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Female oviposition preferences and larval behavior of the *Aedes aegypti* mosquito (Linnaeus, 1762) exposed to predator cues (Odonata: Libellulidae)

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ABSTRACT

Female oviposition preferences and larval behavior of the *Aedes aegypti* mosquito (Linnaeus, 1762) exposed to predator cues (Odonata: Libellulidae).

The reproductive success of mosquitoes is heavily influenced by females' ability to select suitable oviposition sites for the proper development of their offspring. Many of these potential habitats may harbor predators, and the detection of these predators is crucial for assessing the risk of predation, which can, in turn, influence the behavior of the prey. However, there are often trade-offs to consider, which can impact the life history of these organisms. In our study, we investigated the oviposition preferences and larval behavioral responses of the *Aedes aegypti* mosquito when exposed to cues from predators and the actual presence of predation by Libellulidae (Odonata) larvae. We observed the mosquito's choices regarding oviposition with and without predation cues for a duration of ten weeks, following 48 hours of exposure. Additionally, we measured larval behavior across various treatments, including those with predation cues, predator cues, the presence of larvae, and controls with only water. Our findings revealed that the highest preference for oviposition occurred in containers with high organic matter content, regardless the presence of both predator cues and actual predators. Furthermore, we noted significant changes in larval behavior in response to predation cues, including evasive behaviors. When facing the risk of predation, larvae spent more time near the container's edges and exhibited discrete feeding behaviors or avoidance of the risk of predation. Conversely, in the absence of predation risk, larvae explored the entire container, swimming vigorously. These results highlight the significance of anti-predatory behaviors triggered by the recognition of predation cues in the context of *Ae. aegypti*'s reproductive success.

KEY WORDS: anti-predatory responses, behavioral ecology, mosquito, predation, reproductive success.

RESUMO

***Preferências de oviposição das fêmeas e comportamento das larvas do mosquito** Aedes aegypti (Linnaeus, 1762) expostas a sinais de predadores (Odonata: Libellulidae).*

O sucesso reprodutivo dos mosquitos é fortemente influenciado pela capacidade das fêmeas de selecionar locais de oviposição adequados para o desenvolvimento apropriado de seus filhotes. Muitos desses habitats potenciais podem abrigar predadores, e a detecção desses predadores é fundamental para avaliar o risco de predação, o que, por sua vez, pode influenciar o comportamento da presa. No entanto, muitas vezes há compensações a serem consideradas, o que pode afetar o histórico de vida desses organismos. Em nosso estudo, investigamos as preferências de oviposição e as respostas comportamentais das larvas do mosquito Aedes aegypti *quando expostas a sinais de predadores e à presença real de predação por larvas de Libellulidae (Odonata). Observamos as escolhas do mosquito com relação à oviposição com e sem sinais de predação por*

um período de dez semanas, após 48 horas de exposição. Além disso, medimos o comportamento das larvas em vários tratamentos, incluindo aqueles com sinais de predação, sinais de predador, a presença de larvas e controles com apenas água. Nossas descobertas revelaram que a maior preferência para oviposição ocorreu em recipientes com alto teor de matéria orgânica, independentemente da presença de sinais de predadores e predadores reais. Além disso, observamos mudanças significativas no comportamento das larvas em resposta a sinais de predação, incluindo comportamentos evasivos. Quando enfrentavam o risco de predação, as larvas passavam mais tempo perto das bordas do recipiente e apresentavam comportamentos discretos de alimentação ou de evitação do risco da predação. Por outro lado, na ausência de risco de predação, as larvas exploraram todo o ambiente nadando vigorosamente. Esses resultados destacam a importância dos comportamentos antipredatórios desencadeados pelo reconhecimento de sinais de predação no contexto do sucesso reprodutivo do Ae. aegypti.

PALAVRAS-CHAVE: *ecologia comportamental, mosquito, predação, respostas antipredatórias, sucesso reprodutivo.*

INTRODUCTION

A predator is defined as an organism that hunts and uses another organism as a source of food (Becker et al., 2010). In aquatic microhabitats, the presence of an apex predator (e.g. immature forms of Odonata) is recognized as an important factor in structuring these communities (Lima, 1998). Living at the bottom of aquatic environments, semi-buried in the substrate, they use this camouflage to attack and capture prey (e.g. mosquito larvae) that enter their feeding grounds at the slightest movement (Thompson et al., 2003). But can alter the hunt behavior and become active hunters depending on situation (Thompson et al., 2003). Considered generalist predators, they distribute predation pressure over their prey, carrying out population control according to the behavioral reaction adopted by the prey in the quest to ensure its survival (Thompson et al., 2003).

The reproductive success of mosquitoes is often linked to the ability of gravid females to select oviposition sites that ensure the development of their offspring (Albeny-Simões et al., 2014; Santos et al., 2017; Westby & Juliano, 2017). One of the factors influencing the decision to oviposit in a particular aquatic micro-habitat is the presence of predator cues and predation (Lutz et al., 2017). Predator cues are any chemical substance released into the environment (e.g. exudates, saliva, urine) that can be detected by the prey (Brugman et al., 2018). Kairomones are a very specific group of these substances, which only favored species is the receiver, not the emitter of the chemical signal (Brugman et al., 2018). Anti-predatory behaviors triggered by both predator and predation

cues, represent a crucial adaptation shaped by natural selection (Sih, 1986). Additionally, the availability of enough food resources is another crucial factor (Dieng et al., 2003). While some mosquito species do not discriminate against sites where predators are present (Torres-Estrada et al., 2001), others actively avoid ovipositing in such locations (Arav & Blaustein, 2006; Santos et al., 2017; Westby & Juliano, 2017).

Oviposition preference behavior can be influenced by innate olfaction or acquired olfactory experiences (Lutz et al., 2017). Mosquito species differ in their oviposition behaviors in response to predators, including positive, negative, or neutral responses (Torres-Estrada et al., 2001; Ohba et al., 2012; Santos et al., 2017). For example, certain *Culex* species avoid ovipositing in containers with abundant predators, such as *Chaoborus* species and Odonatas. However, species like *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) continue to oviposit even in the presence of predators like *Toxorhynchites splendens* and copepods (Dieng et al., 2017; Torres-Estrada et al., 2001). *Aedes albopictus* females, on the other hand, do not seem to alter their oviposition behavior in response to predator or predation cues, suggesting that they may either not detect these chemical signals or are not sensitive to them (Ohba et al., 2012). *Aedes aegypti* deserves special attention due to public health issues in the Neotropics (OECD, 2018; Marsaro et al., 2023).

The *Aedes aegypti* mosquito, originally hailing from sub-Saharan Africa, has thrived in environments characterized by anthropogenic influence and urbanization. It has emerged as the primary vector responsible for transmitting diseases such
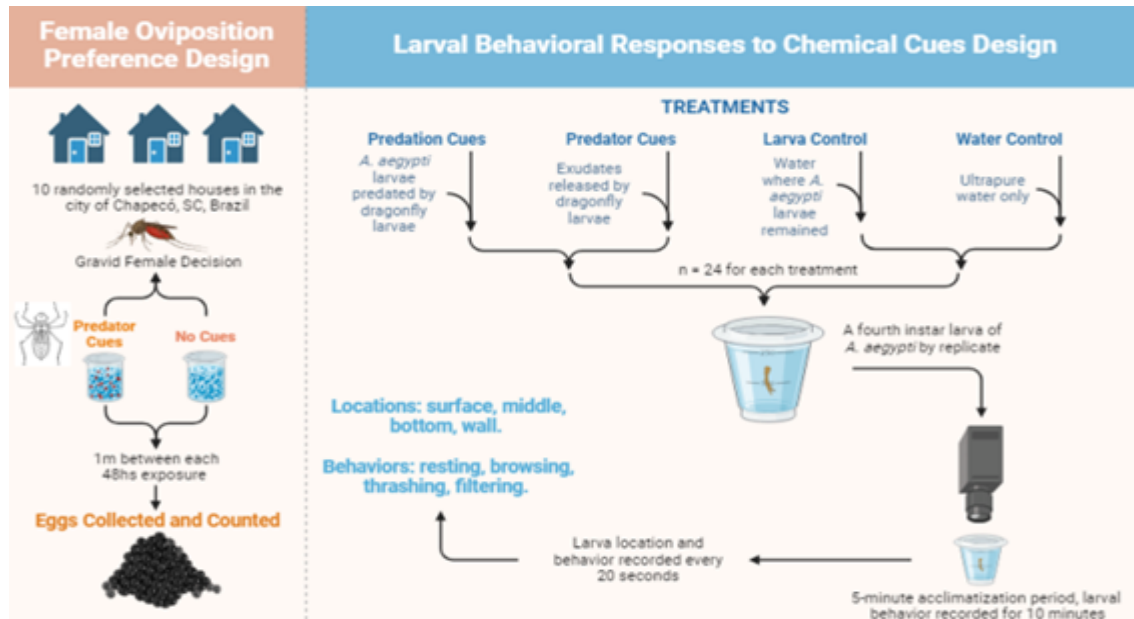
Figure 1. Experimental design of the experiments on oviposition preference by *Ae. aegypti* females and larval behavior to predator signals and predation by dragonfly nymph. *Desenho experimental dos experimentos sobre preferência de oviposição por fêmeas de* Ae. aegypti *e comportamento de larvas em relação a sinais de predadores e predação por larvas de libélulas.*

as dengue, Chikungunya, Yellow Fever, and Zika viruses (OECD, 2018). *Ae. aegypti* is an invasive species with a wide global distribution, found on every continent except Antarctica (Murray et al., 2013). The oviposition behavior of *Ae. aegypti* is a pivotal trait contributing to its adaptability in urban settings, facilitating its dispersal among densely populated areas (Day, 2016).

Mosquito larvae can recognize potential threats and adjust their behavior in response to the predation risk (Bellamy & Alto, 2018). They are capable of detecting predators through chemical signals known as kairomones released by these predators into the water. In the presence of kairomones, larvae behavior changes based on the perceived level of predation risk (Kesavaraju et al., 2007). This behavioral adjustment may be influenced by the specific ability of each mosquito species to detect the signals of particular predators, especially in cases where co-evolution has occurred (Lutz et al., 2017). Some larvae exhibit antipredator behaviors in response to predation cues (Kesavaraju et al., 2007; Kesavaraju & Juliano, 2010; Costanzo et al., 2011). However, *Ae.*

aegypti larvae do not significantly alter their behavior in the presence of belostomatids (Valbon et al., 2019) or *Notonecta undulata* predators (Lutz et al., 2017). Similarly, *Aedes albopictus* larvae behavior do not change when exposed to predation signals from *Culex appendiculata* (Kesavaraju et al., 2007), which do not display a co-evolutionary history with the prey (Kesavaraju et al., 2007; Lutz et al., 2017; Valbon et al., 2019).

Therefore, the objectives of this study were twofold: (i) to assess whether gravid female *Ae. aegypti* mosquitoes display oviposition preferences for environments with cues from predators and predation, and (ii) to investigate whether *Ae. aegypti* mosquito larvae can detect predator and predation cues and respond differently by modifying their behavior upon exposure to these chemical signals. Based on the following premises: (i) gravid female *Ae. aegypti* mosquitoes prefer to oviposit in locations containing larger amounts of organic matter (Dieng et al., 2017; Torres-Estrada et al., 2001); (ii) *Ae. aegypti* larvae have a limited ability to detect kairomones and respond differently when they detect them (Andrade et al.,

2017; Valbon et al., 2019); and (iii) *Ae. aegypti* and Odonata do not have a history of co-evolution (Lutz et al., 2017; Valbon et al., 2019). Our hypothesis is that: (i) adult females did not detect the presence of Odonata as predators, and (ii) *Ae. aegypti* mosquito larvae can detect predator and predation cues and adjust their behavior to maximize survivorship when exposed to these chemical signals.

MATERIAL AND METHODS

Oviposition preference experiment

Study site

We randomly selected ten homes within the urban perimeter of the Chapecó city, Santa Catarina State, Brazil, following Kroth et al. (2019). This region is known for having high adult *Ae. aegypti* population densities throughout most of the year (DIVE, 2019). We aimed to cover various neighborhoods within the city in our choice of sampling locations (residences). These selected residences shared similar characteristics, such as the absence of mosquito nets on windows and the presence of backyards with at least some covered area. Local vector control authorities did not employ any chemical control methods or sampling techniques in these areas for two consecutive weeks early February, before and during the duration of the experiment. The residents were asked not to use insecticides (for home control or skin repellents) during the experimental period.

Experimental design

Each household was equipped with two oviposition containers (Fig. 1). To prevent rainwater from potentially diluting the chemical cues associated with the predator and predation (see below), these containers were strategically placed beneath covered areas in the yards, with approximately one meter of separation between them. They remained in the residences for a 48-hour period every week over the course of 10 weeks. This duration was chosen to account for the potential degradation or volatilization of the chemical cues related to predation over time.

Extracting predator and predation cues

All laboratory procedures were conducted within the controlled environmental conditions of the Ecology Entomology Laboratory at Unochapecó, with a temperature set at $27 \pm 2°C$, a relative humidity maintained at 70-80%, and a 12:12-hour light-dark cycle. The extraction of chemical cues associated with predation was carried out on a weekly basis. During each week, for a period of 96 hours, we introduced 50 ml of distilled water into a sterilized plastic container and a dragonfly nymph (Odonata, Libellulidae) measuring approximately 15 mm in length, fed with 20 fourth-instar *Ae. aegypti* larvae daily (Treatment with Cues). In contrast, the Treatment without Cues received only 50 ml of distilled water and remained in the same location for the same duration (aged water). This precaution was taken to eliminate any potential influence of specific environmental factors from the extraction site on our results. To ensure consistency, all treatments were covered with mesh fabric.

Oviposition containers

We used one-liter capacity black plastic oviposition containers. The internal walls of containers were lined with white seed germination paper filters (5×15 cm) to serve as an oviposition substrate (Albeny-Simões et al., 2014). Each oviposition container was filled with 500 ml of solution. The treatment with cues received 450 ml of distilled water and 50 ml of water from the extraction container. Conversely, the treatment without cues received 450 ml of distilled water and 50 ml of aged water collected from the same environment where the extraction took place.

Egg sampling

After the 48-hour we collected the eggs by removing the oviposition paper from each container. These eggs were transported to the laboratory, counted, allowed to hatch and the larvae identified (unhatched eggs were not considered in our counts). The oviposition containers were thoroughly rinsed and cleaned with running water to ensure any remaining eggs were removed

and placed again in the same location for a new sampling period.

Larval behavior experiment

Experimental treatments and extraction of chemical cues

The larval behavior test included four treatments: (i) Mosquito larvae; (ii) Predation cues; (iii) Predator cues; and (iv) Water control. All the mosquito larvae used in this experiment were in the fourth instar of development and were randomly selected from the total cohort of larvae reared for this purpose. In the Larva treatment, we introduced 50 ml of distilled water and 20 *Ae. aegypti* larvae (with daily replacements to maintain the original density) into a plastic container. In the Predation cues treatment, we introduced 50 ml of distilled water, along with a dragonfly nymph (Odonata, Libellulidae) measuring approximately 15 mm in length, and 20 *Ae. aegypti* larvae (with daily replacements) into a plastic container. All dragonfly nymphs were identified to family level using the following identification keys: Costa et al. (2006) and Mugnai et al. (2010). Odonata nymphs were collected in the Chapecó National Forest, Guatambu, SC/Brazil, by passing the contents of 24 previously allocated and naturally colonized buckets through a granulometric sieve (250μm). This setup allowed us to extract the predation cues generated when the predator consumed *Ae. aegypti* larvae. For the Predator cues treatment, we placed 50 ml of distilled water into a plastic container and added a dragonfly nymph (Odonata, Libellulidae) of similar size. However, this larva was not provided with any food during the experimental period. Lastly, the Water control treatment consisted solely of 50 ml of distilled water in a plastic container. All treatments were covered with fine mesh (tulle) and maintained in a controlled environment for four days (96 hours).

Larval behavioral assay

Fourth-instar *Ae. aegypti* larvae were placed in individual experimental units, consisting of a 100 ml plastic cup containing 50 ml of water (5 cm diameter x 2.5 cm height) from one of the treatment groups: Larva, Predation cues, Predator cues, and Water control (n = 24 for each treatment). Each larva was used only once, making individual larvae the experimental units. Each experimental unit was individually positioned under a camera (Sony HDR-XR160). After a 5-minute acclimatization period, larval behavior was recorded for 10 minutes. During this time, each video sequence was reviewed, and larval activities and positions were recorded every 20 seconds for a total of 600 seconds in instantaneous scan samples, resulting in 30 position and 30 behavior samples per experimental unit (Fig. 1).

Behavioral and positioning categorization

The following behavioral activities were categorized: (i) resting, where larvae remained motionless and did not feed; (ii) browsing, where larvae moved along the inner surfaces of the cup propelled by mouthpart movements associated with feeding; (iii) thrashing, involving larvae moving through the water column by vigorous lateral body flexion; and (iv) filtering, characterized by larvae moving through the water column, propelled by mouthpart movements, creating filtering currents.

The positions within the cup were categorized as: (1) surface - when the larva's spiracular siphon was in contact with the surface; (2) bottom - when the larva was near the cup's bottom, up to 2 mm away; (3) wall - when the larva was up to 2 mm from the cup's side wall; and (4) middle - when the larva was not in contact with any surface and was more than 2 mm away from the cup's inner surfaces. Both, categories and behaviors, were define based on Awasthi et al. (2015).

Statistical Analysis

Oviposition

To evaluate oviposition preference, we employed the Oviposition Activity Index (OAI) (Kramer & Mulla, 1979). The OAI standardizes the data by comparing the number of eggs laid in containers without predator cues (SP) to those with predator cues (CP) using the following formula: OAI = (nCP - nSP) / (nCP + nSP).

Here, nCP represents the number of eggs laid

Table 1. Total number of eggs ± standard deviation in treatments with predation cues (With cues) and without predation cues (Without cues) in each week of the *Ae. aegypti* gravid female oviposition preference experiment. The Oviposition Activity Index (OAI) values indicate preference for the Without cues treatment (negative values) or the With cues treatment (positive values), degrees of freedom (df), F-values, and *p*-values. Significant ANOVA results comparing oviposition means between treatments are in bold. *Número total de ovos ± desvio padrão em tratamentos com sinais de predação (Com sinais) e sem sinais de predação (Sem sinais) em cada semana do experimento de preferência de oviposição de fêmeas grávidas de* Ae. aegypti. *Os valores do Índice de Atividade de Oviposição (IAO) indicam preferência pelo tratamento Sem pistas (valores negativos) ou pelo tratamento Com pistas (valores positivos), graus de liberdade (df), valores de F e valores de* p. *Os resultados significativos da ANOVA comparando as médias de oviposição entre os tratamentos estão em negrito.*

Weeks	With cues	Without cues	OAI	df	F-value	*p*-value
W1	581 (±73.86)	188 (±28.18)	0.511	1,13	24.60	**<0.001**
W2	82 (±4.61)	110 (±32.02)	-0.146	1,5	0.02	0.87
W3	730 (±81.81)	214 (±31.52)	0.547	1,15	28.90	**<0.001**
W4	705 (±64.70)	165 (±18.41)	0.620	1,15	6.84	**0.02**
W5	31 (±17.67)	31 (±21.92)	0.000	1,3	0.27	0.32
W6	32 (±9.71)	31 (±17.89)	0.015	1,5	0.45	0.53
W7	23 (±7.23)	17 (±7.37)	0.150	1,5	0.93	0.38
W8	210 (±19.51)	165 (±24.03)	0.120	1,13	9.04	**0.01**
W9	184 (±31.85)	289 (±48.49)	-0.222	1,11	0.004	0.94
W10	453 (±96.84)	193 (±42.55)	0.402	1,11	18.73	**<0.01**
All	3031 (±64.57)	1383 (±30.01)	0.373	1,105	44.73	**<0.001**

in CP containers, while nSP is the number of eggs laid in SP containers. The resulting values can be positive (+) or negative (-), and a value of 0 indicates no preference. Positive values suggest a preference for CP containers, while negative values imply a preference for SP containers. The magnitude of the value, whether positive or negative, reflects the strength of preference exhibited by gravid *Ae. aegypti* females for a particular environment.

In addition to the OAI, we assessed the impact of the independent variables SP and CP on the proportion of eggs laid (the response variable) through a one-way ANOVA. We utilized generalized linear models to examine error distribution and address potential overdispersion, adjusting data distribution for the quasi-binomial family (Crawley, 1993). These analyses were conducted separately for each week across treatments and a combined analysis that incorporated treatments and weeks at the conclusion of the study. All statistical analyses were performed using R (R Development Core Team R, 2014).

Larval behavioral

To analyze differences in behavior across various positions within the microcosms, we employed a Generalized Linear Model (GLM) with a quasi-binomial distribution (link = log, test = chi-squared; Crawley, 2007). We examined behavior data in different microcosm positions (surface, middle, bottom, and wall) and different activities (rapid movements, navigation, resting, and filtering) as response variables. These variables were analyzed across predation, predator, water control, and larva control treatments as categorical variables, using eight GLMs. It's worth noting that we adjusted all GLMs to correct for potential cases of underdispersion or overdispersion (Crawley, 2007).

Furthermore, we assessed differences among categorical variables through a contrast analysis (Crawley, 2007). In this orthogonal contrast analysis, we ordered the dependent variable for different treatments and tested them in pairs, considering values that were closest to each other. Subsequently, these data were added to the model values and tested with the next pair, simplifying the stepwise model (for more details, refer to Chapter 9 of Crawley, 2007).

Additionally, we conducted a Principal Component Analysis (PCA) based on the eigenvalues and eigenvectors correlation matrix. This analysis helped organize the behavior data, considering different positions within the microcosms

Female oviposition and larval behavior of the *Aedes aegypti* mosquito
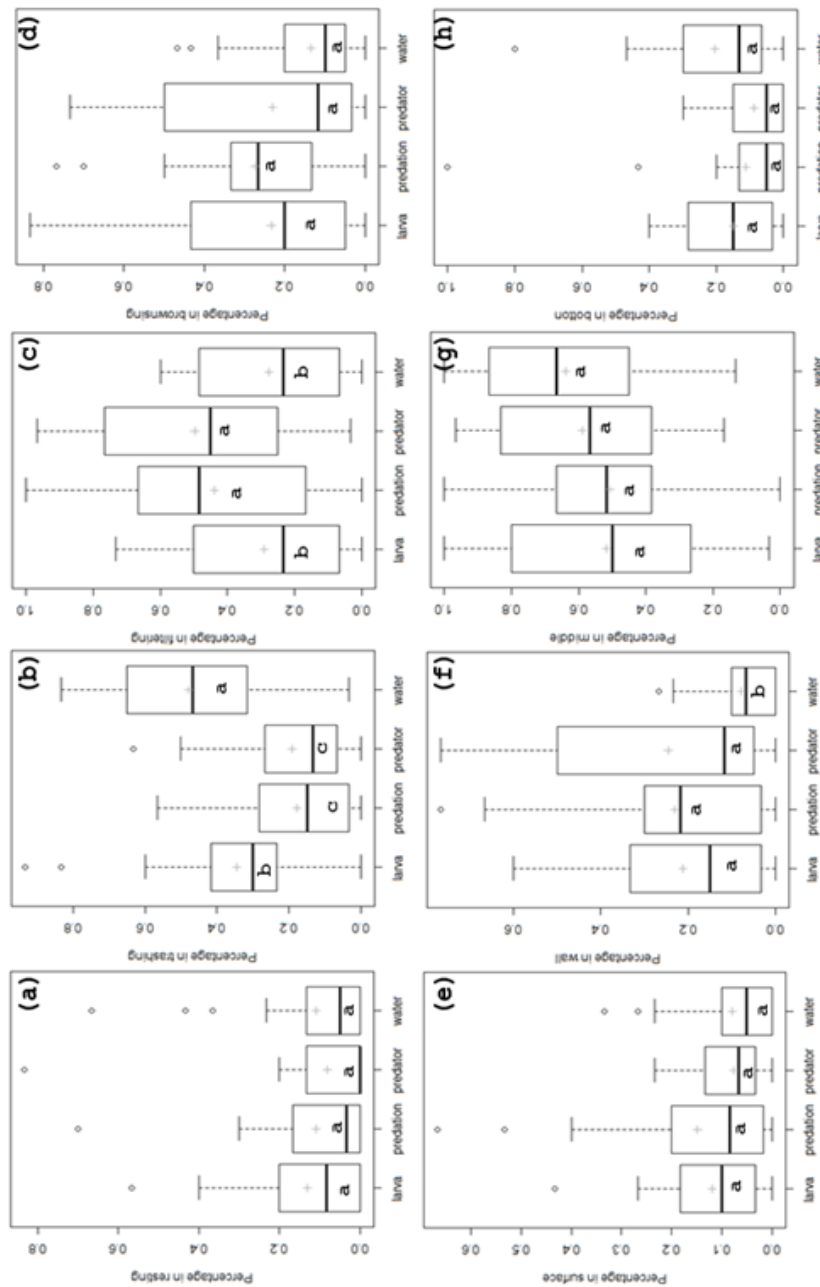
Figure 2. Percentage of time in resting (a) trashing (b) filtering (c), browsing (d) surface (e), wall (f), middle (g) and bottom (h) for order the behavior data at different position among predation, predator, water controls and larva controls treatments on microcosms. Boxes represent the quartiles, the bold line represents the median, cross gray are the mean, the vertical dashed line represents the upper and lower limits and circles the outliers. *Porcentagem de tempo em repouso (a), debatendo-se (b), filtragem (c), navegação (d), superfície (e), parede (f), meio (g) e fundo (h) para ordenar os dados de comportamento em diferentes posições entre os tratamentos de predação, predador, controles de água e controles de larva em microcosmos. As caixas representam os quartis, a linha em negrito representa a mediana, os cinzas cruzados representam a média, a linha tracejada vertical representa os limites superior e inferior e os círculos representam os outliers.*

(surface, middle, bottom, and wall) and various activities (rapid movements, navigation, resting, and filtering). To determine the significance of the axes in the PCA, we applied the Broken-Stick method (Legendre & Legendre, 1998). To use the data in the PCA, we converted behavior data into proportions of time spent on each activity. We then tested the data using Permutational Multivariate Analysis of Variance (PerMANOVA) to determine whether the observed variation was statistically significant. PerMANOVA was carried out with paired comparisons, corrected for Bonferroni adjustments, using an Euclidean distance matrix, 10 000 permutations, and pseudo-F statistics. These analyses were conducted using the Adonis function from the vegan package for R (Oksanen et al., 2008).

Table 2. Results from GLM and contrast analysis ($p < 0.05$) of resting (a) trashing (b) filtering (c), browsing (d) surface (e), wall (f), middle (g) and bottom (h) for order the behavior data at different position among predation, predator, water controls and larva controls treatments on microcosms. R*esultados do GLM e da análise de contraste (*p *< 0.05) de repouso (a), debatendo-se (b), filtragem (c), navegação (d), superfície (e), parede (f), meio (g) e fundo (h) para ordenar os dados de comportamento em diferentes posições entre os tratamentos de predação, predador, controles de água e controles de larva em microcosmos.*

GLM	DF	Resid. Dev.	Pr(>Chi)	Contrast analysis
a) Resting				
Null	95	23.2		
Treatments	3	22.8	0.782	
Residual	92	0.4		
b) Trashing				
Null	95	26.7		
Treatments	3	19.74	**< 0.001**	predation = predator < larva < water
Residual	92	6.96		
c) Filtering				
Null	95	36.5		
Treatments	3	32.8	**0.008**	larva = water < predation = predator
Residual	92	3.7		
d) Browsing				
Null	95	24.9		
Treatments	3	23.4	0.084	
Residual	92	1.5		
e) Surface				
Null	95	13.5		
Treatments	3	12.7	0.089	
Residual	92	0.8		
f) Wall				
Null	95	26.3		
Treatments	3	23.2	**0.003**	water < larva = predation = predator
Residual	92	3.1		
g) Middle				
Null	95	27.9		
Treatments	3	26.8	0.224	
Residual	92	1.1		
h) Bottom				
Null	95	19.7		
Treatments	3	18.3	0.082	
Residual	92	1.4		

RESULTS

Oviposition

A total of 4414 *Ae. aegypti* eggs were collected during the study. Among these, 3031 (69%) were deposited in the treatment with predation cues, while 1383 (31%) were laid in the treatment without predation cues. Oviposition preferences varied across the weeks of the experiment. Significant preferences were observed during the first ($p < 0.001$; OAI = 0.511), third ($p < 0.001$; OAI = 0.547), fourth ($p = 0.02$; OAI = 0.620), eighth ($p = 0.01$; OAI = 0.420), and tenth weeks ($p < 0.01$; OAI = 0.102) in favor of the predation cue treatment.

However, there was a preference for the treatment without predation cues in the second (OAI = -0.146) and ninth weeks (OAI = -0.222), although these preferences were not statistically significant. When considering the cumulative preference across all weeks, it remained statistically significant ($p < 0.001$) and showed a higher preference (OAI = 0.373) for the treatment with predation cues (Table 1).

Larval behavior

There were differences in larvae behavior across various positions and activities among the predation, predator, larva, and water control treatments. These differences were particularly pronounced for the wall position and the activities of rapid movement and filtering (Figs. 2b, 2c, 2f; Tables 2b, 2c, 2f). Rapid movement activity was prevalent in the water control and larva treatments when compared to the predation and predator treatments (Fig. 2b; Table 2b). Filtering activity, on the other hand, was considerably more frequent in the predation and predator treatments in contrast to the water control and larva treatments (Fig. 2c; Table 2c). Moreover, the larvae were more frequent in the contained walls in the presence of predation and predator treatments compared to the water control (Fig. 2f; Table 2f).

Larvae behavior was significantly affected by different treatments (PerMANOVA, F(3,93) = 3.77, $p < 0.001$). The total variance in behavioral data accounted for 66.1%, with the first and second axes explaining 39.3% and 26.8%, respectively. Both the first (3.2) and the second axis (2.2) eigenvalues proved significant after comparison with the Broken-stick model (which yielded values of 2.9 and 1.8 for the first and second axes, respectively; as shown in Fig. 3a).

The PCA (Fig. 3a) and bivariate least squares means (Fig. 3b) highlighted correlations between the predator cues treatment and wall position and filtering activity. Additionally, there was a correlation between the predation cues treatment and surface position, as well as browsing activity. Moreover, the water control treatment correlated with the middle position within the container, particularly involving rapid movement activity. Lastly, the larva control treatment correlated with the bottom position and activities related to rapid movement and rest.

DISCUSSION

Oviposition

The preference of *Ae. aegypti* females for oviposition was significantly higher in the treatment containing predator and predation cues. The female mosquitoes cannot differentiate the chemical cues of the presence of the predator from more organic matter in the system (Albeny-Simões et al., 2014). This finding probably occurred because the presence of cues tends to have higher concentrations of residues related to excretion and predation, which promote the proliferation of bacteria and zooplankton (Kneitel & Miller, 2002; Schmitz et al., 2010). These microbial communities and zooplankton serve as the primary food resources for *Ae. aegypti* larvae, along with organic matter (Kroth et al., 2022; Cozzer et al., 2024). Therefore, breeding sites with predator cues may be more attractive to gravid females due to the greater availability of food resources to support the development of their offspring (Torres-Estrada et al., 2001; Albeny-Simões et al., 2014). This finding agrees with previous studies that have demonstrated the influence of predator-related cues on the oviposition behavior of *Ae. aegypti* (Valbon et al., 2019). Although, female mosquitoes end up losing a percentage of their offspring in exchange for more food resources, even though they exhib-
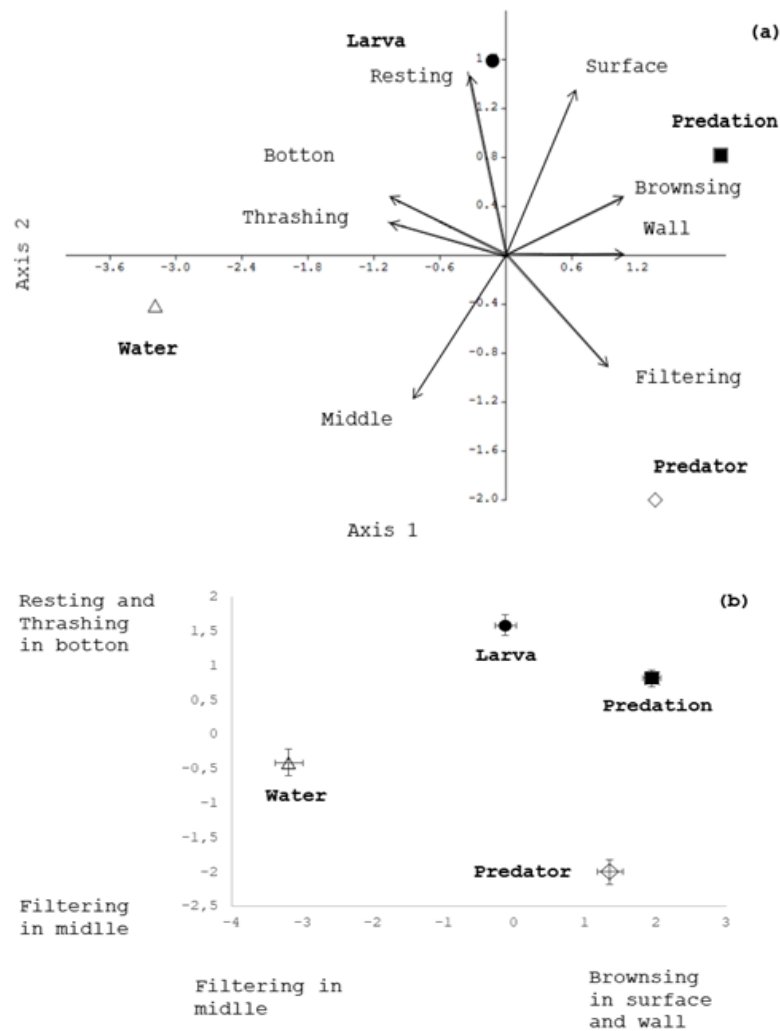
Figure 3. Principal component (PC - a) and bivariate least-squares means (PC - b; ± SE) for order the behavior data at different position on microcosms (Surface, Middle, Bottom and Wall) and different activity (trashing, browsing, resting and filtering) among predation (square) and predator (diamond) compared to controls of water (triangle) and larva (circle) treatments. *Componente principal (PC - a) e médias bivariadas de mínimos quadrados (PC - b; ± SE) para ordenar os dados de comportamento em diferentes posições nos microcosmos (Superfície, Meio, Fundo e Parede) e diferentes atividades (debater-se, navegar, descansar e filtrar) entre predação (quadrado) e predador (diamante) em comparação com os tratamentos controle de água (triângulo) e larva (círculo).*

it anti-predatory behaviors (Valbon et al., 2019). Also, highlights the complex ecological interactions that shape the selection of breeding sites by the mosquito (Kesavaraju et al., 2007; Kesavaraju & Juliano, 2010; Costanzo et al., 2011).

The benefits from more organic matter in sites with predators may be overridden by the costs of modified behavior to mitigate predation risk, resulting in the inefficient allocation of resources (Sih, 1980). This process may undermine growth and development, ultimately leading to prolonged emergence times (Bellamy & Alto, 2018; Cozzer et al., 2023). The stress imposed by predators within the developmental environment can exert a direct influence on the life history of these organisms (Andrade et al., 2017; Bellamy & Alto, 2018). This influence may manifest as alterations in body size and lifespan, subsequently impacting

adult survival (Andrade et al., 2017; Borges et al., 2023).

Larval behavioral

The presence of predators affected the activity and the spatial distribution of *Ae. aegypti*. Larvae tended to stay closer to the wall in treatments with predation and predator signals. *Ae. aegypti* larvae have the ability to detect the risk of predation through chemical cues, a characteristic shared with other mosquito species (Burks & Lodge, 2002; Kesavaraju & Juliano, 2010). When predation occurs, pheromones are released into the water by alarmed, injured, or deceased conspecifics, which are then sensed by the larvae (Chivers & Smith, 1998; Dicke & Grostal, 2001; Ferreira et al., 2023). Additionally, predators release substances called kairomones into the water column, which are also detected by *Ae. aegypti* larvae (Bellamy & Alto, 2018); larvae respond strongly to these predatory signals, reducing their movement in the water column (Anholt et al., 2000; Kesavaraju & Juliano, 2004), as an avoidance behavior or exhibiting vigorous movement to escape (Kesavaraju & Juliano, 2004; Chandrasegaran et al., 2017). This can result in a reduced foraging time (Bellamy & Alto, 2018), consistent with the behaviors observed in this experiment.

The presence of predator cues, *Ae. aegypti* increased its filtering rate. Despite being an avoidance behavior, larvae expose themselves to potential risks while searching for resources, creating a trade-off scenario (Bellamy & Alto, 2018): they must forage to secure necessary resources for normal development, even if it means assuming some risk. It's crucial to note that anti-predatory responses vary depending on the perceived level of risk (Ower & Juliano, 2019) and carry associated costs (Bellamy & Alto, 2018). This balance between foraging and avoidance behaviors can impact their overall development (Merrit et al., 1992; Kneitel & Miller, 2002; Schmitz et al., 2010). Foraging activity, even when approached cautiously, may elevate the chances of larvae falling prey to predators, but it remains a vital aspect of their development (Chandrasegaran et al., 2017; Borges et al., 2023), observations align with the findings of our experiment.

Rapid movements of *Ae. aegypti* were observed more frequently in the middle of the containers in the water and larva treatments. The behavioral patterns exhibited by prey organisms play a crucial role in determining their likelihood of falling victim to predators (Andrade et al., 2016; Chandrasegaran et al., 2017). Mosquito larvae possess the remarkable ability to adapt their behavioral responses depending on the perceived risk of predation (Kesavaraju et al., 2007). In environments devoid of predation threats, these larvae can freely explore their surroundings without the need to curtail their movements or confine themselves to areas near the container's walls to evade potential predators (Bellamy & Alto, 2018). These results further support the idea that the presence of predator cues and the experience of predation by Odonata larvae instigate anti-predatory behaviors in *Ae. aegypti* larvae.

CONCLUSIONS

We found that gravid *Ae. aegypti* females exhibited a preference for laying their eggs in locations with predator and predation cues, likely due to the increased availability of food resources for their offspring's development. This preference may be because these chemical cues didn't pose a significant threat to gravid *Ae. aegypti* females, possibly due to the lack of a co-evolutionary history between the two organisms.

Ae. aegypti larvae adjusted their behavior when sensing the threat of Odonata larvae predation. They reduced their usual foraging activities, staying close to the container's wall instead of moving rapidly in the middle. Furthermore, they increased their filtration activity when exposed to predator and predation cues, indicating a willingness to take some risks to find resources for their development. Therefore, these anti-predatory behaviors in response to predation signals are vital for *Ae. aegypti* larvae's survival and successful growth, shedding light on their preference for ovipositing in such environments.

Our results contributed to a to better understand predator-prey interactions and their implications for the community's well-being and public health through biological control strategies.

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