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Bionomics of Adult Anopheles pseudopunctipennis females (Diptera: Culicidae) in the Historically Malaria- Endemic Focus of Batopilas, Chihuahua, Northwest Mexico

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Abstract

Background. Anopheles pseudopunctipennis

is an important vector of *Plasmodium vivax* in mountainous regions of Mesoamerica and the Andes, but its bionomics remain poorly characterized in northern Mexico. We characterized seasonal abundance, nocturnal host-seeking activity, parity structure, and parity-derived survival in San José de Valenzuela, Batopilas, Chihuahua.

Methods.

Adult mosquitoes were sampled by outdoor human landing catches from August 2024 to July 2025 during five consecutive nights per month, with two intensive campaigns in August–September 2024 and June–July 2025. Parity was determined by ovarian tracheolation. Gonotrophic periodicity was assessed from daily parity series using cross-correlation after AR (1) filtering, and daily survival was estimated across assumed gonotrophic-cycle durations of 3.0–4.0 days. Monthly abundance was evaluated against regional rainfall and temperature using Spearman correlations with false discovery rate adjustment; on-site temperature and relative humidity in 2025 were summarized descriptively.

Results.

Mean monthly host-seeking rates ranged from 2.4 females/person/night in February 2025 to 51.2 in November 2024. Nocturnal timing varied seasonally: 95.3% of November captures occurred before midnight, whereas 86.8% and 78.0% occurred from midnight onward in June and July 2025, respectively. Parous females represented 69.2% of successfully age-graded specimens in 2024 and 68.3% in 2025, with no difference between campaigns ($p = 0.842$). No robust gonotrophic periodicity remained after accounting for temporal autocorrelation. Across assumed 3.0–4.0-day gonotrophic cycles, parity-derived daily survival ranged from 0.885 to 0.912 in 2024 and 0.881 to 0.909 in 2025. No regional climatic association remained significant after multiple-comparison adjustment.

Conclusions. Anopheles pseudopunctipennis

showed pronounced seasonal variation in abundance and substantial flexibility in within-night host-seeking timing. Similar parity structure and conditional survival estimate across intensive campaigns indicate a substantial representation of older females, although a site-specific gonotrophic-cycle duration could not be resolved. These findings provide baseline entomological evidence relevant to residual malaria surveillance in northern Mexico.

Background

Malaria remains an important public health problem in several regions of Latin America, where *Anopheles pseudopunctipennis* Theobald is recognized as an important vector in mountainous and foothill environments [1]. In Mexico, malaria transmission has historically been associated primarily with *An. albimanus* in coastal areas and *An. pseudopunctipennis* across many inland and mountainous endemic regions [2]. *Anopheles pseudopunctipennis* has a broad geographic distribution, extending from the southern United States to northern Argentina, and has been implicated in malaria transmission in several countries in Mesoamerica and South America [3, 4]. Despite its broad distribution and epidemiological importance, the bionomics of *An. pseudopunctipennis* remain poorly documented in northern Mexico, particularly in persistent or residual malaria transmission foci.

The malaria-endemic region associated with the Sierra Madre Occidental in northwestern Mexico encompasses portions of Chihuahua, Sinaloa, Sonora, and Durango and has historically contributed substantially to the national malaria burden [5, 6]. Within this region, the municipality of Batopilas, Chihuahua, has long been recognized as an important malaria focus and has experienced persistent *Plasmodium vivax* transmission [5]. Its rugged canyon topography, warm climate, perennial waterways, and river-associated habitats provide environmental conditions suitable for *Anopheles* populations, resembling foothill transmission environments described elsewhere in Mexico [7–9]. However, detailed entomological information on seasonal abundance and adult host-seeking behavior in Batopilas remains limited, and the contribution of *An. pseudopunctipennis* to malaria transmission in this ecological setting is not well defined.

Entomological studies of *An. pseudopunctipennis* in Mexico have largely focused on populations from southern and central regions, where the species has shown substantial ecological and behavioral variation across climatic, seasonal, larval-habitat, and host-seeking conditions [7–9]. Genetic structuring among populations has also been reported [10], and recent molecular evidence supports at least two putative species within *An. pseudopunctipennis* s.l. on the American mainland [11]. In contrast, systematic entomological information from Chihuahua remains scarce. Available records document the occurrence of *An. pseudopunctipennis*, *An. punctipennis*, and *An. franciscanus* in the state [9, 12], highlighting the need for locally generated bionomic data from malaria-endemic areas of northern Mexico.

This study characterized the adult bionomics of *An. pseudopunctipennis* in San José de Valenzuela, Batopilas, a malaria-endemic canyon locality in northern Mexico. Specifically, we evaluated seasonal abundance and nocturnal host-seeking activity, characterized parity structure, investigated temporal periodicity potentially associated with the gonotrophic cycle, and estimated parity-derived adult survival across biologically plausible gonotrophic-cycle durations. We additionally examined regional climatic covariation with seasonal abundance and described on-site microclimatic conditions associated with host-seeking activity during the 2025 intensive campaign. These data provide a baseline characterization of demographic and behavioral traits relevant to understanding human–vector contact and the potential contribution of *An. pseudopunctipennis* to residual malaria transmission in northern Mexico.

Methods

Study area.

San José de Valenzuela (SJV) is a small community in the municipality of Batopilas, located in the lower canyon region of the Sierra Madre Occidental, Chihuahua, Mexico (26°55'36" N, 107°43'51" W; 468 m a.s.l.; Fig. 1). The village contains approximately 142 inhabitants. The climate is warm and semi-arid, with a rainy season spanning from July to September and a dry season from November to May. During the rainy season, temperatures often range from 25–35°C with high humidity (50–70%), whereas the dry season is characterized by lower humidity (25–30%) and temperatures of approximately 10–30°C [13]. Malaria transmission in the area has historically been associated with *Plasmodium vivax* and with riverine breeding sites of *Anopheles pseudopunctipennis*.

Ethical considerations.

The Research Ethics Committee of the Autonomous University of Ciudad Juárez approved this entomological study on January 12, 2024 (document no. CEI-2024-1-1140). Written informed consent was obtained from all mosquito collectors prior to their participation. Collectors received standardized training before study initiation and were informed of the potential risks associated with mosquito exposure. Because human landing catches involve potential exposure to malaria-infected mosquitoes, collectors received appropriate antimalarial prophylaxis in accordance with WHO recommendations and the procedures of the participating health authorities [14].

During collections, collectors wore protective clothing covering all non-exposed body areas and could withdraw from the study at any time without consequence. Access to the participating household and peridomestic sampling area was granted with the permission of an adult resident.

An. pseudopunctipennis collections.

Human landing catches (HLC) were conducted in San José de Valenzuela from August 2024 to July 2025 in collaboration with personnel from Servicios de Salud de Chihuahua (SSA-Chihuahua) and the Centro Nacional de Programas Preventivos y Control de Enfermedades (CENAPRECE), with the aim of characterizing the seasonal abundance and host-seeking activity of *An. pseudopunctipennis*. For the seasonal survey, adult mosquitoes were sampled on five consecutive nights per month, from Monday to Friday, between 19:00 and 06:00 h, with collections restricted to the first 30 min of each hourly interval.

All HLCs were conducted outdoors at a single fixed peridomestic sampling site. Two trained adult collectors performed collections and were not rotated between hourly sampling intervals. Collectors remained seated with their lower legs exposed and captured *Anopheles* females as they landed using mouth aspirators. No intradisciplinary collections were conducted; therefore, the sampling design aimed to characterize outdoor peridomestic host-seeking activity rather than compare indoor and outdoor biting behavior.

In addition to the monthly seasonal surveys, two intensive sampling campaigns were conducted during the warm season to obtain detailed information on nocturnal host-seeking dynamics and to generate consecutive daily parity data to assess gonotrophic periodicity and adult survival. The first intensive campaign comprised 29 sampling nights during August–September 2024, with HLC conducted between 19:00 and 06:00 h. *Anopheles* females landing on collectors during the first 30 min of each hourly interval were captured.

The second intensive campaign comprised 23 sampling nights during June–July 2025, also conducted between 19:00 and 06:00 h, with collections restricted to the first 30 min of each hourly interval. In addition, the exact landing time of each captured mosquito was recorded as hh:mm to provide a higher-resolution description of nocturnal host-seeking activity and to link individual captures with on-site microclimatic measurements.

Anopheles specimens were initially identified morphologically in the field using the taxonomic keys of Darsie and Ward [15]. Specimens not selected for parity assessment were preserved in 1.8-mL cryovials containing 96% ethanol and subsequently transported to the Universidad Autónoma de Ciudad Juárez (UACJ). In the laboratory, species-level identifications were re-examined and confirmed using the morphological descriptions and taxonomic keys of Carpenter and LaCasse [16]. Only female mosquitoes confirmed as *An. pseudopunctipennis* were included in the analyses.

Parity determination and specimen preservation.

During the intensive sampling campaigns of August–September 2024 and June–July 2025, captured females selected for parity assessment were maintained alive until dissection. Prior to dissection, females were euthanized, generally by exposure to chloroform vapors and occasionally by cooling at −18°C. Because the euthanasia method used for individual specimens was not systematically recorded, it was not considered as a variable in subsequent analyses.

Ovarian dissections were performed in sterile 1% phosphate-buffered saline (PBS). The thorax was stabilized with a dissecting needle while a second needle was used to gently separate the terminal abdominal segments and expose and withdraw the ovaries. Ovarian tissues were cleaned of adhering material under an Olympus SZ61 stereomicroscope and subsequently examined under a LABOMED Lx 300 compound microscope.

Parity status was determined using the ovarian tracheolation method described by Detinova [17]. Females were classified as nulliparous when the ovarian tracheoles remained thick and tightly coiled, and as parous when the tracheoles were uncoiled, stretched, or fragmented, consistent with morphological criteria used for mosquito age grading [17–19].

After determining parity, the dissected ovaries and associated tracheoles were transferred to a cryovial containing 96% ethanol. The remainder of each mosquito was preserved separately in a second cryovial containing 96% ethanol. Ovarian material and the corresponding mosquito body were linked using the

same individual dissection number and collection date, allowing traceability between parity status and the corresponding specimen during subsequent laboratory processing and morphological confirmation.

For each sampling night, the total number of females dissected and successfully classified as parous or nulliparous was recorded. Females for which parity status could not be reliably determined were retained in the total number of dissected specimens but excluded from calculations of the parous rate. The nightly parity rate (PR) was calculated as:

$$PR = \frac{N_{parous}}{N_{parous} + N_{nulliparous}}$$

where N_{parous} and $N_{nulliparous}$ represent the numbers of females successfully classified as parous and nulliparous, respectively. An overall PR was subsequently calculated for each intensive sampling campaign by pooling all females with a successfully determined parity status.

Assessment of gonotrophic periodicity and parity-derived adult survival.

To assess whether daily changes in parity structure contained a periodic signal consistent with the gonotrophic cycle of *An. pseudopunctipennis*, consecutive time series were constructed separately for the August–September 2024 and June–July 2025 intensive sampling campaigns. The 2024 series comprised 29 consecutive sampling nights, and the 2025 series comprised 23 sampling nights.

For each sampling night (t), the total number of successfully age-graded females was defined as:

$$T_t = P_t + N_t$$

where T_t is the total number of successfully age-graded females on day t, P_t is the number classified as parous, and N_t is the number classified as nulliparous. Females with undetermined parity status were excluded from the time-series analysis.

Gonotrophic periodicity was evaluated by cross-correlation between the daily parous series and the total successfully age-graded series at temporal lags of 0–6 days, following Holmes and Birley [20]. Because serial autocorrelation and day-to-day fluctuations in mosquito abundance may generate spurious cross-correlation peaks, first-order autoregressive [AR(1)] models were fitted separately to both time series, and cross-correlations were recalculated using the resulting filtered series. A site-specific gonotrophic-cycle duration was considered supported only when a biologically plausible positive post-zero correlation peak remained statistically significant after autoregressive filtering. When this criterion was not met, gonotrophic-cycle duration was considered not directly estimable from the field time series.

Parity-derived adult survival.

Because a site-specific gonotrophic-cycle duration could not be assumed a priori, adult daily survival was evaluated using a sensitivity analysis rather than assigning a single gonotrophic-cycle duration to the local population. Three biologically plausible gonotrophic-cycle durations (g) were evaluated: 3.0, 3.3,

and 4.0 days. These values encompass field observations for wild *An. pseudopunctipennis* in southern Mexico and physiological time estimates showing temperature-dependent variation in gonotrophic cycle duration [21, 22].

For each sampling campaign and assumed value of g, adult daily survival probability (p) was calculated using Davidson's parity-survival relationship [23]:

$$p = PR^{1/g}$$

where p is the estimated probability that an adult female survives from one day to the next, PR is the observed overall parous rate for the corresponding sampling campaign, and g is the assumed gonotrophic-cycle duration in days. Because a site-specific value of g could not be resolved from the SJV time series, survival estimates for g= 3.0, 3.3, and 4.0 days were interpreted as sensitivity analyses and conditional parity-derived survival estimates rather than direct measurements of adult longevity.

Climatic and microclimatic data.

Seasonal climatic conditions during the study period were characterized using records from climatological station No. 25018, Choix (SMN) [24], located in Choix, Sinaloa, Mexico (26.743264° N, 108.336919° W; 311 m a.s.l.). This station was selected as a regional climatic reference because it provided an official, temporally consistent meteorological record covering the entire entomological study period from August 2024 to July 2025.

For each month, total rainfall (mm) and mean maximum and minimum air temperatures (°C) were extracted from the climatological database of the Mexican National Meteorological Service (Servicio Meteorológico Nacional, SMN). These variables were used to describe regional seasonal climatic patterns and to evaluate their temporal association with the monthly abundance of *An. pseudopunctipennis*. Because the meteorological station is located outside the immediate study site, its records were interpreted as indicators of regional climatic variation rather than as direct measurements of microclimatic conditions in San José de Valenzuela.

On-site microclimatic conditions were monitored during the June–July 2025 intensive sampling campaign using a SoliTec THC-4 data logger. Air temperature (°C) and relative humidity (RH, %) were recorded at approximately hourly intervals throughout nocturnal HLC. During the standardized monitoring period from 28 June to 15 July 2025, environmental measurements were generally obtained at approximately 30 min past each hour, corresponding to the end of each 30-min HLC sampling interval. Rainfall and episodes of strong wind were also recorded qualitatively when observed.

Environmental and entomological records were quality-checked for temporal consistency before analysis. Each standardized HLC interval was linked to the temperature and RH measurement corresponding to the same sampling interval, and only intervals with complete environmental measurements were included in the microclimatic analysis. Temperature was grouped into < 24, 24–<28, and ≥ 28°C, and RH into < 70, 70–<80, and ≥ 80%. For each class, the number of monitored intervals,

number of *An. pseudopunctipennis* females collected, and standardized host-seeking rate were summarized. Because two collectors sampled for 30 min per interval, each interval represented 1 person-hour of sampling effort.

Microclimatic patterns were analyzed descriptively. Conditions before midnight and from midnight onward were also summarized to characterize temporal covariation among host-seeking activity, temperature, and RH. No inferential tests were used to estimate independent effects of temperature or RH because both variables changed systematically with time of night.

Data analysis.

Standardization of host-seeking activity.

Host-seeking activity was standardized according to collector effort and expressed as females/person/hour. For each hourly sampling interval h, the standardized host-seeking rate (HSR_h) was calculated as:

$$HSR_h = \frac{N_h}{C \times d}$$

where HSR_h is the standardized host-seeking rate for hourly interval h, N_h is the combined number of female *An. pseudopunctipennis* captured by all collectors during that interval, C is the number of collectors, and d is the active sampling duration per collector in hours. Because two collectors sampled for 0.5 h during each interval, the total sampling effort was 1 person-hour. Consequently, the combined number of females captured during each interval was numerically equivalent to the standardized hourly rate in females/person/hour.

Nightly and monthly host-seeking abundance.

Nightly host-seeking rates were obtained by summing the 12 standardized hourly rates recorded between 19:00 and 06:00 h and were expressed as females/person/night. A nightly rate was calculated independently for each sampling night. Monthly mean host-seeking rates were then calculated as the arithmetic mean of the five independently sampled nights. Standard deviations and standard errors of the mean were calculated using the nightly host-seeking rates as the observational units.

Monthly nocturnal host-seeking distribution.

Nocturnal host-seeking patterns were evaluated independently within each month to distinguish temporal distribution from absolute mosquito abundance. For each month, the number of females captured during each hourly interval was divided by the total number of females captured during the five monthly sampling nights and expressed as a percentage. Thus, hourly percentages within each month summed to 100%. These percentages were used to characterize seasonal changes in the timing of host-

seeking activity and visualized them as a month-by-hour heatmap. Patterns from months with low total mosquito numbers were interpreted cautiously.

Regional climatic associations.

Associations between monthly mosquito abundance and regional climatic variables were evaluated using Spearman rank correlation coefficients (ρ). Variables included same-month total rainfall; rainfall lagged by 1, 2, and 3 months; cumulative rainfall during the preceding 2 and 3 months; mean maximum temperature; and mean minimum temperature. Analyses used months for which complete mosquito and climatic observations were available. Because multiple environmental associations were evaluated, raw p-values were additionally adjusted using the Benjamini–Hochberg false discovery rate (FDR) procedure [25]. These analyses were interpreted as exploratory associations with broad regional seasonal climatic variation rather than as evidence of direct meteorological effects at SJV.

Microclimatic analysis.

Microclimatic conditions were analyzed descriptively at the standardized HLC-interval level. Each 30-min sampling interval with complete temperature and relative-humidity measurements was considered an analytical unit. Temperature was categorized as < 24, 24–<28, and ≥ 28°C, and relative humidity as < 70, 70–<80, and ≥ 80%. For each environmental class, the number of monitored intervals, number of female *An. pseudopunctipennis* collected, and standardized host-seeking rate were summarized. Microclimatic conditions and host-seeking rates were also summarized before midnight and from midnight onward to characterize their temporal covariation over the course of the night. Because temperature and relative humidity varied systematically with time of night, these analyses were considered descriptive, and no inferential tests were used to estimate their independent effects on host-seeking activity.

Comparison of parity structure.

Overall parity structure was compared between the 2024 and 2025 intensive campaigns using a Pearson chi-square test with Yates' continuity correction on a 2×2 contingency table of parous and nulliparous females in each campaign. Females with undetermined parity status were excluded from this analysis.

Gonotrophic periodicity and survival analysis.

Gonotrophic periodicity was evaluated separately for the 29-night 2024 and 23-night 2025 time series using the cross-correlation and AR (1)-filtering procedure described above. Cross-correlation functions derived from both the original and filtered series were examined. Parity-derived adult daily survival was subsequently evaluated using the overall parous rate for each campaign and assumed gonotrophic cycle durations of 3.0, 3.3, and 4.0 days.

Software and reproducibility.

Statistical significance was assessed at α = 0.05. Data processing and statistical analyses were performed in Python 3.13.5. Data management and aggregation were conducted using pandas 2.2.3 and NumPy 2.3.5; statistical tests and correlation analyses were performed using SciPy 1.17.0; autoregressive model fitting and Benjamini–Hochberg false discovery rate corrections were implemented using statsmodels 0.14.6. Figures were generated using Matplotlib 3.10.8.

The curated entomological and environmental data underlying the reported summaries, statistical analyses, tables, and figures are provided in Additional file 1. The supporting workbook includes analysis-ready data, derived summaries, and quality-control information used throughout the study. The reproducible Python analysis script is provided separately as Additional file 2.

Results

Parity structure and gonotrophic periodicity.

During the August–September 2024 intensive sampling campaign, 640 female *Anopheles pseudopunctipennis* were dissected to determine parity. Parity status could be reliably determined for 634 females, of which 69.2% were parous (439/634), and 30.8% were nulliparous (195/634). Six females for which parity status could not be reliably determined were excluded from parity-rate calculations.

During the June–July 2025 intensive sampling campaign, parity status was successfully determined for 249 females, of which 68.3% were parous (170/249), and 31.7% were nulliparous (79/249). The proportion of parous females did not differ significantly between the 2024 and 2025 campaigns (Pearson's chi-square test with Yates' continuity correction: $\chi^2 = 0.040$, df = 1, $p = 0.842$), indicating a similar parity structure between sampling periods.

Daily parity series from the 29-night 2024 and 23-night 2025 intensive campaigns were evaluated for temporal periodicity potentially associated with the gonotrophic cycle. Positive post-zero cross-correlation peaks were observed in the unfiltered time series; however, these patterns were not retained after removal of temporal autocorrelation. Consequently, no statistically robust post-zero cross-correlation peak was detected in either campaign, and a site-specific gonotrophic-cycle duration could not be directly estimated from the field time series.

Parity-derived estimates of adult daily survival.

Because gonotrophic-cycle duration could not be directly resolved from the daily parity series, adult daily survival was evaluated across biologically plausible gonotrophic-cycle durations of 3.0, 3.3, and 4.0 days. For the 2024 campaign, with an overall parous rate of 69.2% (PR = 0.692), estimated daily survival probabilities ranged from 0.885, assuming a 3.0-day gonotrophic cycle, to 0.912, assuming a 4.0-day cycle. At an intermediate gonotrophic-cycle duration of 3.3 days, estimated daily survival was 0.895.

A similar pattern was observed during the 2025 campaign, with an overall parous rate of 68.3% (PR = 0.683). Estimated daily survival probabilities ranged from 0.881 assuming a 3.0-day gonotrophic cycle to

0.909 assuming a 4.0-day cycle, with an intermediate estimate of 0.891 for a 3.3-day cycle.

Overall, parity structure and parity-derived survival estimates were highly similar between the two intensive sampling campaigns. Across the range of gonotrophic-cycle durations evaluated, estimated daily survival remained consistently high (Table 1). These estimates are conditional on the assumed gonotrophic-cycle duration and should therefore not be interpreted as direct measurements of adult longevity.

Table 1
Parity structure and parity-derived daily survival during the 2024 and 2025 campaigns.

Sampling campaign	Successfully age-graded, n	Parous, % (n)	Assumed gonotrophic cycle, g (days)	Estimated daily survival, p
Aug–Sep 2024	634	69.2 (439)	3.0	0.885
			3.3	0.895
			4.0	0.912
Jun–Jul 2025	249	68.3 (170)	3.0	0.881
			3.3	0.891
			4.0	0.909

Note. Parous rate (PR) was calculated as the proportion of successfully age-graded females classified as parous. Nulliparous females represented 30.8% (195/634) in 2024 and 31.7% (79/249) in 2025. Females for which parity status could not be reliably determined were excluded from PR calculations. Adult daily survival probability (*p*) was estimated as $p = PR^{1/g}$, where g represents the assumed gonotrophic-cycle duration. Gonotrophic-cycle durations of 3.0, 3.3, and 4.0 days were evaluated as a sensitivity analysis. Because the duration of the site-specific gonotrophic cycle could not be directly inferred from the field time series, survival probabilities should be interpreted as conditional estimates rather than direct measurements of adult longevity.

Nocturnal host-seeking activity.

The timing of *Anopheles pseudopunctipennis* host-seeking activity varied substantially across the annual sampling period (Fig. 2). During August 2024, activity was predominantly concentrated after midnight, with 66.4% of monthly captures occurring between 00:00 and 06:00 h and the highest proportion recorded during the 01:00 sampling interval (21.3%). In September, captures were evenly distributed before and after midnight, with the largest proportion occurring at 22:00 h (19.0%). By October, activity had shifted toward the first half of the night, with 62.8% of captures occurring before midnight and a peak at 22:00 h (16.8%).

The strongest early-evening concentration occurred in November 2024, when 95.3% of all captures occurred before midnight. The 19:00 sampling interval alone accounted for 46.1% of the monthly total. This pronounced early-evening pattern diminished during subsequent months, when mosquito abundance was also substantially lower.

A contrasting late-night pattern was observed at the beginning of the 2025 rainy season. During June 2025, 86.8% of captures occurred from midnight onward, with the highest proportion during the 02:00 sampling interval (27.9%). A similar pattern persisted in July, with 78.0% of captures occurring from midnight onward, and the highest proportion again recorded at 02:00 h (19.5%).

Overall, these observations indicate substantial seasonal variation in the timing of host-seeking activity rather than a single consistent nocturnal peak throughout the year. Hourly distributions during months with low mosquito abundance, particularly January and February 2025, were interpreted with caution due to the small number of captures.

Seasonal abundance and regional climatic variation.

Monthly host-seeking rates showed marked seasonal variation throughout the August 2024–July 2025 sampling period (Fig. 3). The highest mean abundance was recorded in November 2024 (51.2 ± 9.8 SE females/person/night). Relatively high host-seeking rates were also observed in October (27.4 ± 5.1), August (24.4 ± 4.6), and September 2024 (20.0 ± 3.8 females/person/night). Abundance subsequently declined in December and reached its lowest levels in January and February 2025, at 3.6 ± 1.2 and 2.4 ± 0.2 females/person/night, respectively.

Host-seeking activity increased again during the subsequent warm season, reaching 12.6 ± 2.6 females/person/night in April and 13.6 ± 1.5 in June 2025, before decreasing to 8.2 ± 1.9 females/person/night in July. Overall, the seasonal pattern was characterized by a pronounced abundance peak during the late rainy–early dry-season transition of 2024, followed by substantially lower densities during the cooler months and a moderate increase during the following warm season.

Regional rainfall records from climatological station No. 25018 at Choix (SMN) showed substantial temporal variation during the study period. Total monthly rainfall was highest in August 2024 (103.1 mm) and July 2025 (90.2 mm), whereas little or no rainfall was recorded during several intervening months. Because the Choix station was used as a regional climatic reference rather than a direct measure of conditions in San José de Valenzuela, associations between these records and mosquito abundance were interpreted with caution.

Monthly mosquito abundance was not significantly associated with same-month rainfall (Spearman's $\rho = 0.261$, $p = 0.412$). Rainfall lagged by two months showed the strongest positive association with mosquito abundance ($\rho = 0.639$, $p = 0.034$); however, this association was not statistically significant after adjustment for multiple comparisons using the Benjamini–Hochberg false discovery rate procedure (adjusted $p = 0.273$). Rainfall lagged by one or three months and cumulative rainfall over the preceding two or three months were also not significantly associated with mosquito abundance after adjustment.

Mean maximum and minimum regional temperatures were positive but not significantly associated with mosquito abundance (maximum temperature: $\rho = 0.343$, $p = 0.276$; minimum temperature: $\rho = 0.406$, $p = 0.191$). Thus, although mosquito abundance exhibited pronounced seasonal variation and peaked after

the principal regional rainfall event of 2024, the exploratory correlation analyses did not identify a robust independent association between monthly abundance and the evaluated regional climatic variables.

Microclimatic conditions during the 2025 intensive campaign.

Standardized microclimatic monitoring from 28 June to 15 July 2025 provided complete temperature and RH measurements for 205 of 216 expected HLC intervals (94.9%) across 18 sampling nights. Temperature during valid intervals ranged from 21.6 to 36.1°C and RH from 46.8 to 98.4%. A total of 127 *An. pseudopunctipennis* females were collected during standardized HLC intervals with complete microclimatic measurements.

Most females were collected at temperatures of 24–<28°C (82.7%; 105/127), with an interval-specific host-seeking rate of 0.87 females/person/hour. In comparison, 16.5% of females were collected at ≥ 28°C (HSR = 0.27) and 0.8% at < 24°C (HSR = 0.14). Similarly, 73.2% of females were collected when RH was ≥ 80%, although host-seeking rates did not increase monotonically across RH classes.

Microclimatic conditions changed markedly overnight. Before midnight, mean temperature and RH were 30.1°C and 72.7%, respectively, and the host-seeking rate was 0.22 females/person/hour. From midnight onward, mean temperature decreased to 26.0°C and RH increased to 85.8%, while the host-seeking rate increased to 0.88 females/person/hour. These patterns indicate that the cooler and more humid conditions accompanying most mosquito activity were also strongly associated with the later portion of the night.

Discussion

Parity structure and implications for adult survival.

The consistently high proportion of parous *Anopheles pseudopunctipennis* females observed during both intensive sampling campaigns indicates that a substantial component of the host-seeking population had completed at least one gonotrophic cycle. Parous females accounted for 69.2% of successfully age-graded mosquitoes in August–September 2024 and 68.3% in June–July 2025, with no significant difference between campaigns. The close agreement between sampling periods suggests that the adult age structure of the host-seeking population was relatively similar across the two warm-season campaigns, despite their occurring at different times in the annual cycle.

Parity rates of this magnitude are consistent with the presence of relatively long-lived adult females in the population and are broadly comparable with observations reported for *An. pseudopunctipennis* in other endemic settings [21]. However, translating parity directly into daily survival requires knowledge of the gonotrophic cycle duration and assumptions about the stability of the sampled population. These considerations are particularly important under field conditions, where recruitment, mortality, host-seeking behavior, and environmental variation may change over relatively short time scales.

In the present study, temporal analysis of the daily parity series did not yield a statistically robust estimate of gonotrophic cycle duration. Although positive post-zero cross-correlation peaks were apparent in the unfiltered series, these patterns were not retained after accounting for temporal autocorrelation. Therefore, the absence of a robust periodic signal should not be interpreted as evidence that a defined gonotrophic rhythm was absent, but rather that a site-specific gonotrophic-cycle duration could not be resolved reliably from the available field time series. This distinction is important because previous physiological and field studies have reported gonotrophic cycle durations of approximately 3–4 days in *An. pseudopunctipennis* under favorable thermal conditions [21, 22].

Given this uncertainty, we evaluated parity-derived adult survival across gonotrophic-cycle durations of 3.0, 3.3, and 4.0 days rather than assigning a single fixed value to the SJV population. Across this range, estimated daily survival was similar between campaigns, with values of 0.885 to 0.912 in 2024 and 0.881 to 0.909 in 2025. The similarity of these estimates reflects the nearly identical parity structure observed between years and suggests that adult survival during both intensive sampling periods was relatively stable within the assumptions of the parity-based framework.

These results should nevertheless be interpreted as conditional estimates rather than direct measurements of mosquito longevity. Parity provides information on reproductive history, whereas estimates of daily survival depend on the assumed duration of the gonotrophic cycle and on demographic assumptions that may not be fully satisfied in natural populations. Consequently, mark–release–recapture studies, longitudinal age-grading approaches, or other independent estimates of adult survival would be valuable for validating the survival patterns suggested by the present data.

Seasonal abundance and regional climatic variation.

Anopheles pseudopunctipennis was detected throughout the annual sampling period, but adult host-seeking abundance varied markedly among months. The highest mean rate occurred in November 2024, when abundance reached 51.2 females/person/night, whereas the lowest rates were recorded during January and February 2025. This pronounced seasonal variation is consistent with previous observations that populations of *An. pseudopunctipennis* can fluctuate substantially in response to seasonal changes in the availability and productivity of larval habitats [8, 26–28].

The November abundance peak occurred after the principal regional rainfall event recorded during August 2024. A delayed increase in adult abundance following rainfall is biologically plausible because precipitation may modify river margins, seepage areas, and other aquatic habitats used by immature *An. pseudopunctipennis*, while subsequent larval development necessarily introduces a temporal delay before changes are reflected in the adult population. Similar post-rainfall increases have been described in other regions where this species occurs [8, 26, 27]. However, the climatic associations observed in the present study provide only limited statistical support for this interpretation.

Rainfall, lagged by two months, showed the strongest positive correlation with adult abundance, but the association did not remain statistically significant after correction for multiple environmental

comparisons. Same-month rainfall, the other lag periods, cumulative rainfall, and regional maximum and minimum temperatures were likewise not significantly associated with abundance after adjustment. Therefore, the pronounced November peak should not be attributed to rainfall alone. Mosquito abundance in this canyon environment is likely determined by multiple interacting processes, potentially including the persistence and suitability of aquatic habitats, river flow, temperature, local humidity, and other environmental factors not resolved by monthly regional meteorological records.

This interpretation is further constrained by the spatial scale of the climatic data. Meteorological records from the Choix station provided a homogeneous regional climatic series, but they do not represent direct measurements of conditions at San José de Valenzuela. Consequently, the climatic analyses are most appropriately interpreted as describing broad seasonal covariation rather than demonstrating direct meteorological control of local mosquito abundance. The clear seasonal signal in adult density nevertheless identifies late 2024, particularly October–November, as a period of substantially greater potential human–vector contact than the low-abundance months of the subsequent cool season.

Seasonal variation in nocturnal host-seeking activity.

The temporal distribution of host-seeking activity varied considerably across the annual cycle. Rather than exhibiting a single consistent nocturnal peak, *An. pseudopunctipennis* shifted between early- and late-night activity depending on the sampling month. This contrasts with the pattern obtained when monthly data were pooled, in which the large number of early-evening captures recorded in November disproportionately emphasized the 19:00 h interval. Examination of month-specific distributions therefore provides a more informative representation of temporal host-seeking behavior in this population.

During November 2024, host-seeking activity was strongly concentrated during the first part of the night, with 95.3% of captures occurring before midnight and 46.1% during the 19:00 sampling interval alone. In contrast, activity occurred substantially later during August 2024 and again during June–July 2025. In June, 86.8% of captures occurred from midnight onward, with the highest proportion recorded at 02:00 h, and July showed a similarly late-night distribution. Together, these observations indicate considerable temporal flexibility in host-seeking behavior within the same population over relatively short seasonal time scales.

Variation in host-seeking schedules has been reported previously for *An. pseudopunctipennis* across its geographic range, with nocturnal peaks differing among localities, sampling conditions, and environmental settings [3, 7, 29–31]. The present findings extend this variability to the seasonal scale within a single locality and caution against characterizing the SJV population as having a fixed early-evening or late-night host-seeking pattern.

Microclimatic observations during the June–July 2025 intensive campaign provided additional context for the pronounced post-midnight activity recorded during this period. Most females associated with complete environmental measurements were collected at temperatures of 24–<28°C and RH ≥ 80%, and

interval-specific host-seeking rates were highest within the 24–<28°C temperature range. However, these conditions also characterized the later portion of the night: temperature decreased, and RH increased as the night progressed, coinciding with the marked increase in mosquito activity after midnight. Thus, the concentration of captures under relatively cooler and more humid conditions cannot be separated from the temporal structure of host-seeking activity in this observational design. Moreover, host-seeking rates did not increase monotonically across RH classes, providing little support for interpreting humidity alone as a direct driver of the observed nocturnal pattern. The microclimatic results therefore best describe the environmental conditions accompanying late-night host-seeking rather than demonstrating independent effects of temperature or humidity.

From an epidemiological perspective, this temporal flexibility is relevant because human–vector contact cannot be assumed to occur within a single fixed portion of the night. Surveillance or control strategies based exclusively on an expected early-evening peak could underestimate contact during periods when host-seeking shifts toward post-midnight hours. Conversely, the pronounced early-evening activity observed in November indicates that exposure before typical sleeping hours may be particularly relevant during periods of high mosquito abundance. These seasonal shifts reinforce the importance of considering both mosquito density and within-night host-seeking phenology when characterizing potential human exposure in this malaria-endemic setting.

Epidemiological implications and local vector role.

Anopheles pseudopunctipennis is recognized as an important malaria vector in several mountainous and foothill regions of Mexico, Central America, and South America [3, 28, 32–34]. The present study did not evaluate *Plasmodium* infection in mosquitoes and therefore cannot directly establish the SJV population's contribution to local malaria transmission. Nevertheless, several characteristics documented here are relevant to its potential epidemiological importance.

First, the species was collected throughout the annual survey and reached high host-seeking densities during particular months, demonstrating substantial opportunities for human–mosquito contact. Second, approximately two-thirds of successfully age-graded females were parous during both intensive campaigns, indicating that a large proportion of the host-seeking population had survived at least one gonotrophic cycle. Third, host-seeking occurred outdoors in the peridomestic environment throughout the night, with pronounced seasonal shifts in timing. Together, these findings demonstrate the persistence of an adult population with demographic and behavioral characteristics relevant to malaria transmission.

These observations should not, however, be interpreted as a direct estimate of vectorial capacity. Vectorial capacity depends on several components that were not measured in this study, including mosquito infection prevalence, human infectiousness, host preference, feeding success, and parasite development. Similarly, because all HLCs were conducted outdoors at a fixed peridomestic site, the present data characterize outdoor human-seeking activity but do not establish whether this population is predominantly exophagic or how its activity compares with indoor biting.

The combination of pronounced seasonal abundance and variable nocturnal host-seeking suggests that entomological surveillance in this region would benefit from accounting for both dimensions. Sampling restricted to a single season or a narrow interval of the night could substantially underestimate either population density or the timing of human exposure. In particular, the contrast between the intense early-evening activity observed in November and the predominantly post-midnight activity recorded during June–July illustrates the potential limitations of assuming a temporally fixed pattern of vector–human contact.

Limitations and perspectives.

Several limitations should be considered when interpreting these results. The study was conducted at a single community and encompassed one complete annual sampling cycle; therefore, the seasonal patterns observed here cannot yet be assumed to represent year-to-year dynamics throughout the lower Tarahumara region. Monthly seasonal estimates were based on five consecutive sampling nights, and hourly distributions during months with very low mosquito abundance, particularly January and February, are consequently based on relatively small numbers of females.

Two collectors conducted human landing catches at one fixed outdoor peridomestic location. This standardized design improved comparability across sampling periods, but it does not capture spatial heterogeneity within the community or allow comparisons between indoor and outdoor host-seeking. Furthermore, HLC quantifies mosquito landing activity and potential human contact rather than successful blood feeding.

The parity-based survival analysis also has inherent limitations. Parity status distinguishes females that have completed at least one gonotrophic cycle from nulliparous females but does not provide their exact chronological age. A site-specific gonotrophic-cycle duration could not be resolved from the temporal parity series; therefore, daily survival estimates depended on assumed biologically plausible gonotrophic-cycle durations. These estimates should be viewed as sensitivity-based demographic approximations rather than direct measurements of longevity. Mark–release–recapture approaches or complementary molecular and physiological age-grading methods could provide independent survival estimates in future studies.

Environmental analyses were similarly constrained by data resolution. The Choix meteorological station provided a consistent regional record but was located outside the immediate study area, and monthly values cannot capture the fine-scale environmental conditions mosquitoes experience within the canyon. On-site temperature and relative humidity measurements were available only during the June–July 2025 intensive campaign and were analyzed descriptively. Future studies incorporating continuous local environmental monitoring, hydrological characteristics of larval habitats, and repeated sampling across multiple annual cycles would allow stronger evaluation of the environmental drivers of seasonal abundance and host-seeking behavior. Because temperature and relative humidity covaried strongly with time of night, their independent effects on host-seeking activity could not be assessed.

Finally, mosquito infection with *Plasmodium* was not assessed, and no entomological inoculation rate or direct measure of vectorial capacity could therefore be calculated. Integration of sporozoite detection, blood-meal analysis, longitudinal parity or age-grading, and local epidemiological surveillance would be particularly valuable for determining the specific contribution of *An. pseudopunctipennis* to residual malaria transmission in this region.

Conclusions

This study provides a detailed characterization of the seasonal abundance, nocturnal host-seeking activity, parity structure, and parity-derived survival of *Anopheles pseudopunctipennis* in San José de Valenzuela, a malaria-endemic locality in northern Mexico. Adult abundance varied markedly throughout the annual sampling period, with the highest host-seeking densities recorded in late 2024, while the timing of nocturnal activity shifted substantially among months rather than following a single consistent host-seeking schedule. Similar parous proportions were observed during the 2024 and 2025 intensive campaigns, and sensitivity analyses indicated consistently high parity-derived daily survival across biologically plausible gonotrophic-cycle durations, although the field time series could not directly resolve a site-specific gonotrophic interval. Regional climatic variables did not show robust associations with mosquito abundance after adjustment for multiple comparisons, emphasizing the likely contribution of local ecological processes not captured by monthly regional meteorological records. Together, these findings highlight pronounced seasonal and nocturnal variation in human–vector contact and provide a stronger empirical basis for future entomological surveillance and studies aimed at defining the contribution of *An. pseudopunctipennis* to residual malaria transmission in the lower Tarahumara region.

Abbreviations

AR(1)
first-order autoregressive model
CENAPRECE
Centro Nacional de Programas Preventivos y Control de Enfermedades
FDR
false discovery rate
HLC
human landing catch
HSR
host-seeking rate
PBS
phosphate-buffered saline
PR
parous rate
RH
relative humidity

SD
standard deviation
SE
standard error
SJV
San José de Valenzuela
SMN
Servicio Meteorológico Nacional
SSA-Chihuahua
Servicios de Salud de Chihuahua
UACJ
Universidad Autónoma de Ciudad Juárez
WHO
World Health Organization.

Declarations

Ethics approval and consent to participate.

The Research Ethics Committee of the Autonomous University of Ciudad Juárez approved this entomological study on January 12, 2024 (document no. CEI-2024-1-1140). Written informed consent was obtained from all mosquito collectors prior to participation.

Consent for publication.

Not applicable.

Availability of data and materials.

The curated datasets supporting this study's findings are provided as supplementary material with the article. Additional file 1 contains the entomological, parity, climatic, and microclimatic data used to generate the reported analyses, tables, and figures, together with derived summaries and quality-control information. Additional file 2 contains the reproducible analytical code used for data processing and statistical analyses.

Competing interests.

The authors declare no competing interests.

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Authors' contributions.

R.G.-P. , S.V.L.-T., and J.A.G.-H. conceived and designed the study. R.G.-P., S.V.L.-T., E.J.L.-S., J.R.A.-G., E.R.-T., C.A.R.-A., R.U.-R., E.V.-B. and J.A.G.-H. performed the mosquito collections. C.K. and C.F. performed the final statistical analysis. J.C.A.-M. performed the logistical of field annual collections. J.E.G.-R. and C.M.B.-B. reviewed the initial and final draft. J.A.G.-H. and E.J.L.-S. performed the financial support. All authors reviewed the manuscript.

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Figures

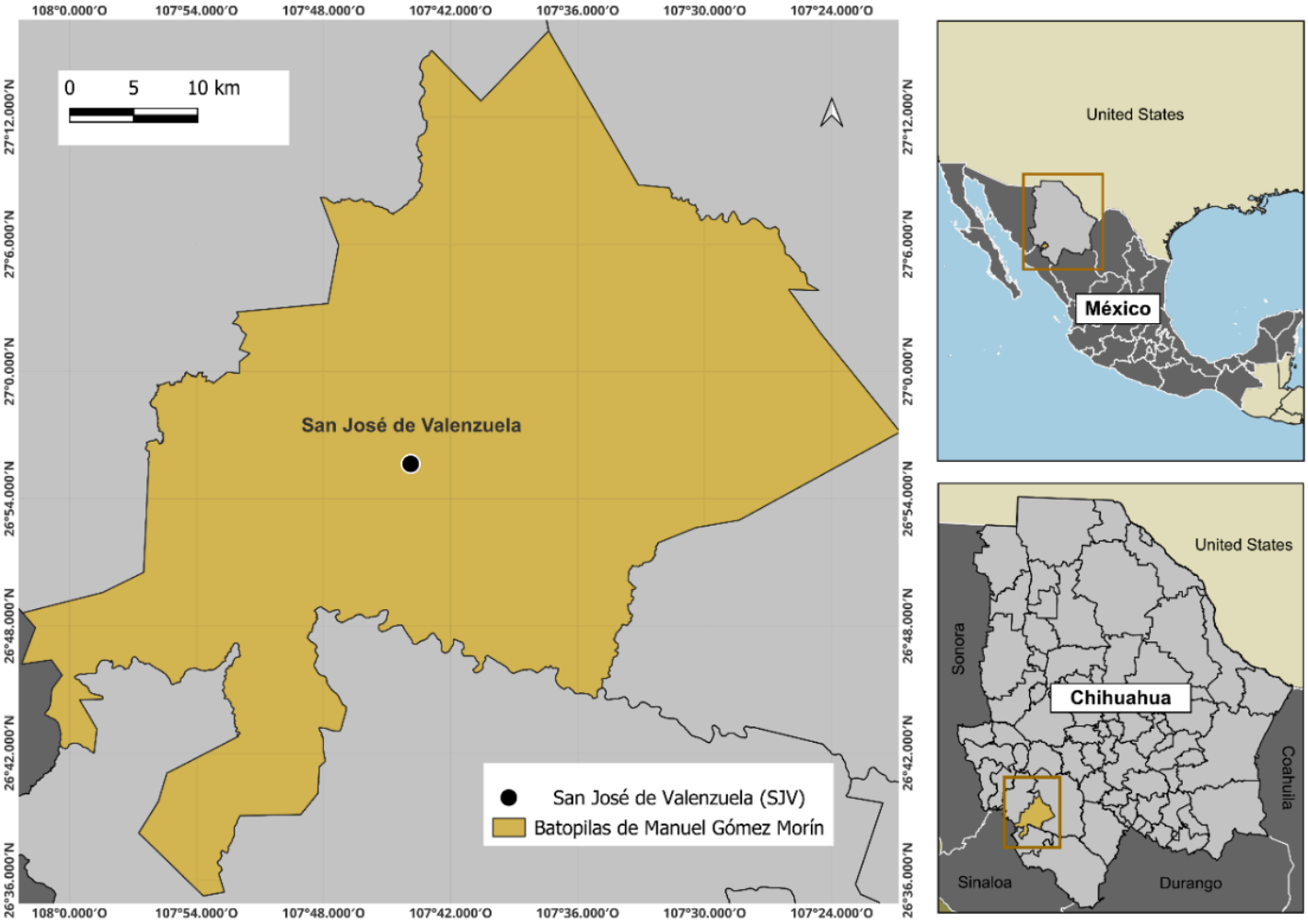


Figure 1

Location of San José de Valenzuela, Batopilas, Chihuahua, Mexico.

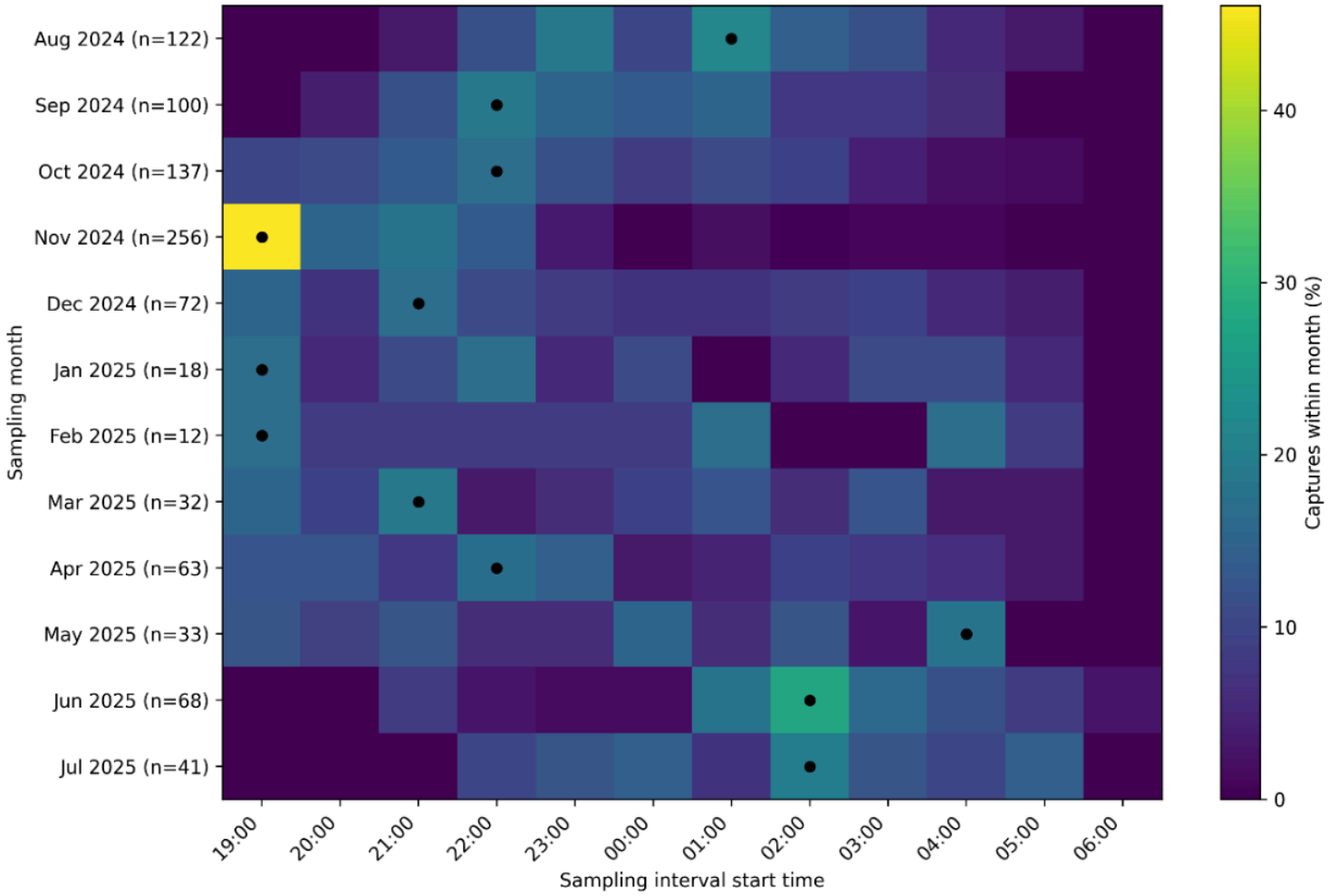


Figure 2

Monthly nocturnal host-seeking activity of Anopheles pseudopunctipennis in San José de Valenzuela. Each cell represents the percentage of the total monthly captures recorded during the first 30 min of each hourly sampling interval. Percentages were calculated independently within each month so that each row represents the temporal distribution of host-seeking activity rather than absolute mosquito abundance. Black dots indicate the sampling interval with the highest capture percentage in each month. Values in parentheses beside each month indicate the total number of females captured during the five monthly sampling nights.

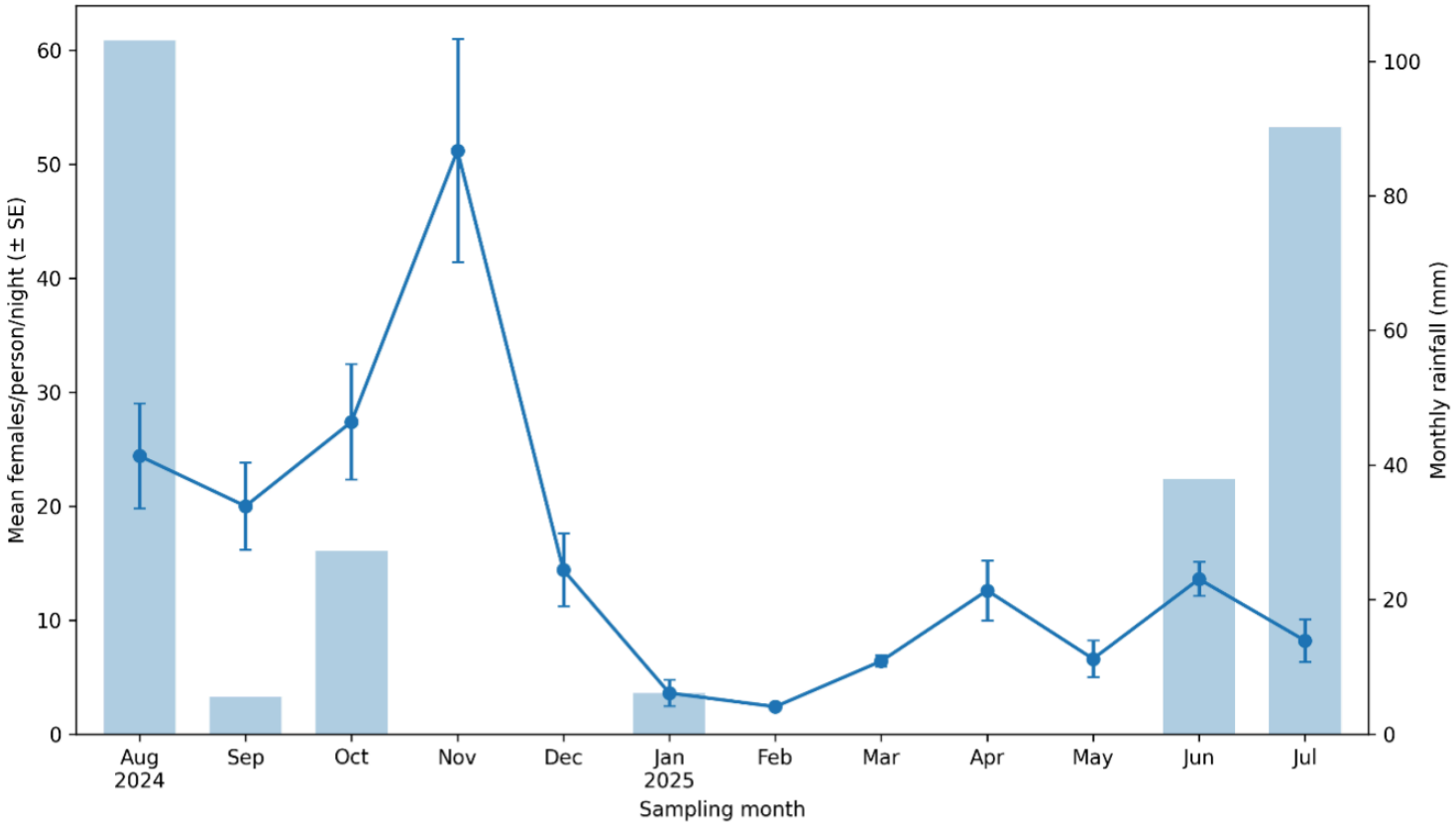


Figure 3

Seasonal host-seeking abundance and regional rainfall in San José de Valenzuela. Points and error bars represent the monthly mean (± SE) number of host-seeking females/person/night calculated from five consecutive sampling nights per month. Bars represent total monthly rainfall recorded at climatological station No. 25018, Choix (SMN), Sinaloa, which was used as a regional climatic reference. Rainfall records should therefore be interpreted as indicators of broad seasonal climatic variation rather than direct measurements of conditions at the mosquito-collection site.