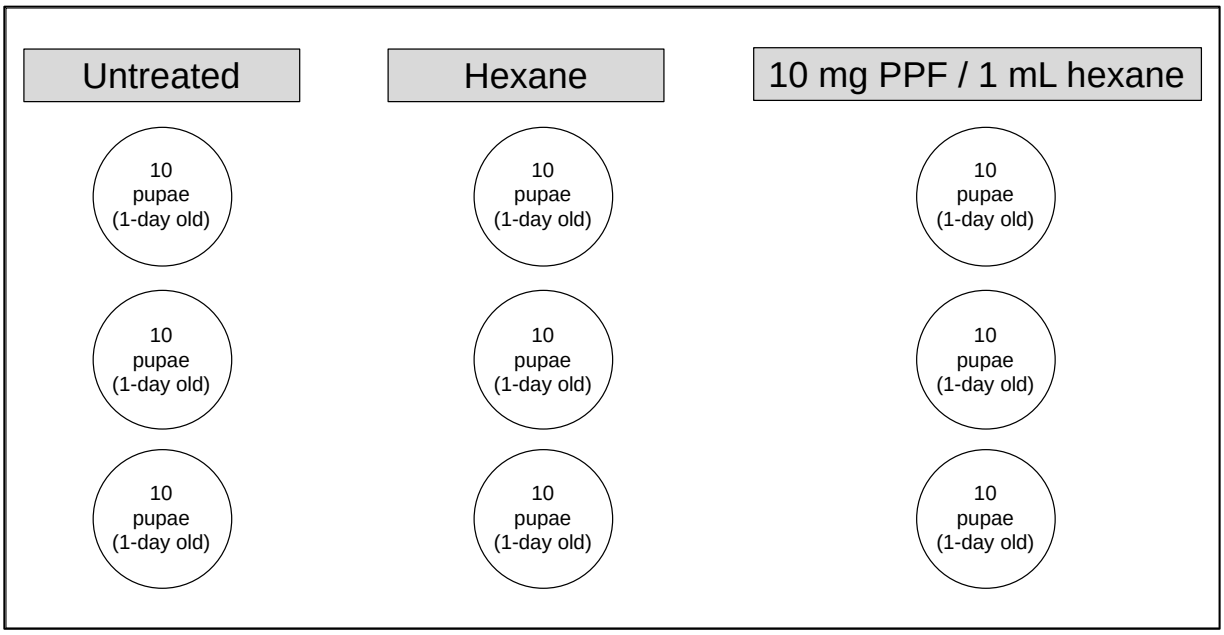

Figure 2: Exposure layout for experiments conducted to test susceptibility of *Ae. albopictus* pupae to adult emergence inhibition through PPF exposure.

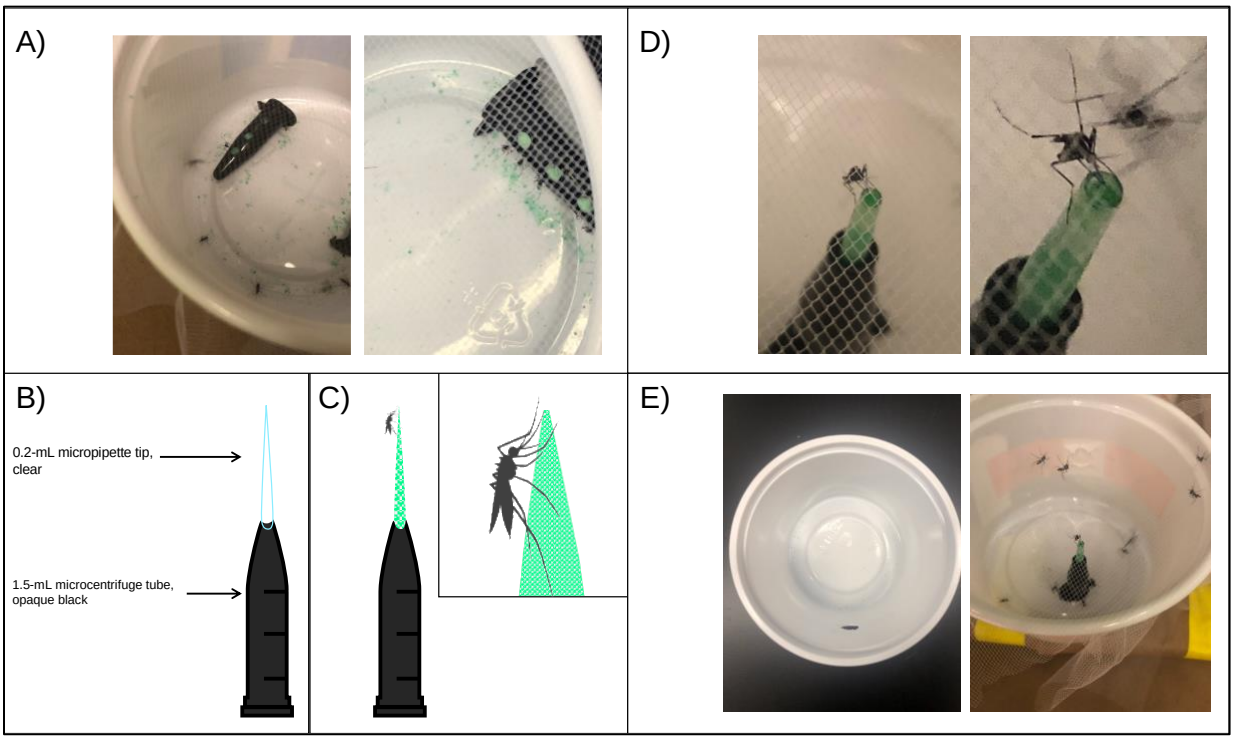

Figure 3: Diagram of final sugar feeder design.

A) Prototype of sugar feeder design; evidence of tarsal exposure can be seen in the transfer of green dye from the sugar solution to the legs of mosquitoes by the green-tinted footprints in their enclosure.

B) Empty sugar feeder constructed from a 0.2-mL micropipette tip protruding from a 1.5-mL microcentrifuge tube.

C) Sugar feeder in use by mosquito after it has been prepared with sugar-soaked cotton. Each sugar feeder holds 1 mL of solution.

D) Mosquitoes are able to rest on the plastic of the micropipette tip and access the sugar-soaked cotton through the top opening.

E) The final design for the sugar feeder did not result in physical contact between the mosquitoes and the sugar solution.

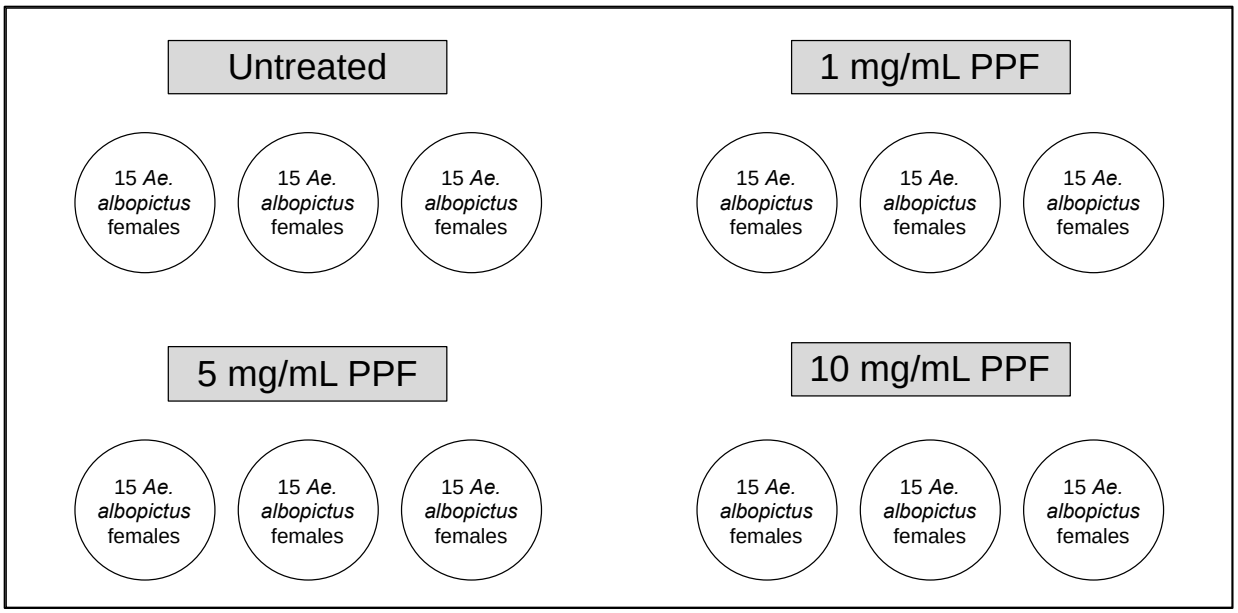

Figure 4: Exposure layout for experiments conducted to test susceptibility of *Ae. albopictus* females tested across four doses of dietary PPF (untreated controls treated with 10 mg/mL sugar solution with no PPF). One-day old females were used. In total, 45 females were used per treatment.

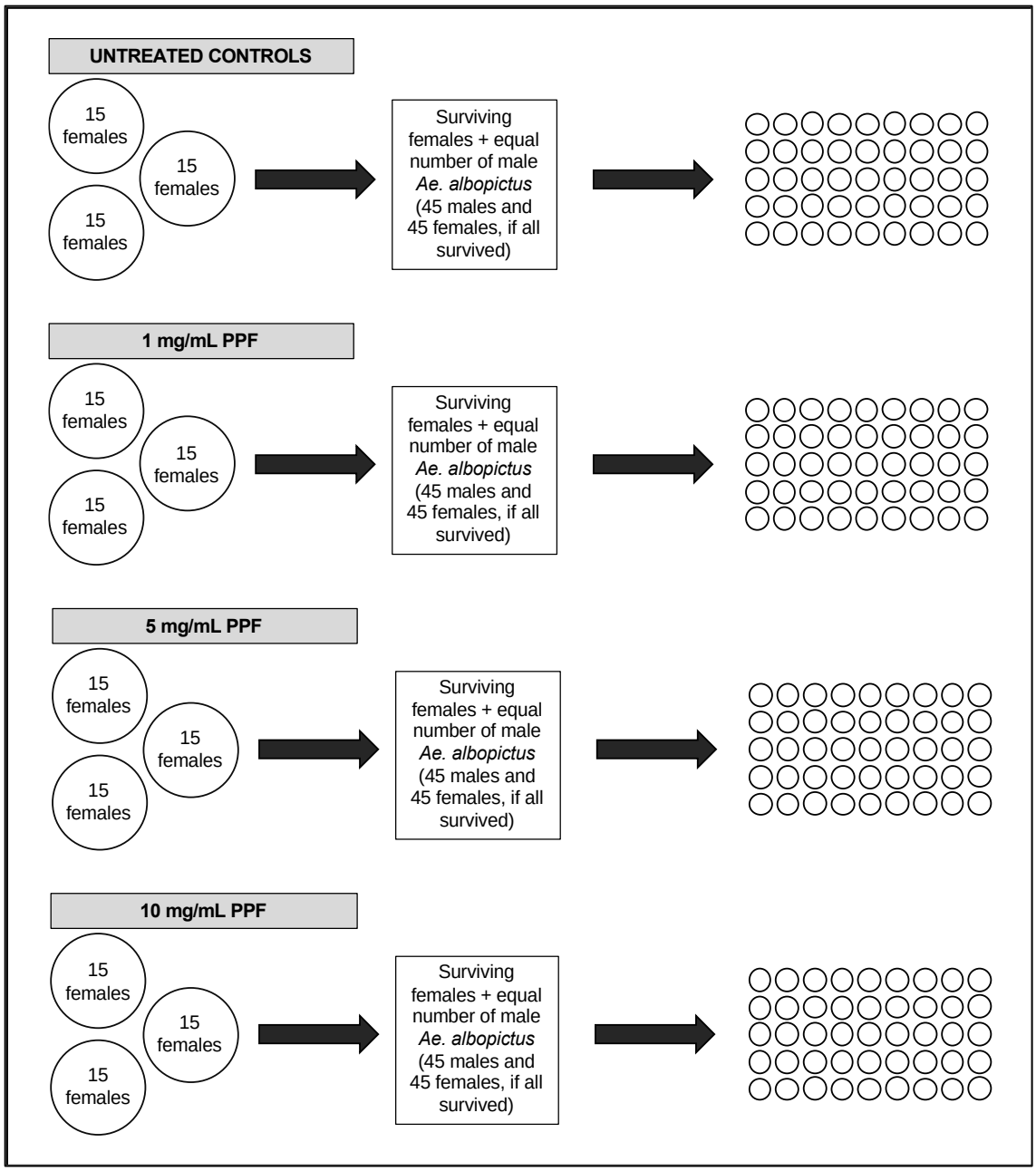

Figure 5: Experimental design of PPF and control trials. Females were given access to PPF/sugar feeders in cups (15 females per cup, 3 cups per treatment). After exposure, all surviving females were moved to Bugdorms containing an equal number of males (1 Bugdorm per treatment). After cohabitating for 5 days, females were individualized in Falcon tubes to lay eggs (1 female per tube, separated by treatment).

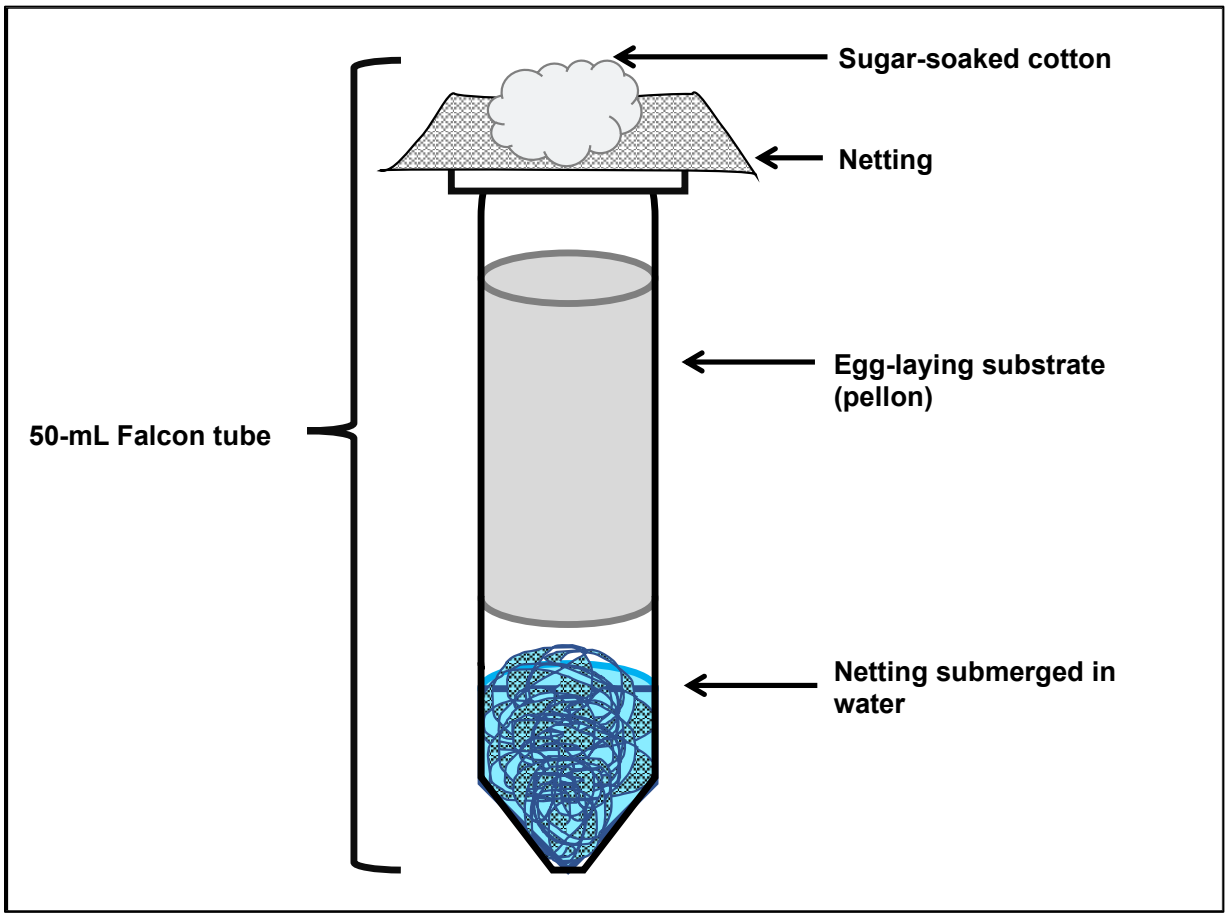

Figure 6: Diagram of tubes used to isolate females for oviposition.

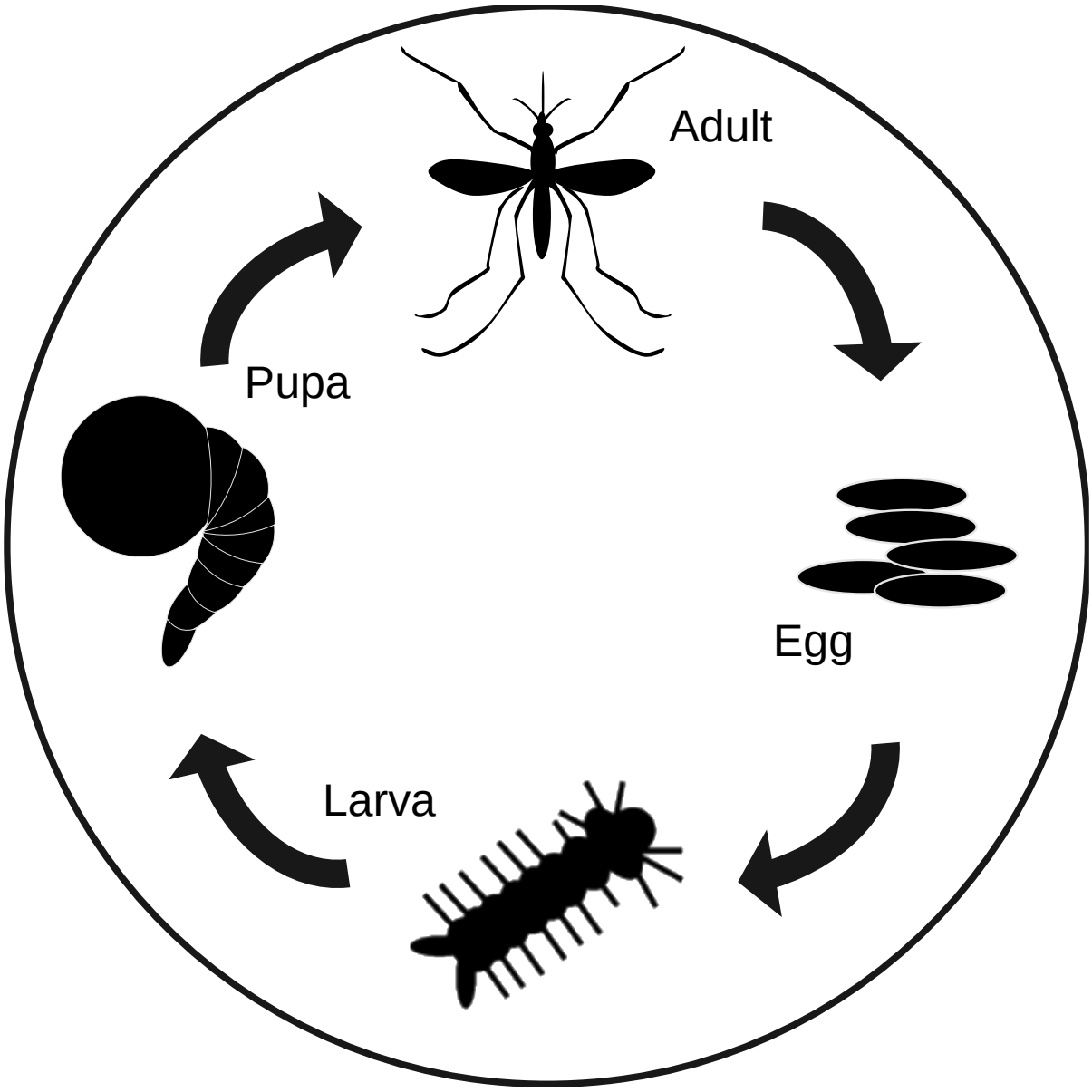

Figure 7: Life cycle of *Ae. albopictus* mosquito.

A)

$$N_{E,t+1} = EE * N_{E,t} + FE * N_{F,t}$$

$$N_{L,t+1} = LL * N_{L,t} + EL * N_{E,t}$$

$$N_{P,t+1} = PP * N_{P,t} + LP * N_{L,t}$$

$$N_{A,t+1} = AA * N_{A,t} + PA * N_{P,t}$$

$$N_{F,t+1} = FF * N_{F,t} + AF * N_{A,t}$$

$$A = \begin{array}{c} \\ E \\ L \\ P \\ A \\ F \end{array} \begin{array}{c} \begin{array}{ccccc} E & L & P & A & F \end{array} \\ \begin{bmatrix} EE & 0 & 0 & 0 & FE \\ EL & LL & 0 & 0 & 0 \\ 0 & LP & PP & 0 & 0 \\ 0 & 0 & PA & AA & FA \\ 0 & 0 & 0 & AF & FF \end{bmatrix} \end{array} \quad N = \begin{bmatrix} N_E \\ N_L \\ N_P \\ N_A \\ N_F \end{bmatrix}$$

$$N_{t+1} = A * N_t$$

B) Naïve Model

	E	L	P	A	F
E	0.33	0	0	0	40
L	0.67	0.013	0	0	0
P	0	0.15	0.014	0	0
A	0	0	0.44	0.81	0.45
F	0	0	0	0.01	0.05

C) Alternate Model

	E	L	P	A	F
E	0.33	0	0	0	31
L	0.67	0.013	0	0	0
P	0	0.078	0.014	0	0
A	0	0	0.25	0.81	0.45
F	0	0	0	0.01	0.05

Figure 8: Equations and matrices used in calculations of parameters for stage-based population projections.

A) System of equations to determine the change in the number of individuals within each life stage that will be present in a population at the next projected time step. Matrices are based on the variables included in the system of equations. Matrix A is a transition matrix constructed from the transition probabilities from the equations. Matrix N is a vector of population size giving the number of individuals in each life stage in the population. Equation indicates how the system of equations can be rewritten as the product of the transition matrix and the population vector to project how the population will look at the next time step.

B) Values used in transition matrices for the naïve model and the alternate model **(C)** in each projection cycle. Blue cells show daily probabilities of a mosquito remaining in its current life stage. Orange cells show daily probabilities of a mosquito developing into the next life stage. Green cells show the number of eggs laid per oviposition by females.

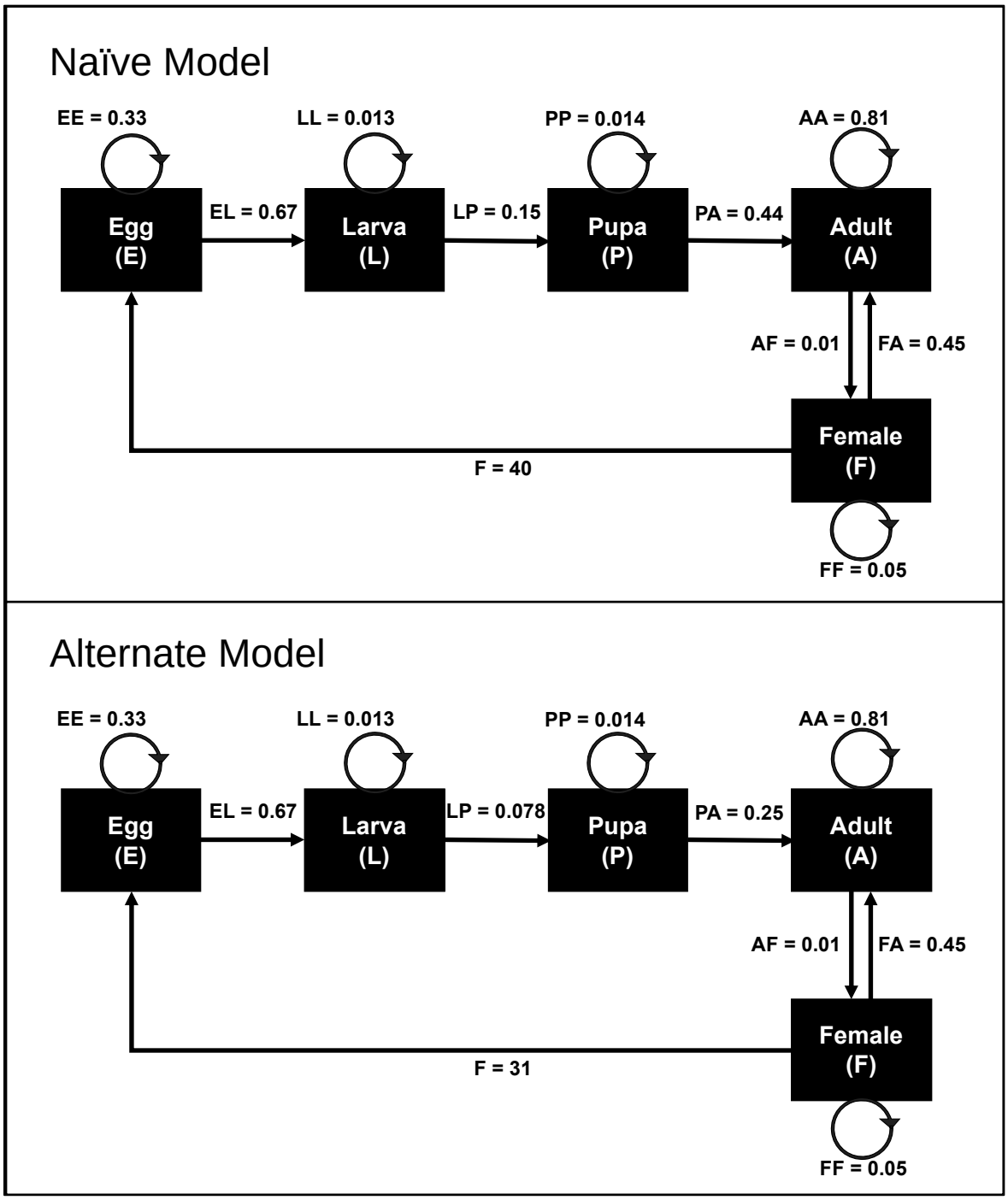

Figure 9: Diagrams of the life cycle graphs used to structure of the two matrix projection models used in this study. The naïve model (above) is based on the Lefkovitch stage-structured model, adjusted for a 1-day time step. The alternate model (below) is a modified version of the Naïve model to reflect the significant reductions in mosquito growth and development at each life stage found in our experiments with ingestion of 5 mg/mL PPF.

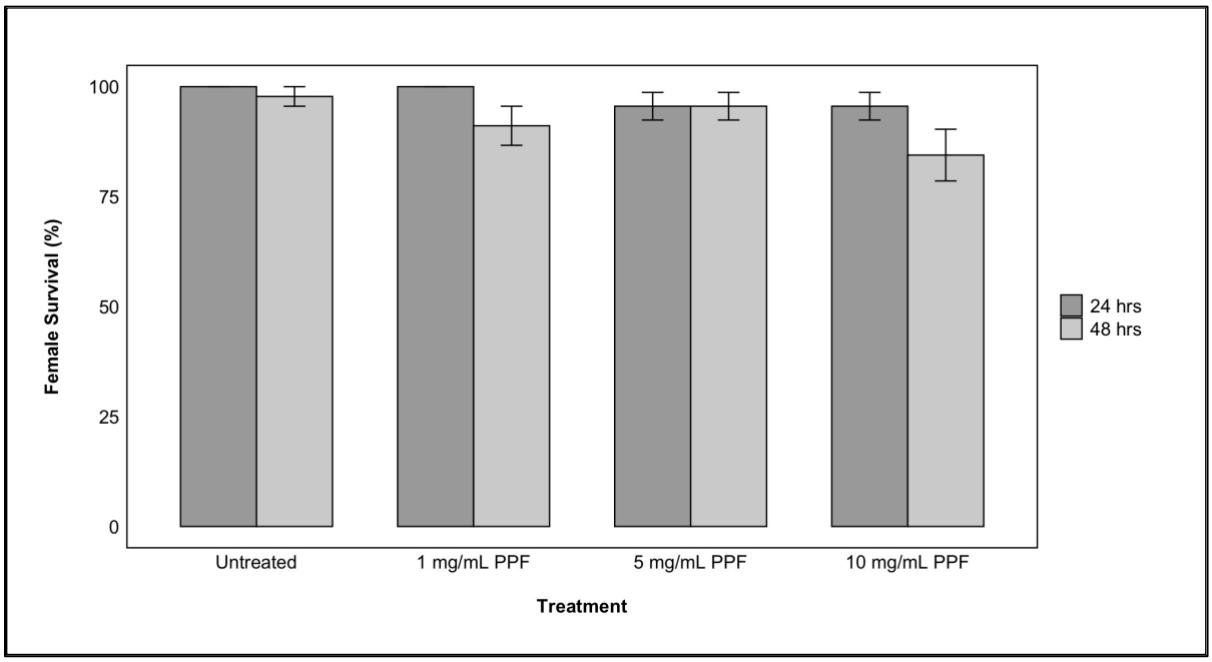

Figure 10: Bar chart indication percentage of *Ae. albopictus* females surviving ingestion of dietary PPF by treatment level (untreated controls treated with 10 mg/mL sugar solution with no PPF). Observations were recorded 24 and 48 hours after treatment was initiated. Percent survival calculated from the proportion of females surviving at each time stamp; standard error calculated from the binomial distribution for a treatment group and its proportional survival. No statistically significant differences were observed between treatments and control ($p > 0.05$).

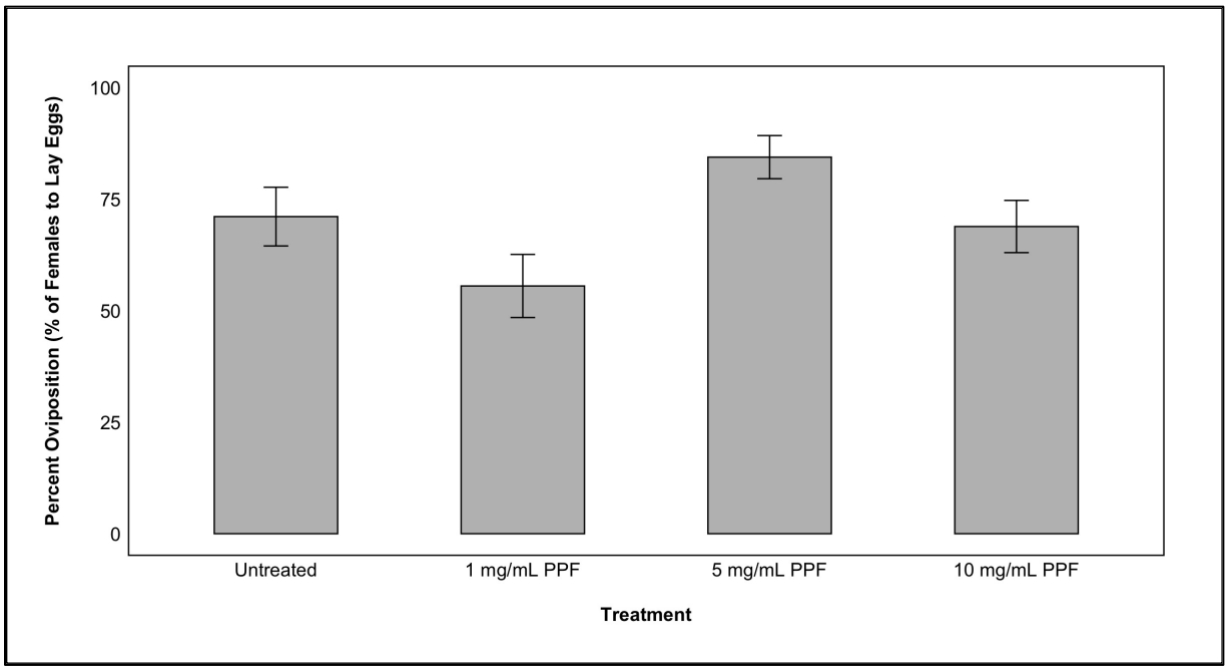

Figure 11: Bar chart indicating percent of *Ae. albopictus* females to lay eggs following ingestion of dietary PPF by treatment level (untreated controls treated with 10 mg/mL sugar solution with no PPF). Percent oviposition calculated from the proportion of surviving females that laid eggs; standard error calculated from the binomial distribution for a treatment group and its proportional oviposition. No statistically significant differences were observed between treatments and control ($p > 0.05$)

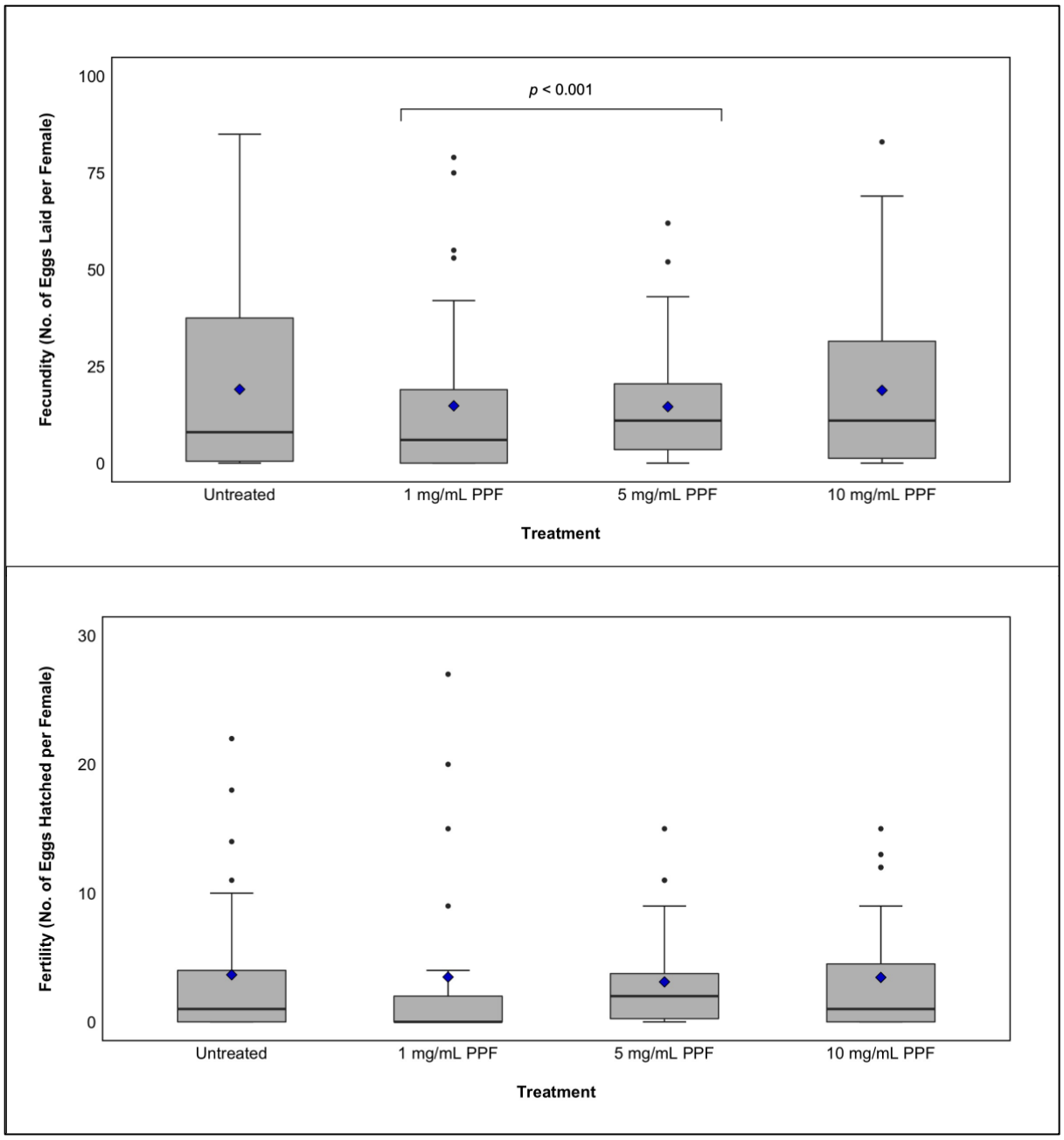

Figure 12: Box plots indicating the distribution of fecundity (above) and fertility (below) of *Ae. albopictus* females following ingestion of dietary PPF by treatment level (untreated controls treated with 10 mg/mL sugar solution with no PPF). Statistically significant differences in fecundity were observed between 1 mg/mL and 5 mg/mL treatments and control ($p < 0.001$). No statistically significant differences in fertility were observed between treatments and control ($p > 0.05$).

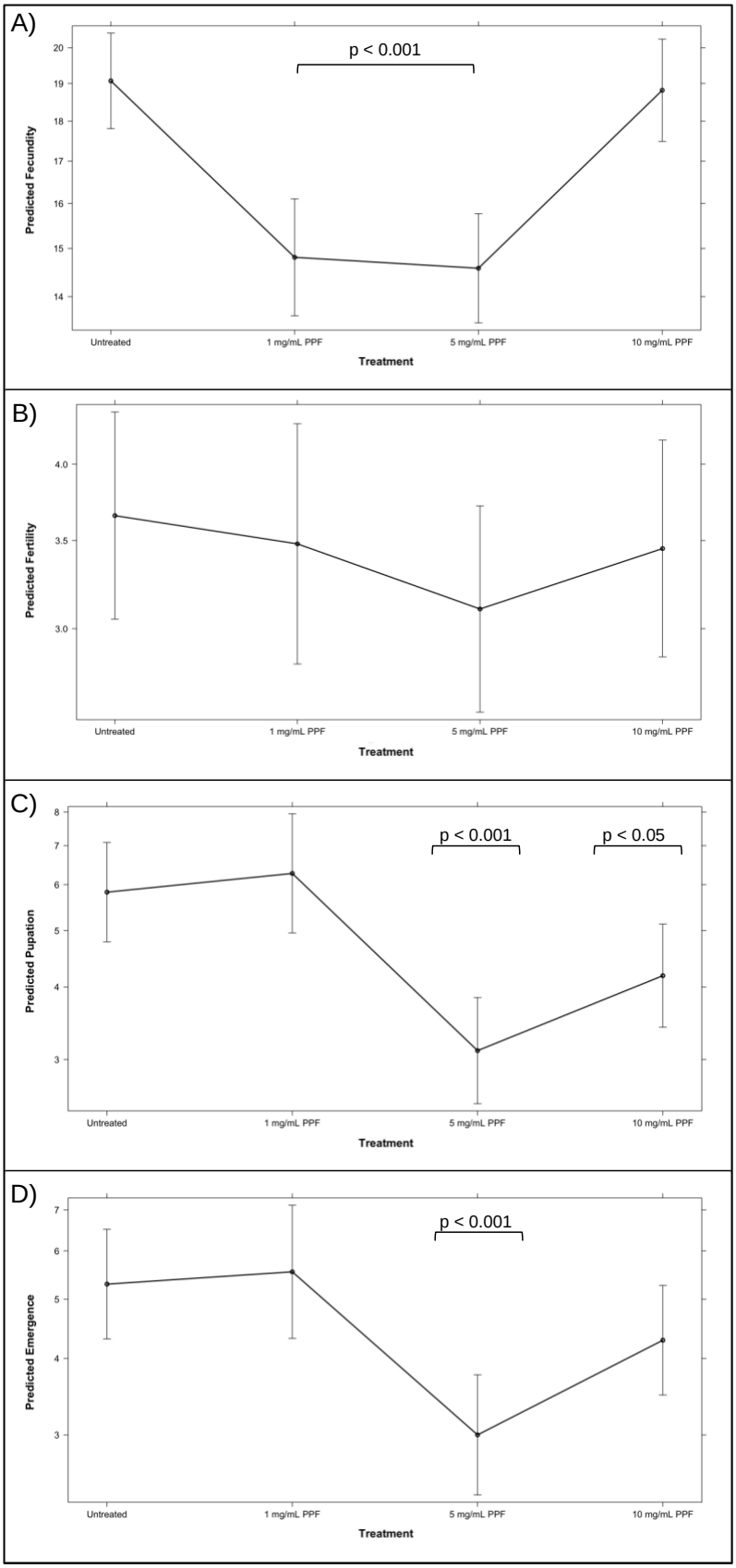

Figure 13: Predictor effect plots indicating the predicted fecundity (A) and fertility (B) of *Ae. albopictus* females following ingestion of dietary PPF by treatment level and predicted pupation (C) and adult emergence (D) of offspring of treated females (untreated controls treated with 10 mg/mL sugar solution with no PPF).

A) Statistically significant differences in fecundity observed between 1 mg/mL and 5 mg/mL treatments and control ($p < 0.001$).

B) No statistically significant differences in fertility were observed between treatments and control ($p > 0.05$).

C) Statistically significant differences in pupation were observed between 5 mg/mL ($p < 0.001$). and 10 mg/mL ($p < 0.05$) treatments and control.

D) Statistically significant differences in adult emergence were observed between 5 mg/mL ($p < 0.001$). and 10 mg/mL ($p < 0.05$) treatments and control.

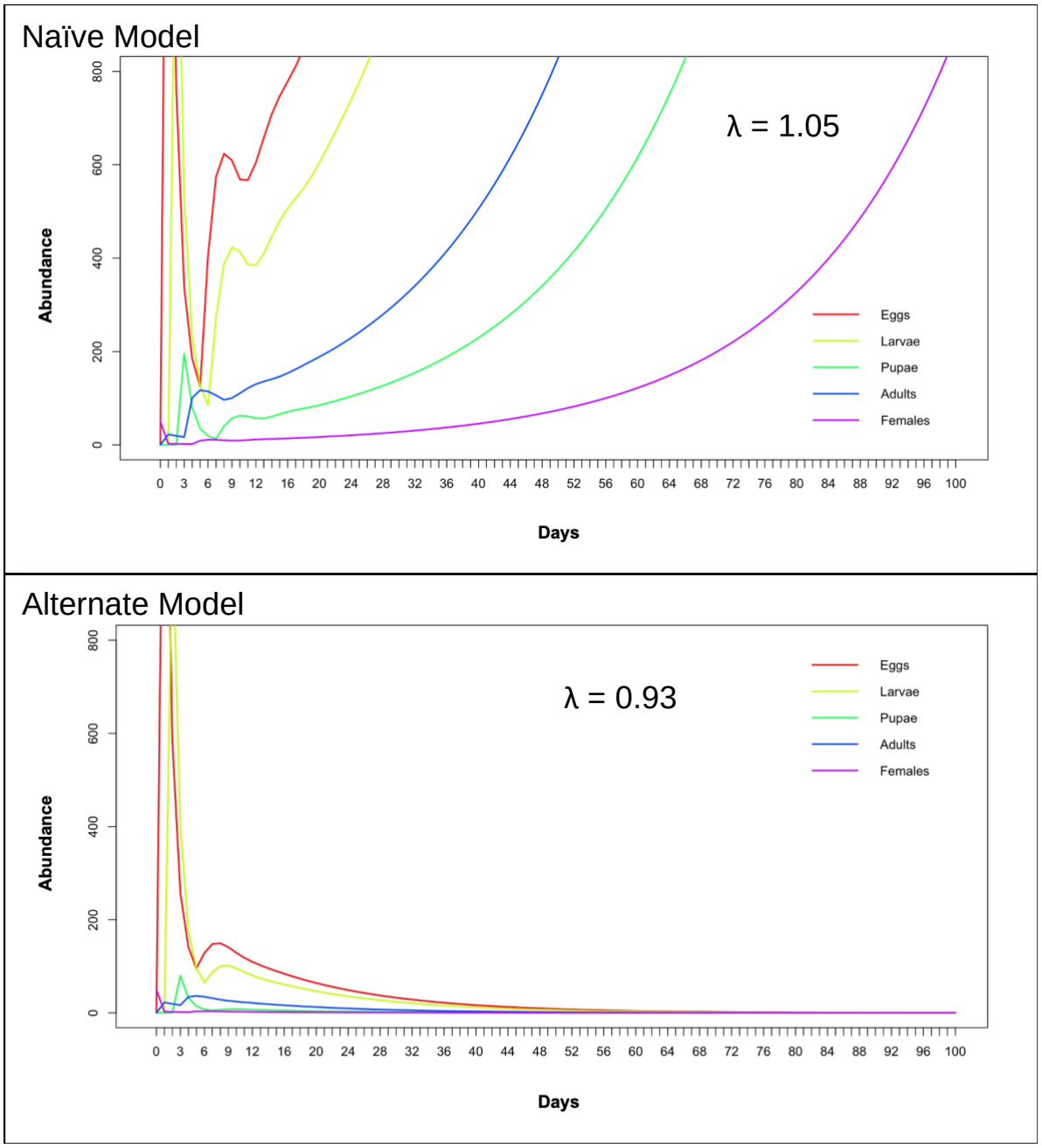

Figure 14: Population projections for *Ae. albopictus* separated by life stage using iterations generated by the naïve model (top) and alternate model (bottom). The naïve model projects a local population with no intervention; the alternate model projects a local population after intervention with 5 mg/mL dietary PPF.

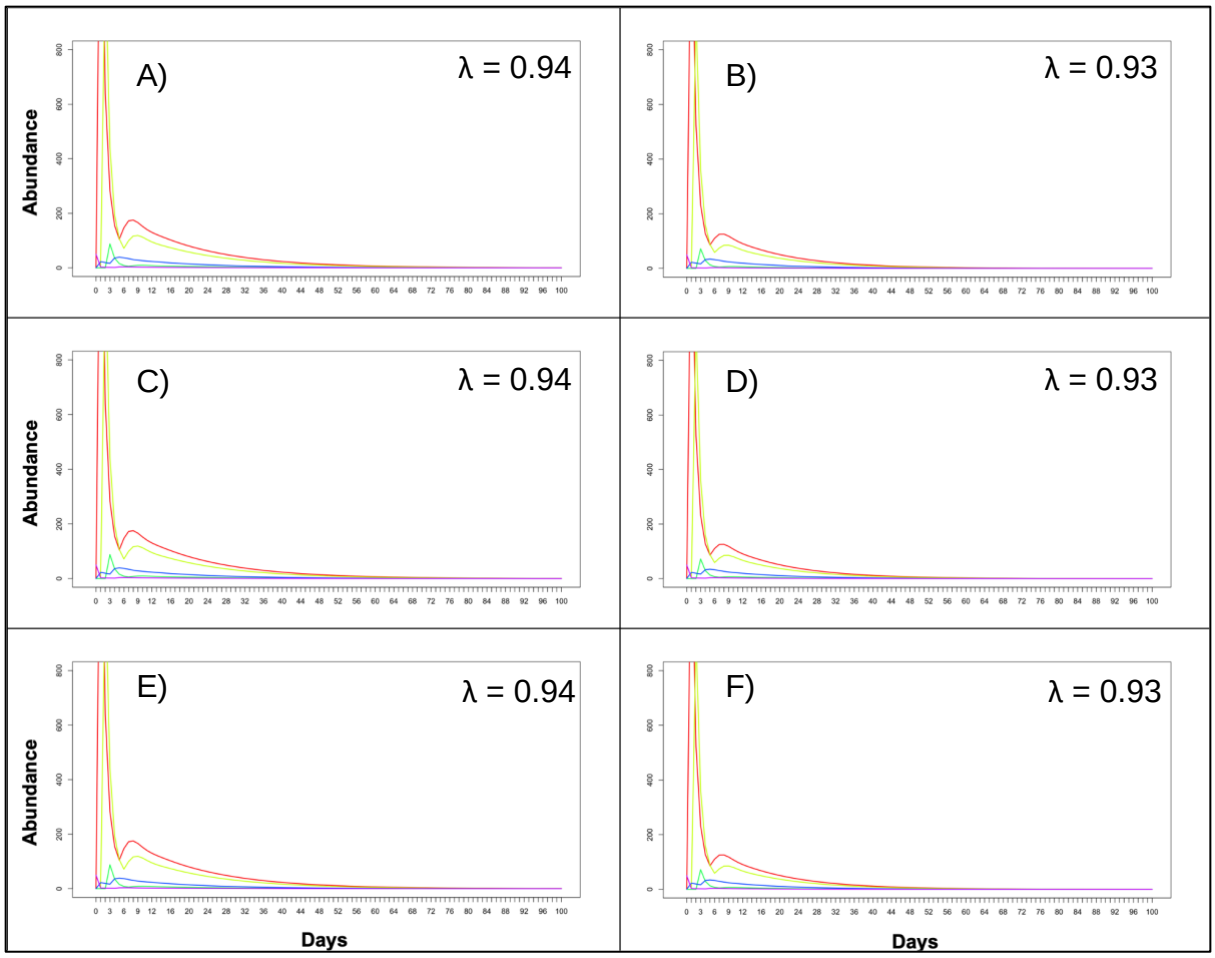

Figure 15: Sensitivity models for the alternate model.
A) 10% increase in fecundity.
B) 10% reduction in fecundity.
C) 10% increase in pupation.
D) 10% reduction in pupation.
E) 10% increase in adult emergence.
F) 10% reduction in adult emergence