



UNIVERSIDAD PERUANA
CAYETANO HEREDIA

FACULTAD DE CIENCIAS E INGENIERÍA

**Mosquito (Diptera: Culicidae) oviposition site selection: interplay of
water chemistry and polarized light cues**

Tesis en formato artículo científico para optar el Título Profesional de Químico
Farmacéutico

DOI: <https://doi.org/10.52707/1081-1710-50.1-89>

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Lima, Perú

2025

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Índice

RESUMEN	1
ABSTRACT	2
I. INTRODUCTION	3
II. MATERIALS AND METHODS	5
III. RESULTS	8
IV. DISCUSSION	17
V. ACKNOWLEDGMENTS	21
VI. REFERENCES CITED	22

RESUMEN

Se investigó la asociación entre parámetros fisicoquímicos del agua y patrones de luz polarizada con la oviposición de mosquitos en áreas urbanas de la costa central peruana. Analizamos muestras de agua de diversas ubicaciones, evaluando conductividad, sólidos disueltos totales, dureza, pH, alcalinidad, fosfato y niveles de nitrato.

El análisis de componentes principales demostró que la conductividad, los sólidos disueltos totales y la dureza están significativamente asociados con la presencia de oviposición de mosquitos. Los modelos lineales mixtos generalizados confirmaron estos resultados, revelando efectos específicos según la ubicación y variaciones estacionales en los parámetros fisicoquímicos vinculados a la oviposición.

También se exploró la luz polarizada como posible señal para la oviposición. Si bien no se definió un vínculo causal directo entre los patrones de luz polarizada y la oviposición, se identificó una correlación positiva entre los niveles de pH y los patrones de luz polarizada circular, lo que sugiere una potencial interacción entre la química del agua y esta señal óptica.

Los resultados subrayan la compleja interacción de factores ambientales asociados con la oviposición de mosquitos en entornos urbanos costeros. Los resultados indican que ciertas propiedades fisicoquímicas del agua, especialmente la conductividad, los sólidos disueltos totales y la dureza, están asociadas con la selección de sitios de oviposición por parte de los mosquitos, mientras que la luz polarizada podría desempeñar un papel complementario en este proceso. Esta investigación contribuye a la comprensión de los factores que influyen en la reproducción de mosquitos en entornos urbanos peruanos.

Palabras claves: oviposición, larvae, Culicidae, polarización, comportamiento del mosquito, hábitat larval, cuerpos de agua

ABSTRACT

We investigated the association between water physicochemical parameters and polarized light patterns with mosquito oviposition in central coast urban areas of Peru. Water samples from diverse locations were analyzed, assessing key parameters, including conductivity, total dissolved solids, hardness, pH, alkalinity, phosphate, and nitrate levels. Principal component analysis revealed that conductivity, total dissolved solids, and hardness were significantly associated with the presence of mosquito oviposition. These findings were corroborated by generalized linear mixed models, which highlighted location-specific effects and seasonal variations in physicochemical parameters associated with oviposition. Our study also explored polarized light as a potential oviposition cue. While a direct causal link between polarized light patterns and oviposition requires further investigation, a positive correlation between pH levels and circularly polarized light patterns suggests a potential interaction between water chemistry and this optical cue. These findings underscore the complex interplay of environmental factors associated with mosquito oviposition. Our results suggest an association between physicochemical cues, particularly conductivity, total dissolved solids, and hardness, and the presence of mosquito oviposition, with polarized light potentially playing a role.

Keyword Index: Culicidae, larvae, oviposition, polarotaxis, larval site characteristics, mosquito behavior.

I. Introduction

Mosquito-borne diseases are commonly found in habitats with specific environmental conditions, such as high temperature and humidity. In Peru, mosquito-borne diseases such as malaria, dengue, and Zika pose a significant public health challenge, contributing to increased morbidity and mortality rates (Silver 2007). The economic burden associated with these diseases further underscores the need for effective control strategies (Sanchez-Castro et al. 2022). These diseases are primarily transmitted by *Aedes aegypti*, *Aedes albopictus*, and *Anopheles* species, with varying distributions and prevalence across different regions (Silver 2007). Peru's diverse climate, ranging from coastal deserts to Amazonian rainforests, creates unique ecological niches that influence vector populations and disease transmission patterns (Espinal et al. 2019). Furthermore, rapid urbanization and inadequate sanitation in some areas can create oviposition sites for mosquitoes, increasing the risk of disease outbreaks (Lyu et al. 2022).

Given current weather conditions and degree of pollution, it becomes crucial to understand the interaction between water chemistry and light to better control vector insects, particularly for countries with limited resources, such as Peru. Larval source management strategies have shown promise in mitigating the spread of mosquito-borne illnesses. Thus, we investigated the influence of physicochemical water parameters and polarized light patterns on the oviposition site-selection behavior of female mosquitoes (Diptera: Culicidae).

Mosquito oviposition preferences are influenced by a complex interplay of environmental factors, including water characteristics and habitat features. Gravid female mosquitoes use chemical cues to identify potential oviposition sites. Certain water physicochemical parameters, including temperature, salinity, conductivity, total dissolved solids (TDS), and pH, influence the prevalence of mosquito species (Iman and Deeni 2014). Anophelines and Culicines detect different chemical cues (Mwingira et al. 2020). *Aedes albopictus* and *Anopheles gambiae* oviposition activity is correlated with water conductivity levels and vegetation in oviposition sites (Saifur et al. 2010). However, specific chemical parameters, such as pH, temperature, and

dissolved oxygen (DO), impact larval survival rather than Anopheline oviposition behavior (Patz et al. 2000). *Culex* mosquitoes, such as *Cx. pipiens*, can adapt to various oviposition sites but avoid highly polluted ones (Allgood and Yee 2017). Moreover, *Cx. pipiens quinquefasciatus* is attracted to volatile compounds in urban areas (McCabe et al. 2021). While chemical cues are relevant, visual cues also play a crucial role in guiding gravid females toward suitable oviposition sites. In particular, the polarization of light reflected from water surfaces provides a key visual signal (Schwind 1995, Schwind and Horváth 1993).

Photoreceptors in the ventral eye region capture horizontally polarized light (Hu et al. 2014). Maximum photoreception spectral sensitivity in Culicidae mosquitoes was reported in 340 nm (gray) and 530 nm (green) wavelength spectra (van der Kooi et al. 2021). This mechanism, called polaritaxis, allows the mosquito to visualize the light reflection or polarized light against the water's surface (Horváth and Kriska 2008). Certain mosquitoes are attracted to horizontally polarized light or positive polaritaxis. While both *Aedes aegypti* and *Aedes albopictus* provide the most evidence of polarization sensitivity (Bernáth et al. 2008), studies suggest that other species, such as *Culex* mosquitoes, may also use polarized light cues during oviposition site selection. Further research is needed to confirm the extent of this sensitivity (Bernáth and Meyer-Rochow 2016). A comprehensive understanding of the chemical and visual cues involved in oviposition, including water chemistry and polarized light, is essential for developing effective and targeted vector control strategies. This study analyzes the interplay between the mentioned physicochemical parameters and polarized light patterns in influencing oviposition site selections by Culicidae mosquitoes in urban larval habitats. We hypothesize that specific physicochemical properties (e.g., pH) and the degree of polarized light of aquatic habitats will be associated with the presence of Culicidae mosquito oviposition.

II. Materials and Methods

Study Areas

Sampling was carried out from November to December, 2020 and April to November, 2021 across six regions in Lima, Peru: Cieneguilla, San Vicente de Cañete, Lunahuana, Calango, Pachacamac, and Mala (Figure 1). This region has high humidity and subtropical weather, sporadic rainfall events from December to April, and a dry season from May to November (Sanabria et al. 2018). During the rainy season in Andean regions, the water level in Lima's rivers rises, threatening the population with landslides (Sanabria et al. 2018). All collection sites were near rivers within urbanized (Cieneguilla and Pachacamac) and agricultural (San Vicente de Cañete, Lunahuana, Calango, and Mala) areas. The level of urbanization surrounding each study site was assessed using a 2.5 km radius buffer. This buffer zone was chosen to capture the potential influence of human activity and urban development on mosquito populations while maintaining relevance to the specific location of each site. Within this buffer, the percentage of green areas (e.g., parks, forests, cultivated vegetation, private gardens) was quantified using GIS local data from the Environmental Peruvian Ministry 2015).

These riparian zones exhibited a mix of vegetation types, providing habitat for both aquatic and terrestrial organisms. Dominant plant species observed at the study sites included water primrose (*Ludwigia peploides*, *Ludwigia octovalvis*), shepherd's needles (*Bidens alba*), Japanese honeysuckle (*Lonicera japonica*), and Bougainvillea (*Bougainvillea spectabilis*).

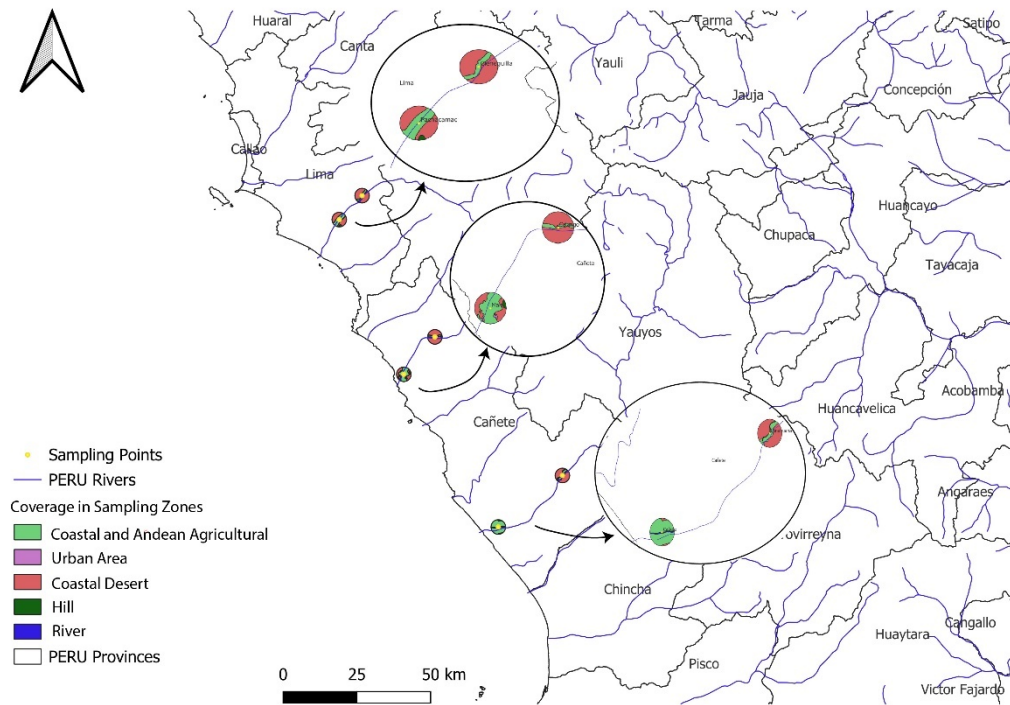


Figure 1. A constructed map that illustrates the study area of Lima. The map illustrates the distribution of sampling points (yellow dots) across various provinces of Peru (Pachacamac, Cieneguilla, Calango, Mala, Lunahuana, and Cañete) with blue lines representing the river network. The circular insets highlight specific sampling zones with pie charts showing the proportional coverage of different ecological zones: Coastal and Andean Agricultural areas (green), Urban Areas (purple), Coastal Desert (red), Hill (dark green), and River (blue). Scale bar indicates 50 km.

Identification of oviposition habitats, water sampling, and analysis

Potential oviposition habitats were identified within 100 m of the central riverbanks across three coastal rivers of central-south Lima, Peru, by systematically searching for standing water bodies (Lawal et al. 2022). Natural water bodies, including stagnant puddles and lagoons, were considered potential oviposition sites. All artificial water containers were excluded. Each potential site was carefully examined for the presence of mosquito larvae or pupae using the 50 ml dipper method. A site was classified as a confirmed oviposition habitat only if larvae or pupae were present. Confirmed oviposition sites were then assigned a unique identifier and their GPS coordinates

recorded prior to the collection of water samples. The collection of water samples followed the Standard Methods for Examining Water and Wastewater in 40 ml tubes (Baird et al. 2017). Water samples were stored at $-3\pm 3^{\circ}$ C. Physicochemical parameters were measured using quantitative instruments in situ. Alkalinity was determined through colorimetry and titration using an Alkalinity Test Kit, while total hardness was measured with a Hardness Test Kit. Phosphate levels were assessed using a Phosphate Reagent Test Kit and nitrogen was analyzed with a Nitrate Reagent Test Kit. Additionally, a multiparameter instrument was employed to record pH, TDS, and conductivity, and a dissolved oxygen meter was used to measure DO levels.

Light polarization in the field

We photographed oviposition habitats in different spectral ranges through CPL and UV filters in front of the objective lens. We minimized the risk of camera movement by using a tripod. Lens adjustment and pictures were manually fixed in three angles (0° , 45° , and 90°), repeating the process for each lens filter as follows in previous works (Horváth and Varjú 1997, Bernáth et al. 2008). These images were captured from 15:00 to 17:00, as evidence suggests that mosquito oviposition and gravid female activity peak at dawn and dusk (Day 2016). Pictures from the camera SD card were uploaded to a personal cloud storage platform for later analysis. Further analysis was conducted using Image J v.1.5# (National Institutes of Health, U.S.A.) and imported to AlgoNet[®] software (Estrato, Budapest, Hungary) to assess polarimetry. The degree and angle of polarization (Horváth and Varjú 1997, Horváth and Varjú 2004) were determined as per the method used by Horváth and Varjú (2004). This method involves capturing multiple images of the same scene through a rotating polarizer. The degree and angle of polarization for each pixel can be calculated by analyzing the intensity variations across these images. This approach is particularly relevant for studying polarization in natural environments, as it allows for the characterization of the polarization patterns of complex scenes (Horváth and Varjú 1997). Mosquitoes have photoreceptors sensitive to different wavelengths of light, including those corresponding to red, green, and blue (Horváth and Varjú 1997). Analyzing polarization across these channels allows for the assessment of potential

polarization-based cues utilized by mosquitoes across their visual spectrum. The outcomes were then processed in ImageJ software using a script to detect and count the pixels from the angle polarization image for each color channel (blue, red, and green) to quantify each image's different angle polarization areas. Subsequently, the package "multcomp" (Hothorn et al. 2008) in R Studio v 1.1.419 was employed to analyze the significance of polarized values, physicochemical parameters, and larval density for each oviposition site.

Data Analysis

The mean levels of physical and chemical parameters were visualized using Microsoft® Excel 16.30v for each sampled location. We conducted a principal component analysis (PCA) using Past v.4 Field software to reduce the physicochemical parameters. The PCA was also used to examine the potential seasonal clustering of these parameters (PC1 and PC2). We developed a generalized linear mixed-effects model (GLMM) that considered only the first two principal components and the spatial locations of oviposition sites and linked the mosquito oviposition choice to the Culicidae family. Analysis was performed using the 'lmer' package (Bates et al. 2015) in R studio v 1.1.419. To assess the significance of observed differences between sites and seasons, we performed a permutational analysis of variance (PERMANOVA) on the variables contributing to PC1.

III. Results

Physicochemical parameters

We analyzed water oviposition sites that were distributed across Calango (9), Cañete (18), Cieneguilla (15), Lunahuana (3), Mala (24), and Pachacamac (25). The mean and standard deviation (SD) of the physicochemical parameters for each site are presented in Table 1. TDS values in Pachacamac displayed a wide range, potentially reflecting occasional contamination from nearby agricultural runoff. All data points were analyzed using a mixed-effects model to account for environmental heterogeneity and potential data noise. Physicochemical parameters with high collinearity were excluded. Conductivity was the highest in Cieneguilla (736.36 ± 466.2

μS), Mala ($730.49 \pm 477.9 \mu\text{S}$), and Lunahuana ($724 \pm 489.0 \mu\text{S}$), and the lowest values in Cañete ($487.21 \pm 242.8 \mu\text{S}$). Higher conductivity can indicate increased nutrient levels, potentially making these sites more attractive for oviposition for *Culex* mosquitoes (Avramov et al. 2024). The observed pH was alkaline in most locations, which is generally favorable for Culicidae mosquito larval development (Ukubuiwe et al. 2020), and thus it could contribute to higher mosquito density. The high TDS in Pachacamac ($2038.77 \pm 6531.8 \text{ ppm}$), despite its wide range, may contribute to the suitability of this location as a breeding site, as *Culex* mosquito species tolerate higher TDS levels (El Ela et al. 2024). Water hardness and alkalinity observed in Mala suggest a potential preference for these conditions by the Culicidae species encountered.

Table 1. Mean levels of the physicochemical parameters by sampling location.

	Conduc tivity (mean \pm SD)	TDS (mean \pm SD)	pH (mean \pm SD)	Hardn ess (mean \pm SD)	Alkalini ty (mean \pm SD)	Phosp hate (mean \pm SD)	Nitrate (mean \pm SD)	DO (mean \pm SD)
Cienegu illa	$736.36 \pm$ 466.2	1859.9 $1 \pm$ 6058.2 (^e)	$8.19 \pm$ 0.8 (^e)	141.02 ± 40.0 (^e)	107.85 ± 29.2	$1.92 \pm$ 1.8 (^e)	$5.38 \pm$ 6.0 (^e)	$8.22 \pm$ 4.3 (^e)
Calango	$507.38 \pm$ 160.0	360.11 \pm 123.9	$7.84 \pm$ 0.8	171.56 \pm 65.9 (^e)	121.23 ± 42.7	$2.66 \pm$ 1.81 (^e)	$9.43 \pm$ 4.82 (^e)	$11.66 \pm$ 5.8
Cañete	$487.21 \pm$ 242.8	353.34 \pm 182.0	$8.25 \pm$ 0.8 (^e)	147.91 \pm 60.4 (^e)	109.88 ± 40.0	$2.05 \pm$ 1.8 (^e)	$6.29 \pm$ 5.7 (^e)	$10.08 \pm$ 5.1
Lunahu ana	$724 \pm$ 489.0	542.67 \pm	$7.61 \pm$ 0.5	$175 \pm$ 31.2 (^e)	$138.3 \pm$ 7.6	0	0	NA

		400.4(°)						
Mala	730.49± 477.9	1932.0 1± 6226.1 (°)	8.22± 0.7(°)	152.63 ± 52.3(°)	113.52 ± 36.1	1.97± 1.8(°)	5.92± 5.9(°)	10.11± 5.2
Pachacamac	676.85± 463.00	2038.7 7± 6531.8 (°)	8.26± 0.7(°)	153.48 ± 57.0(°)	114.33 ± 36.5	2± 1.8(°)	5.77± 6(°)	9.69± 5.1

(*) outside of the environmental standard range (Biggs et al. 2016, Alam et al. 2007).

NA: Not available; DO: Dissolved Oxygen; TDF: Total Dissolved Solids

Correlation between environmental factors and oviposition selection

The first two components (PC1 and PC2) explained 39% and 17%, respectively, of the variance in the physicochemical parameters (Figure 2). PC1 was primarily associated with hardness, and PC2 with conductivity and TDS (Table 2). PCA scores were clustered according to both seasons (rainy and dry seasons) and sites (Figure 3). This analysis underscored the importance of conductivity, TDS, and hardness in differentiating oviposition sites, such as Pachacamac, Mala, and Cieneguilla, particularly during the dry season. Our GLMM analysis (Table 3) revealed significant differences in PC1 values between locations, particularly in Cieneguilla and Lunahuana. Both locations are near coastal desert zones, which may influence their climate and vegetation. Moreover, Lunahuana and Cieneguilla show evidence of human activity, such as agriculture and settlements, which interact with the natural environment.

Table 2. Principal Component Analysis (PCA) loadings related to the eight chemical parameters measured in the study. Two principal components (PC1 and PC2) represent combinations of the variables in the original dataset.

Variable	PC1	PC2
pH	-0.46801	-0.11622
Conductivity	0.26694	0.59864
TDS	0.13765	0.63203
Hardness	0.5345	-0.24454
Alkalinity	0.52763	-0.22945
Phosphate	0.18948	-0.029076
DO	0.21028	-0.17881
Nitrate	0.2122	0.023346

DO: Dissolved Oxygen; TDF: Total Dissolved Solids

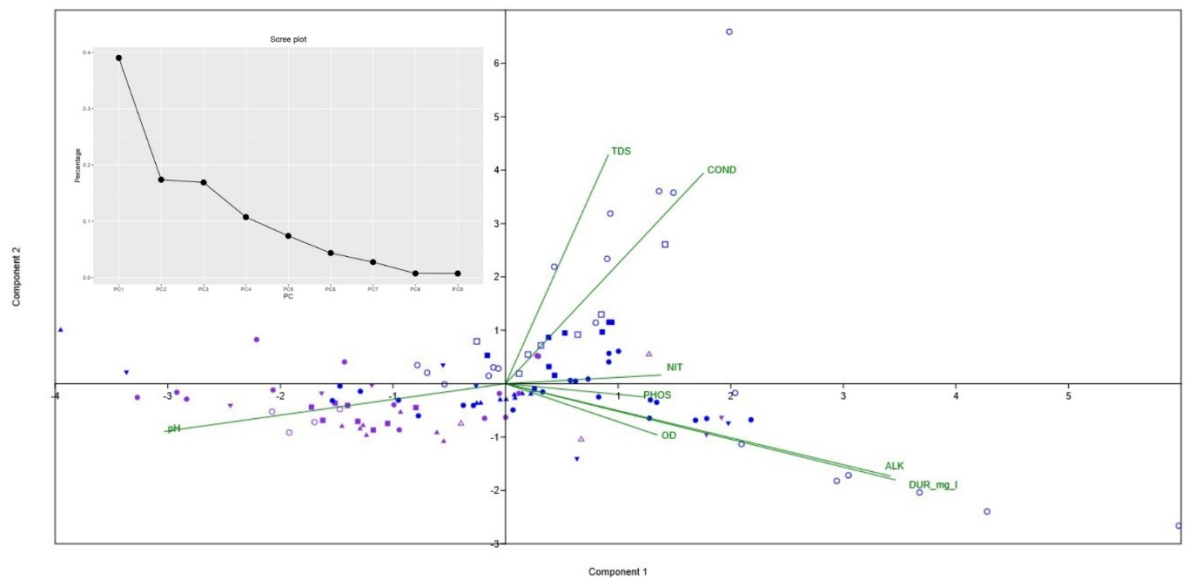


Figure 2. Principal component analysis (PCA) biplot of PCA scores of physicochemical variables [Total Dissolved Solids (TDS), pH, Conductivity (COND), Hardness (DUR_mg_l), nitrate (NIT), phosphate (PHOS), Dissolved Oxygen (OD), and Alkalinity (ALK)] on the PC1

(x-axis) and PC2 (y-axis). The vectors' length (in green) represents each variable's contribution to each PC. Vectors correlated negatively are on opposite sides of the chart, and vectors' proximity with each other defines positive correlation variables. Sampling sites are represented by distinct symbols: Cieneguilla (●), Pachacamac (○), Mala (■), Cañete (▲), Lunahuana (△), and Calango (▼). Seasonal variations are indicated by color, with blue representing the dry season and purple representing the rainy season. The inserted graph is the scree plot showing the percentage of variance explained by each principal component (PC) in the dataset. The x-axis represents the principal components (PC1 to PC9), and the y-axis shows the percentage of variance. The plot illustrates a decreasing trend in variance explained, with PC1 accounting for the highest variance and subsequent components contributing progressively less.

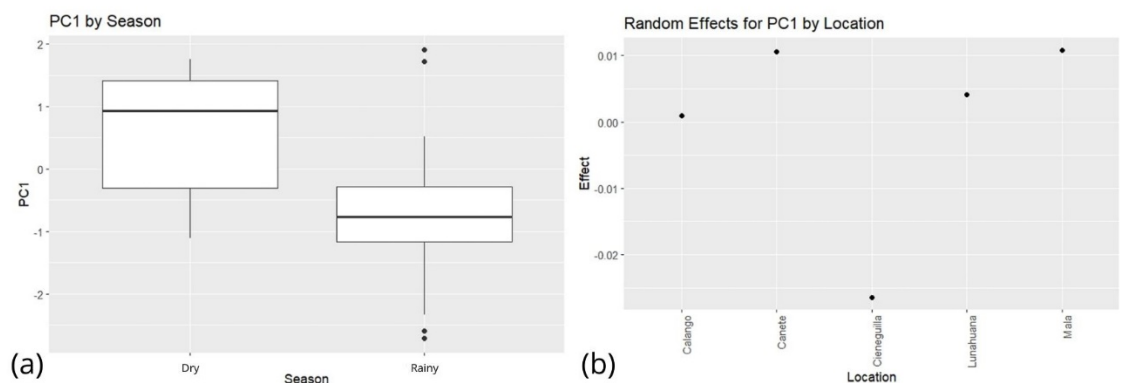


Figure 3. (a) Principal Component 1 (PC1) values comparing seasonal variation between dry and flood/rainy periods. The box plots display the median (horizontal line), interquartile range (box), and whiskers extending to the most extreme data points not considered outliers. This analysis demonstrates the seasonal dynamics in the studied system. (b) Scatter plot showing the random effects for Principal Component 1 (PC1) across different locations. The y-axis represents the effect size, while the x-axis lists the locations: Calango, Cañete, Cieneguilla, Lunahuana, and Mala. Each point indicates the

random effect for PC1 at a specific location, highlighting variations in effect size across these geographical areas.

The GLMM analysis (Table 3) found that PC1, but not PC2, is significantly influenced by seasonal change (p-value=.0423), with the rainy season associated with lower PC1 values and higher variability compared to the dry season (Figure 3a). The rainy season negatively affected PC1 values, while the dry season positively affected PC1 values. The random effects of location on PC1, as estimated by the GLMM, revealed that most locations had effects close to zero, indicating minor deviations from the overall mean effect (Figure 3b). However, specific locations exhibited noticeable deviations. For instance, Cieneguilla displayed a slightly negative effect, suggesting lower PC1 values compared to the average. Meanwhile, Lunahuana and Cañete showed higher and positive PC1 values. To further investigate the observed seasonal differences in PC1, we conducted a PERMANOVA on the physicochemical parameters contributing to PC1. The results confirmed a significant interaction between season and location ($F=9.158$, $p=.0001$), indicating that the influence of these parameters on oviposition site selection varied significantly between the dry and rainy seasons across the different locations. PERMANOVA tests indicated that during the dry season, locations such as Mala ($p<0.0001$) and Cañete ($p<.001$) showed a significant impact of variables contributing to PC1 during this period, such as water hardness.

Table 3. A generalized linear mixed-effects model (GLMM) examining the influence of seasonality and location on the two principal components associated with mosquito oviposition site selection. Significant P values are in boldface.

Model	Estimate	Std. error	t-value	p-value
Principal Component 1 (PC1)				
FIXED EFFECTS				
Season	-1.2943	0.5153	-2.512	<0.05
Intercept	0.5879	0.4639	1.267	0.2963

RANDOM EFFECTS		Variance	SD	
Location		0.008202	0.09057	
Principal Component 2 (PC2)				
FIXED EFFECTS				
Season	0.4817	0.5640	0.854	0.40
Intercept	-0.3693	0.5045	-0.732	0.47
RANDOM EFFECTS		Variance	SD	
Location		0.000	0.000	

Polarization Effect

To explore the potential association of polarized light with oviposition site selection, we measured the polarized patterns from the surface of a subset of our oviposition sites (selected randomly across all locations) using capture polarimetry. The distribution of intensity, degree of polarization, and polarization angle were measured by capture polarimetry through the camera's red, green, and blue channels. While we found a non-significant influence in the degree of polarization between sites or seasons (Figures 4 and 5), this could be due to the limited sample size or the inherent variability in natural water surfaces. However, our analysis revealed a significant influence of location ($p < 0.05$) and pH ($p < 0.01$) on larval presence (Table 4). This suggests that while polarized light may not be the primary factor associated with oviposition site selection, it could act in conjunction with physicochemical factors influencing this behavior. Further research incorporating species-level larvae identification (e.g., using molecular techniques) is needed to investigate potential species-specific responses to polarized light. By identifying the species present at each site, we could better understand the relative importance of polarized light cues for different species within the Culicidae family.

Table 4. Analysis of Variance (ANOVA) for location, pH, phosphate, and nitrate effects on polarized light (CPL) and UV light. Significant P values are in boldface.

	Df	Pillai	Approx F num	Df den	Df	P-value
CPL						
Location	6	5.8676	-11.5197	24	88	-
pH	1	0.5114	4.9715	4	19	0.00650 **
Phosphate	1	0.0069	0.0329	4	19	0.99773
Nitrate	1	0.0407	0.2015	4	19	0.93440
UV						
Location	5	0.60715	0.78738	20	88	0.7216
pH	1	0.02509	0.12222	4	19	0.9728
Phosphate	1	-0.00833	-0.03922	4	19	-
Nitrate	1	0.10761	0.57276	4	19	0.6857

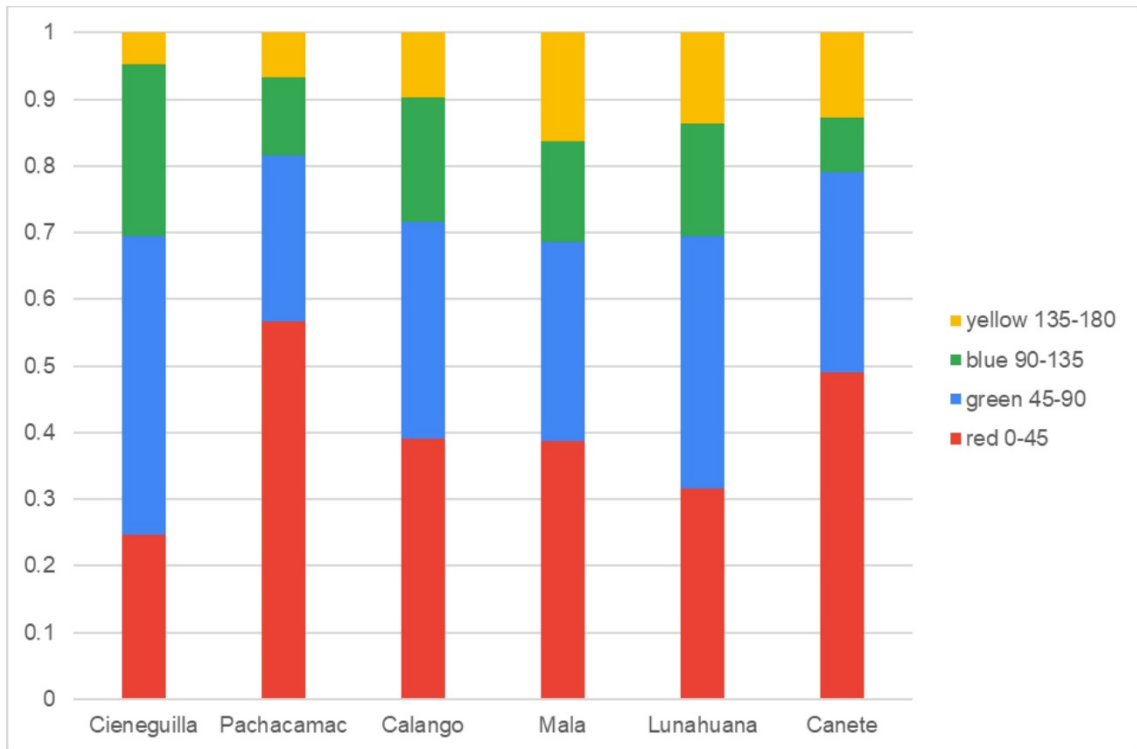


Figura 3. Stacked bar chart showing the proportion of polarized light measured using a CPL (Circular Polarizing) lens filter across different body length ranges (0-45, 45-90, 90-135, 135-180) for various locations: Cieneguilla, Pachacamac, Calango, Mala, Lunahuana, and Cañete. The y-axis represents the proportion of polarized light, while the x-axis lists the locations. Each color in the bars corresponds to a specific body length range, demonstrating the spatial variation in polarized light distribution across these geographical areas.

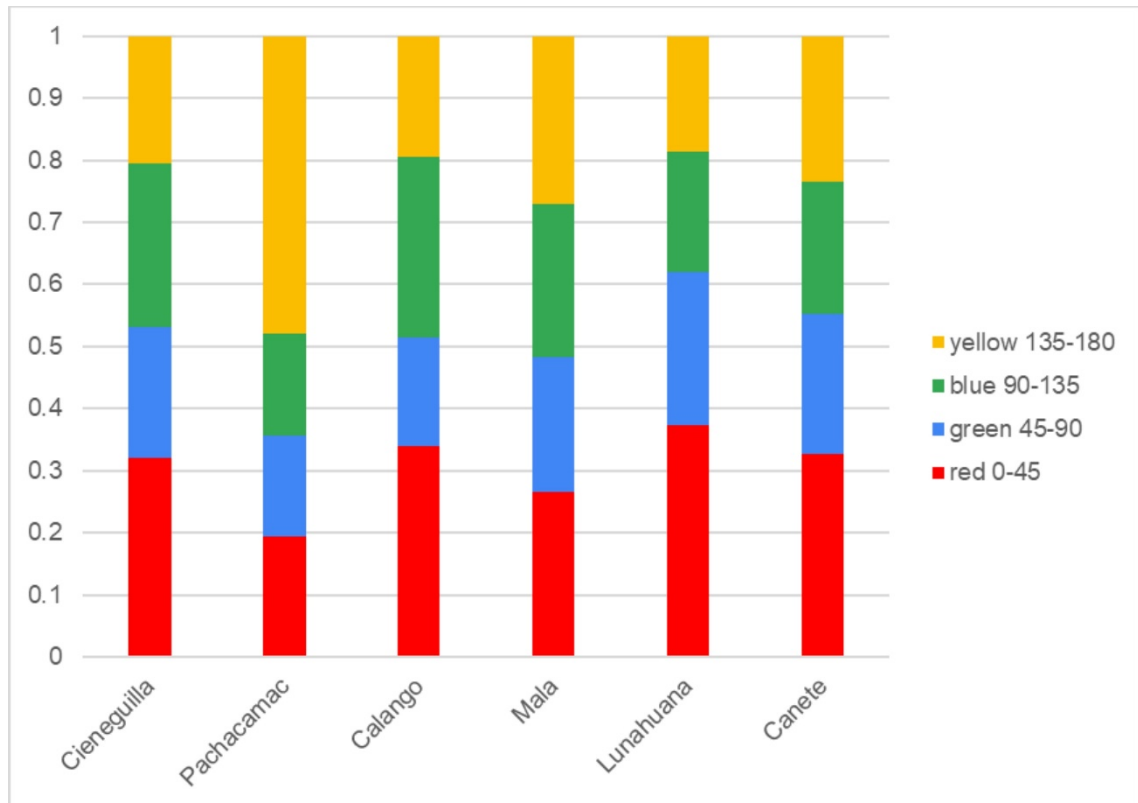


Figura 4. Stacked bar chart showing the proportion of polarized light measured using a UV lens filter across different body length ranges (0-45, 45-90, 90-135, 135-180) for various locations: Cieneguilla, Pachacamac, Calango, Mala, Lunahuana, and Cañete. The y-axis represents the proportion of polarized light, while the x-axis lists the locations. Each color in the bars corresponds to a specific body length range, demonstrating the spatial variation in polarized light distribution across these geographical areas.

IV. Discussion

Our results demonstrate an association among specific physicochemical water parameters, the degree of polarized light of the aquatic habitats, and the presence of mosquito oviposition. Conductivity, TDS, and hardness emerged as significant factors in the PCA and GLMM analyses, indicating their association with Culicidae oviposition patterns. Our PCA analysis (PC1 and PC2) suggests that while these two components capture a substantial portion (56%) of the overall variability, a considerable amount of information resides in the remaining components and other possible environmental

factors not quantified in this study. These findings are consistent with the results found in previous studies that have established that mosquitoes are attracted to water bodies with specific physicochemical properties, likely reflecting the suitability of these habitats for larval development (Emidi et al. 2017). In contrast, we found higher pH values than those reported in urbanized locations mentioned in Emidi et al. (2017). Our finding on pH values contradicts Adebote et al. (2008) that described a preference of larvae for an acid pH. This aligns with growing evidence of mosquito adaptation to various water pollution indicators (Medeiros-Sousa et al. 2020). This discrepancy might be attributed to organic contaminants in our study sites, which can elevate pH levels and serve as a food source for mosquito larvae (Ranasinghe and Amarasinghe, 2020). Potential sources of these contaminants include agricultural runoff from surrounding fields, which may introduce pesticides and fertilizers into the water bodies, as well as untreated sewage discharge from nearby settlements, as seen in Cieneguilla and Lunahuana. The decomposition of these organic materials can release ammonia, increasing pH levels. This might suggest that the suitability of oviposition sites for mosquito larvae may fluctuate seasonally. This elevated pH, coupled with the increased nutrient availability from the organic contaminants, could create a favorable environment for certain mosquito species, even if the pH deviated from their typical preferences (Jeanrenaud et al. 2019). The observed negative deviation in PC1 in Cieneguilla (Figure 4b) could be related to the influence of agricultural runoff, as it is in an urban area containing a minor agricultural area. In contrast, Lunahuana, situated in a more arid region along the Cañete River, is characterized by predominantly rocky terrain and sparse vegetation. This difference in landscape and land use likely contributes to the distinct water quality profiles observed in each location. Further research is needed to characterize the specific types of organic contaminants present in our study sites and to investigate the mechanism by which these contaminants influence mosquito oviposition preference, e.g., increasing feedable algae and/or microbes. This research could advise the development of more effective mosquito control strategies, such as targeted removal of specific contaminants or the development of ovitraps baited with specific organic attractants. The seasonality of water regimes and their association with oviposition site suitability was also observed, as demonstrated by the differential impact of PC1

parameters between the rainy and dry seasons. The seasonal variation observed in the physicochemical parameters and their influence on oviposition site selection aligns with the results reported in certain studies (Santana et al. 2015, Hinne et al. 2021). This suggests that larval mosquito habitats depend not solely on water availability but on specific water quality characteristics, such as organic contaminants, which may vary seasonally (As 2019). The significant location-specific effects on PC1 identified by the GLMM further highlight the importance of spatial heterogeneity in the physicochemical parameters of oviposition sites. Given the association between PC1 and water hardness, these findings suggest that geographical factors inherent to each location, such as underlying geology or water sources, might influence water hardness levels and consequently affect its suitability for mosquito oviposition (Ukubuiwe et al. 2020). Additionally, the use of pesticides and herbicides has been shown to alter the chemical composition of water bodies, which may impact their suitability as mosquito larval habitats (Do Rego et al. 2024). Further research incorporating detailed water flow measurements and analysis of other ecological factors would provide a more comprehensive understanding of the complex interplay between environmental characteristics and mosquito oviposition site selection. In addition to the physicochemical parameters, our study highlights the potential association of polarized light with oviposition sites. To quantify the polarized light patterns, the distribution of intensity, degree of polarization, and polarization angle were measured by capturing polarimetry through the camera's red, green, and blue channels (Horváth and Kriska 2008). However, this cue might be affected by the presence of organic contaminants and their influence on algal growth. Heavy algal blooms, often fueled by organic contaminants (As 2019), can reduce water clarity and alter the polarization patterns perceived by mosquitoes. This could disrupt their ability to assess a water body's suitability for oviposition accurately. While our study did not directly measure algal density, future research should investigate the potential masking effect of algal blooms on polarized light signals and its subsequent impact on mosquito oviposition choices. The significant association of location and pH with oviposition suggests that these factors, potentially detectable through variations in polarized light, may be related to oviposition site choice. Contrary to our initial hypothesis, we did not observe a clear

preference for either UV or CPL sensitivity in association with oviposition sites. This finding suggests that while CPL light might play a role, other factors, such as water chemistry, could be more strongly associated with oviposition. Future studies integrating surveillance entomological data with the physicochemical and optical measurements could provide valuable insights into the relationship between oviposition site characteristics and mosquito distribution and disease transmission dynamics. Additionally, field experiments designed to manipulate specific aspects of polarized light in natural settings would help disentangle the relative importance of this cue in the presence of other environmental factors. The findings of this study suggest promising avenues for developing more effective ovitraps by integrating both physicochemical and optical cues. Future research could focus on designing species-specific ovitraps with tailored water chemistry that mimics the preferred conditions of target species. For instance, ovitraps targeting *Aedes aegypti* could be designed with slightly acidic water and elevated conductivity. Furthermore, incorporating attractive polarization patterns on the ovitrap surface, combined with optimized placement in areas with suitable light environments, could significantly enhance their attractiveness. These advanced ovitraps could be integrated into targeted surveillance programs to monitor specific mosquito vectors and provide early warning of potential outbreaks. By combining our understanding of mosquito sensory ecology with innovative trap designs, we can develop more effective tools for mosquito surveillance and control, ultimately contributing to the reduction of mosquito-borne diseases. For instance, artificial containers and stagnant water in drainage systems are common oviposition sites, and ovitraps could be designed to mimic the specific physicochemical parameters and polarization patterns found in these locations in urban areas. In rural settings, where natural larval sites like marshes and irrigation ditches are prevalent, manipulating the physicochemical parameters of these sites or introducing artificial surfaces with specific polarization patterns could potentially reduce their attractiveness to mosquitoes. However, implementing these strategies in natural environments presents challenges, such as the difficulty of controlling water chemistry in large areas and the potential for unintended environmental impacts. In conclusion, our findings demonstrate an association between specific physicochemical and optical properties of aquatic habitats

and the presence of Culicidae mosquito oviposition. Further research, including manipulative experiments, is needed to determine the causal relationships between these factors and oviposition site selection. While our study highlights the correlation of optical and chemical cues as attractants for gravid female *Culicidae* (Diptera) mosquitoes, their interaction with other compounds acting as repellents and attractants requires further investigation. A deeper understanding of this multi-complex interaction is key to further developing more effective ovitraps.

V. Acknowledgments

We express our appreciation to the Instituto de Medicina Tropical Alexander Von Humboldt [FA5 DGD-ITM 2022-2026] for its support. We are also grateful to Dr. Andras Barta for giving us access to AlgoNet software with polarized features.

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