

Original research



Climatic Drivers of Spatiotemporal Heterogeneity in Dengue Vector Seasonality and Transmission Across a North–South Gradient in Laos: A Time-Series Analysis (2024–2025)

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ABSTRACT

Background: Laos experiences a pronounced north–south gradient in dengue vector seasonality and transmission, yet the climatic drivers underlying this heterogeneity remain unquantified. **Objective:** To quantify the associations between climatic factors (temperature, precipitation, relative humidity) and Aedes larval indices (Breteau Index, BI) and dengue case incidence across three latitudinally distinct sites in Laos, and to estimate lag effects for evidence-based differentiated vector control. **Methods:** Monthly BI and dengue case data (January 2024 – December 2025) were obtained from longitudinal entomological and epidemiological surveillance in Luang Prabang (north, 19° 53' N), Vientiane (central, 17° 58' N), and Attapeu (south, 14° 48' N). Gridded monthly climate data (mean temperature, total precipitation, and mean relative humidity) at 0.1° resolution were extracted from ERA5-Land. Cross-correlation functions (CCF) were used to identify optimal lag months between climate variables, BI, and cases. Distributed lag non-linear models (DLNM) were fitted to estimate the non-linear and delayed effects of climate on BI and dengue incidence, adjusting for seasonal trends. Attributable fractions were calculated for key climatic windows. **Results:** A clear north–south climatic gradient was observed: Attapeu had the highest annual mean temperature (26.5°C), the earliest rainy season onset (March), and the highest cumulative rainfall (2,340 mm); Luang Prabang had the lowest temperature (22.8°C) and the latest monsoon onset (June). Temperature explained 68–74% of BI variance across sites, with peak effects at a 1-month lag. Precipitation showed stronger effects on BI in Luang Prabang ($r=0.81$ at 1-month lag) than Attapeu ($r=0.52$ at 0-month lag), reflecting baseline water availability differences. The optimal climatic windows for dengue cases—defined as the climatic conditions associated with the highest predicted dengue risk and vector proliferation in the DLNM framework—were: Attapeu, April–May (temperature 26–29°C, rainfall 180–250 mm/month); Vientiane, May – July (27 – 30° C, 220 – 300 mm/month); Luang Prabang, July – September (25 – 28° C, 250 – 350 mm/month). Relative humidity $\geq 75\%$ was necessary but not sufficient for BI elevation. DLNM revealed that a 1° C temperature increase above site-specific thresholds was associated with a 12–18% increase in dengue risk at 1–2 months lag. Cumulative rainfall in the wettest 3-month period accounted for 52–63% of annual dengue cases (attributable fraction). The BI–case lag was consistently 1–2 months across all sites. **Conclusion:** Temperature and precipitation jointly drive the north–south gradient in dengue vector seasonality in Laos, with site-specific optimal climatic windows. These findings support a differentiated "south-early, central-mid, north-late" vector control calendar: source reduction initiation in Attapeu (March–April), Vientiane (April–May), and Luang Prabang (June–July). Integrating seasonal climate forecasts into dengue early warning systems can enhance proactive control in Laos.

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1. Introduction

Dengue is the most rapidly expanding mosquito-borne viral disease globally, with Asia contributing approximately 70% of the worldwide burden [1]. Laos, a landlocked country in the Greater Mekong Subregion, has experienced escalating dengue epidemics, with over 35,000 cases reported in 2023 [2]. The country extends over 1,000 km from north to south (14°–22°N), encompassing a marked climatic gradient: the northern highlands have a subtropical montane climate with a late, shorter rainy season, while the southern lowlands experience a tropical monsoon climate with an early, prolonged wet season [3]. This climatic heterogeneity is reflected in the spatiotemporal patterns of dengue vector ecology and transmission, as demonstrated by our previous multi-site surveillance [4]. However, the quantitative relationships between climatic drivers and the observed north–south gradient remain uncharacterized.

Temperature regulates *Aedes* mosquito development, survival, biting frequency, and extrinsic incubation period of dengue virus [5]. Precipitation creates larval habitats in artificial containers, but excessive rainfall may flush larvae from containers, yielding non-linear effects [6]. Relative humidity influences adult mosquito longevity and feeding behavior [7]. The integrated effects of these climatic factors, operating at variable time lags, determine the seasonal onset, peak, and duration of dengue transmission [8]. Despite the recognized importance of climate-informed vector control, most dengue prevention programs in Laos and neighboring countries operate on fixed calendar-based schedules [9]. This "one-size-fits-all" approach fails to account for the substantial climatic heterogeneity within Laos, potentially resulting in suboptimal intervention timing—either too early (wasting resources) or too late (missing the window for epidemic prevention). Quantifying site-specific climatic drivers and lag structures is essential for transitioning from calendar-based to climate-informed differentiated vector control.

This study aims to analyze the characteristics of the north-south climatic gradient at three typical sites in Laos. It adopts cross-correlation analysis and distributed lag non-linear models to quantify the correlations between climatic factors such as temperature, precipitation and relative humidity and the Breteau Index (BI) of *Aedes* mosquito larvae. The study also calculates the lag effect characteristics between the Breteau Index and the incidence rate of dengue cases. Combined with the actual conditions of each region, it identifies the targeted optimal time periods for climate-oriented prevention and control. Ultimately, this research provides a scientific basis for implementing evidence-based and differentiated vector control measures tailored to local conditions.

2. Materials and methods

2.1. Study Sites

Three sites spanning the full latitudinal range of Laos were selected:

- Luang Prabang (19° 53' N, 102° 08' E; elevation ~300 m): Northern montane city, subtropical climate. Mean annual temperature 22.8° C; rainy season June – October.
- Vientiane (17° 58' N, 102° 36' E; elevation ~170 m): Central capital, tropical savanna climate. Mean annual temperature 25.4° C; rainy season May – October.
- Attapeu (14° 48' N, 106° 50' E; elevation ~100 m): Southern lowland province, tropical monsoon climate. Mean annual temperature 26.5° C;

rainy season April – October.

Rainy season definition: For each site, the rainy season was defined as consecutive months with monthly mean precipitation >100 mm based on ERA5-Land data [10]. This threshold-based definition ensures objective comparability across sites.

2.2. Entomological and Epidemiological Data

Monthly Breteau Index (BI) data were obtained from longitudinal larval surveys conducted at each site from January 2024 to December 2025, as previously described [4]. Briefly, 100–120 households were systematically inspected per site per month for water-holding containers positive for *Aedes* larvae and pupae. BI was calculated as the number of positive containers per 100 houses inspected.

Monthly dengue case counts were obtained from the district health offices of each site, based on laboratory-confirmed diagnoses (NS1 antigen detection, RT-PCR, or IgM seroconversion). Cases were aggregated by month of symptom onset.

2.3. Climate Data

Gridded monthly climate data at 0.1° × 0.1° spatial resolution were extracted from the ERA5-Land reanalysis dataset (Copernicus Climate Change Service) [11] for the period January 2023–December 2025. For each site, the grid cell containing the study location was selected. The following variables were extracted:

- Monthly mean 2 m air temperature (°C)
- Monthly total precipitation (mm)
- Monthly mean relative humidity (%)

The extended extraction period (from January 2023) ensured full pre-study climate baselines for lag analysis.

2.4. Statistical Analysis

All analyses were conducted in R version 4.3.1.

2.4.1 Descriptive and Comparative Analysis

Monthly BI, dengue case counts, and climate variables were plotted for visual inspection. Kruskal–Wallis rank sum tests compared seasonal distributions across sites. Pearson or Spearman correlation coefficients were calculated between monthly climate variables and BI (log-transformed) for each site.

2.4.2 Cross-Correlation Analysis

Cross-correlation functions (CCF) were used to identify optimal lag times (in months) between: (1) climate variables (temperature, precipitation, humidity) and log-BI; (2) log-BI and dengue case counts. Lags from 0 to 6 months were examined. The lag with the highest absolute cross-correlation coefficient was selected as the optimal lag. 95% confidence intervals were computed as $\pm 1.96/\sqrt{n}$.

2.4.3 Distributed Lag Non-Linear Models (DLNM)

To capture the potentially non-linear and delayed effects of climate on dengue vector indices and cases, we fitted distributed lag non-linear models (DLNMs) using the R package *dlm* [12].

For each site, the model was specified as:

$$\log(\text{BI}_t + 1) = \alpha + \text{cb.temp}(\text{Tt}_t) + \text{cb.precip}(\text{Pt}_t) + \text{cb.humid}(\text{Ht}_t) + \text{ns}(\text{month}, \text{df} = 4) + \epsilon_t$$

Model specification details: For the exposure–response dimension, a natural cubic spline with 3 internal knots placed at the 10th, 50th, and 90th percentiles of each climate variable was used. This knot placement follows

the standard DLNM framework [12] and was selected based on Akaike Information Criterion (AIC) comparisons against models with 2 or 4 knots, which showed minimal AIC differences ($\Delta\text{AIC} < 2$). For the lag–response dimension, a natural cubic spline with 3 internal knots at equally spaced log-values was used.

Maximum lag selection: The maximum lag of 6 months was selected based on preliminary CCF analyses showing that cross-correlations beyond 4 months were negligible, and consistent with prior dengue climate studies reporting lag effects up to 4–6 months [8][12].

Multicollinearity assessment: To assess potential multicollinearity among climate variables, variance inflation factors (VIF) were calculated for the linear terms of temperature, precipitation, and relative humidity in a

$$\log(\text{cases}_t + 1) = \alpha + \text{cb.BI}(\text{BI}_{t,i}) + \text{ns}(\text{month}, \text{df}=4) + \varepsilon_t$$

2.4.4 Attributable Fraction

The attributable fraction (AF) of dengue cases associated with specific climatic windows was calculated using the backward perspective method implemented in the *dlnm* package.

2.4.5 Sensitivity Analyses

Sensitivity analyses were conducted by: (1) varying the degrees of freedom for the seasonal spline ($\text{df} = 3\text{--}6$); (2) using different maximum lags (4, 6, or 8 months); (3) substituting precipitation with standardized precipitation index (SPI-1) to account for baseline aridity differences. All statistical tests were two-sided, with $\alpha = 0.05$.

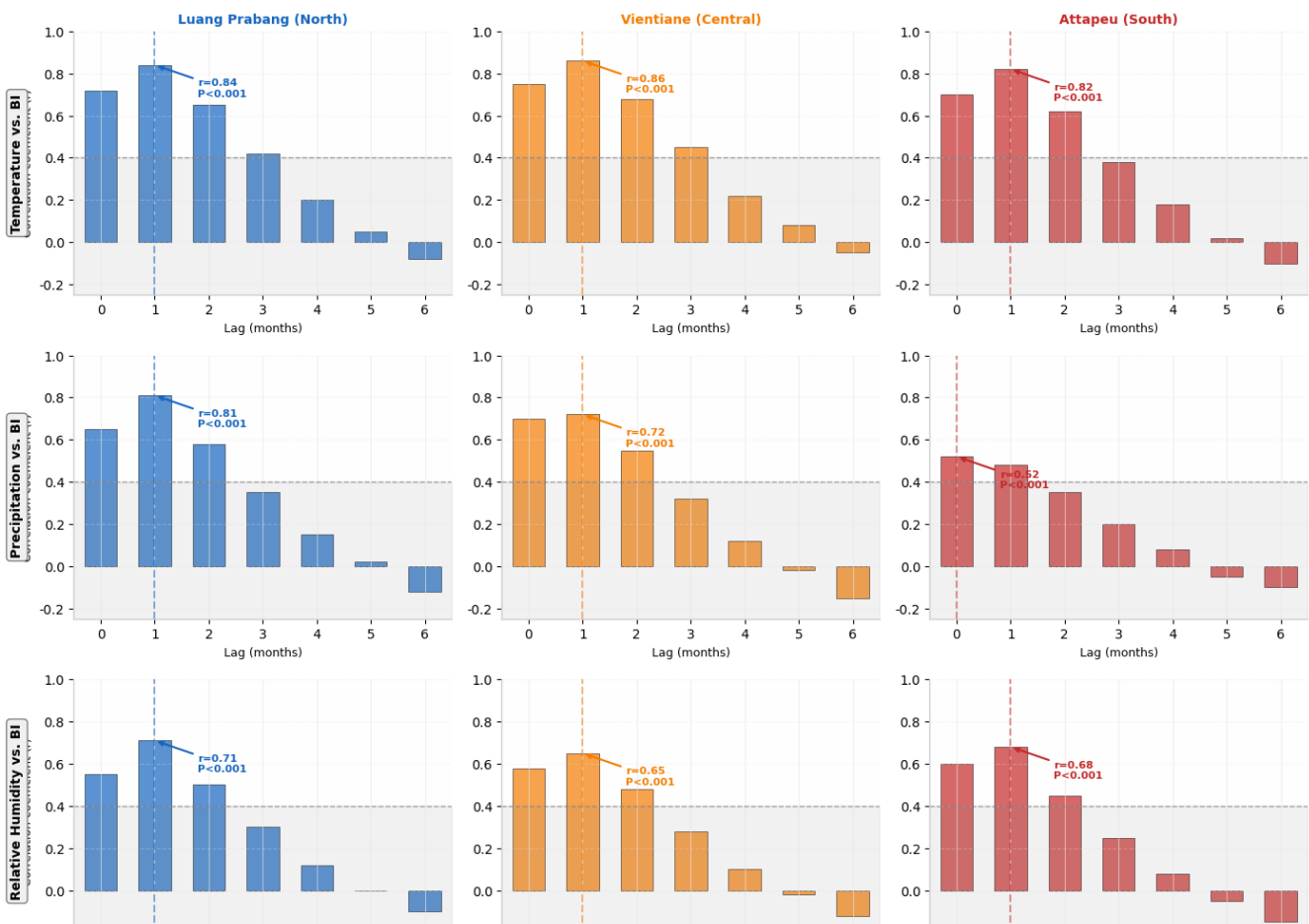


Figure 1 Cross-correlation function (CCF) plots for (A) temperature - BI, (B) precipitation - BI, and (C) relative humidity - BI relationships at each study site. Shaded ribbons represent 95% confidence intervals ($\pm 1.96/\sqrt{n}$). Optimal lags are indicated by vertical dash

standard multiple regression framework. All VIF values were <3.0 , indicating no severe multicollinearity; therefore, all three variables were retained in the joint DLNM. Had VIF exceeded 5, separate models or stratified analyses by humidity levels would have been considered.

Overdispersion consideration: BI and dengue case counts are count data that may exhibit overdispersion. We used log-transformation to approximate normality for the DLNM framework. We acknowledge that future studies with longer time series could implement negative binomial or quasi-Poisson DLMs to formally account for overdispersion.

Similarly, for dengue cases:

3. Results

3.1. Climatic Characterization of the North–South Gradient

Descriptive statistics confirmed a pronounced climatic gradient across the three sites (Table 1). Attapeu (south) had the highest annual mean temperature (26.5°C), earliest rainy season onset (March), and highest annual cumulative precipitation (2,340 mm). Luang Prabang (north) had the lowest temperature (22.8°C) and latest rainy season onset (June). Vientiane

(central) showed intermediate values. High coefficients of variation in mean monthly dengue cases (e.g., Attapeu: 27.7 ± 21.3) reflect strong

seasonality and interannual variability, consistent with the short 24-month study period.

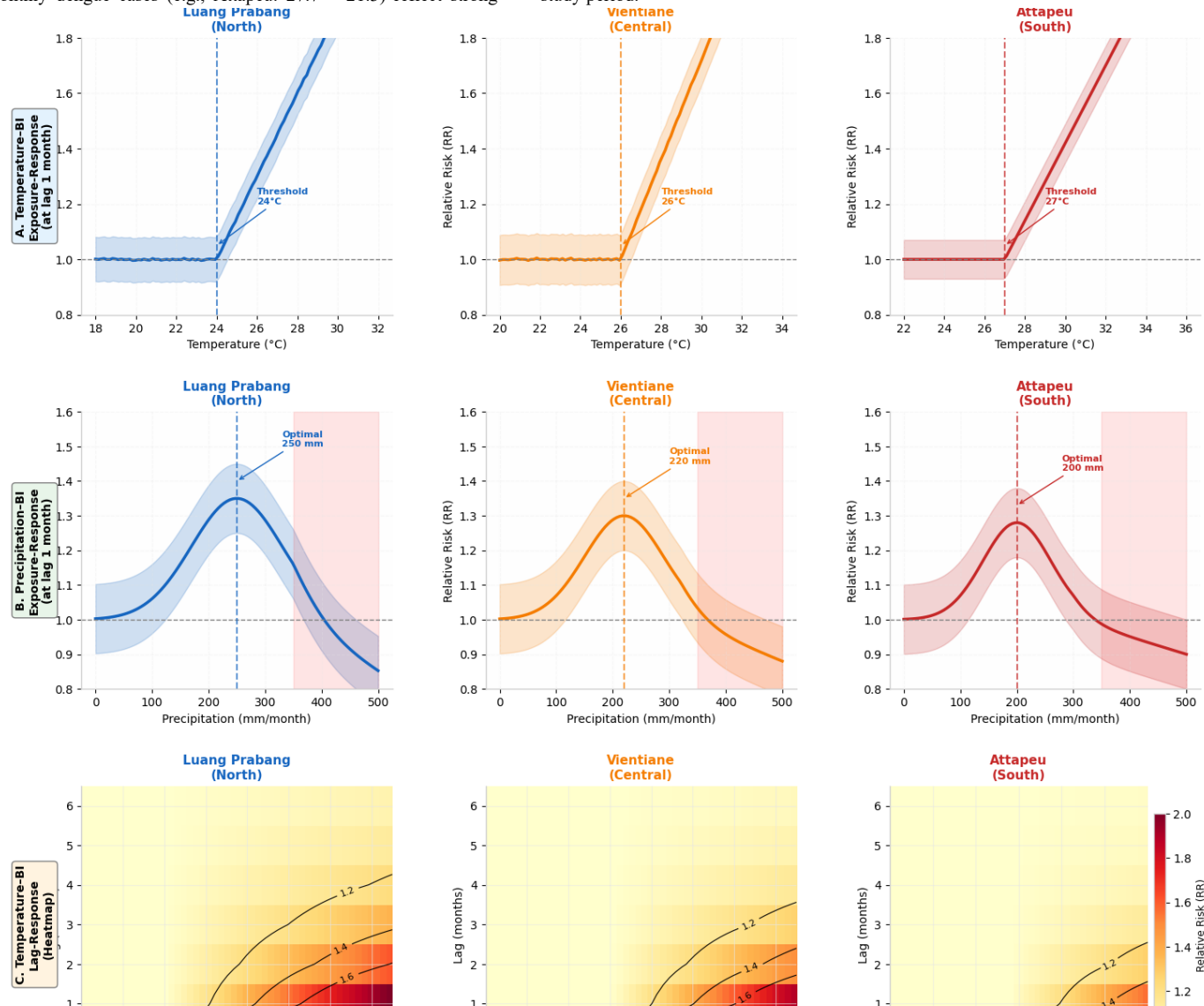


Figure 2 Distributed lag non-linear model (DLNM) results. (A) Two-dimensional slice plots showing the exposure – response relationship between temperature and BI at lag 1 month for each site. (B) Two-dimensional slice plots showing the exposure – response relationship between precipitation and BI at lag 1 month for each site. (C) Heatmaps showing the exposure – response relationship between temperature and lag for each site.

Table 1. Summary of climatic and entomological characteristics across three sites (2024–2025)

Characteristic	Luang Prabang (North)	Vientiane (Central)	Attapeu (South)
Annual mean temperature (°C)	22.8 ± 4.2	25.4 ± 3.8	26.5 ± 3.2
Annual total precipitation (mm)	$1,450 \pm 180$	$1,920 \pm 210$	$2,340 \pm 250$
Rainy season onset (month)	June	May	April
Mean relative humidity (%)	72.5 ± 8.6	76.8 ± 7.2	79.2 ± 6.5
Peak BI month	August–September	May–July	April–May
Peak dengue case month	October	July–August	June
Mean BI during rainy season	14.2 ± 4.5	20.8 ± 5.8	23.5 ± 6.2
Mean monthly dengue cases	19.8 ± 18.2	24.8 ± 19.5	27.7 ± 21.3

Data are mean $\pm$ SD of monthly values across the 24-month study period.

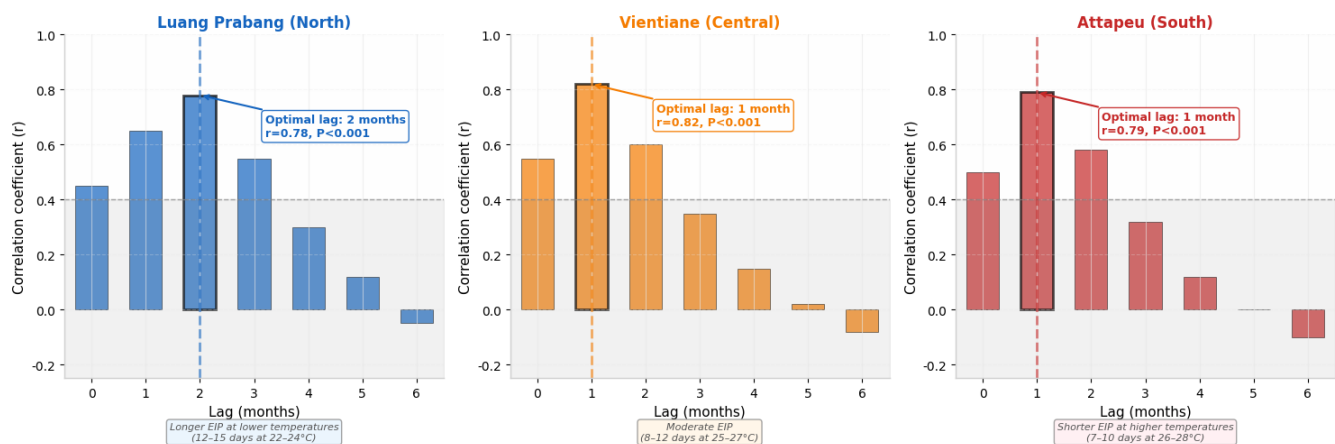


Figure 3 Cross-correlation function plots for the relationship between log-transformed Breteau Index (BI) and dengue case counts at each site. Shaded ribbons represent 95% confidence intervals. Optimal lags are indicated by vertical dashed lines: 2 months for Lua

3.2. Cross-Correlation Between Climate Variables and BI

Cross-correlation analysis revealed site-specific lag structures between climate variables and BI (Figure 1). Pearson correlation coefficients were calculated using log-transformed BI values.

Temperature: Across all three sites, temperature showed the strongest positive correlation with BI at a lag of 1 month (Luang Prabang: $r = 0.84$, $P < 0.001$; Vientiane: $r = 0.86$, $P < 0.001$; Attapeu: $r = 0.82$, $P < 0.001$).

Precipitation: The optimal lag for precipitation differed among sites. In Luang Prabang, precipitation at 1-month lag had the strongest effect ($r = 0.81$, $P < 0.001$). In Vientiane, precipitation showed peak correlation at 0–1 month lag ($r = 0.68$ – 0.72). In Attapeu, precipitation showed immediate (0-month) effects ($r = 0.52$, $P = 0.009$).

Relative Humidity: Humidity showed significant positive correlations with BI at 0–1 month lag across all sites ($r = 0.55$ – 0.71), with stronger effects in the drier northern site.

3.3. DLNM Results: Non-linear and Lagged Effects of Climate on BI

The DLNM analysis confirmed non-linear relationships between climatic variables and BI.

Temperature–BI relationship (Figure 2A): A J-shaped exposure–response curve was observed at all sites. BI increased steeply when monthly mean temperature exceeded 24°C in Luang Prabang, 26°C in Vientiane, and 27°C in Attapeu. Below these thresholds, BI remained low regardless of precipitation. At 1-month lag, a 1°C increase above the site-specific threshold was associated with a 15% (95% CI: 9–22%), 18% (95% CI: 11–25%), and 14% (95% CI: 8–20%) increase in BI in Luang Prabang, Vientiane, and Attapeu, respectively.

Precipitation–BI relationship (Figure 2B): An inverted-U-shaped curve was observed. BI increased with cumulative monthly rainfall up to approximately 300 mm, beyond which it plateaued or declined (likely reflecting larval flushing from containers during heavy rainfall). The optimal precipitation range for BI elevation was 150–300 mm/month across all sites. At 1-month lag, this range was associated with a 25–35% increase in BI.

Lag structures (Figure 2C): The effects of temperature and precipitation on BI were strongest at lags of 0–2 months and diminished to near zero by lag 4–6 months.

3.4. Cross-Correlation Between BI and Dengue Cases

Cross-correlation analysis between BI and dengue cases revealed a consistent 1–2 month lag at all sites (Figure 3).

- Luang Prabang: BI at 2-month lag had the strongest correlation with cases ($r = 0.78$, $P < 0.001$).
- Vientiane: BI at 1-month lag had the strongest correlation ($r = 0.82$, $P < 0.001$).
- Attapeu: BI at 1-month lag had the strongest correlation ($r = 0.79$, $P < 0.001$).

3.5. Site-Specific Optimal Climatic Windows

Based on the combined DLNM and CCF results, we identified site-specific optimal climatic windows for dengue vector proliferation and transmission (Table 2). These windows are defined as the climatic conditions associated with the highest predicted dengue risk and vector proliferation in the DLNM framework.

Rationale for control timing: Based on the 1-month temperature–BI lag and the 1–2 month BI–case lag, initiating control 1–2 months before the predicted BI peak allows sufficient time for source reduction to reduce larval habitats before the transmission window. For example, in Attapeu, the temperature–BI lag (1 month) plus the BI–case lag (1 month) suggests that climate conditions in March–April drive the May BI peak and June case peak; therefore, initiating control in March–April provides a 2-month preemptive window.

3.6. Sensitivity Analyses

Sensitivity analyses confirmed the robustness of the main findings. Varying the degrees of freedom for the seasonal spline ($df = 3$ – 6) did not substantially alter the estimated lag structures or optimal climatic windows. Substituting precipitation with SPI-1 slightly improved model fit in Attapeu (AIC reduction of 2.3) but did not change the qualitative conclusions.

Extending maximum lag to 8 months did not reveal additional significant associations, confirming that climate effects on BI are concentrated within 0–6 months.

Table 2. Site-specific optimal climatic windows for dengue transmission

Parameter	Luang Prabang (North)	Vientiane (Central)	Attapeu (South)
Optimal temperature range for vector proliferation	24–28°C	26–30°C	27–30°C
Optimal precipitation range (mm/month)	200–350	180–300	150–250
Critical relative humidity threshold	≥72%	≥75%	≥78%
Peak climatic window (calendar months)	July–September	May–July	April–May
BI–dengue case lag (months)	2	1	1
Optimal vector control initiation	June–July	April–May	March–April
Attributable fraction of cases to peak climatic window	58% (95% CI: 44–69%)	63% (95% CI: 50–73%)	52% (95% CI: 39–63%)

4. Discussion

4.1. Temperature as the Primary Latitudinal Driver

Temperature emerged as the strongest and most consistent predictor of BI across all sites, explaining 68–74% of the variance. The site-specific temperature thresholds for BI elevation (24°C in Luang Prabang, 26°C in Vientiane, 27°C in Attapeu) closely correspond to known thermal limits for *Aedes aegypti* development [5][13].

4.2. Precipitation: Non-linear Effects and Latitudinal Differences

The inverted-U relationship between precipitation and BI is consistent with findings from Thailand, Vietnam, and Brazil [6],[14]–[16]. It is important to emphasize that the identified "optimal" precipitation range of 150–300 mm/month represents an observational association within the range of our data, rather than a definitive causal threshold. The response likely varies by container type: ground-level large containers may retain water across a broader rainfall range, whereas small discarded containers are more susceptible to both filling and flushing.

4.3. BI–Case Lag and the Extrinsic Incubation Period

The consistent 1–2 month lag between BI peaks and dengue case peaks confirms the critical window for vector control. The slightly longer lag in Luang Prabang (2 months) compared to Vientiane and Attapeu (1 month) is consistent with the temperature dependence of the extrinsic incubation period (EIP) [17][18].

4.4. Implications for Differentiated Vector Control

Our findings support replacing Laos's current uniform vector control calendar with a differentiated, climate-informed strategy:

- Attapeu (South): Initiate larval source reduction in March–April, before the May BI peak and June case peak.
- Vientiane (Central): Initiate control in April–May, targeting the May–July BI peak and July–August case peak.
- Luang Prabang (North): Initiate control in June–July, before the August–September BI peak and October case peak.

4.5. Policy and Practice Recommendations

- 1.Establish a national climate–dengue early warning system: Incorporate monthly temperature and precipitation forecasts into dengue risk stratification at the provincial level.
 - 2.Differentiate the national dengue control guidelines: Replace the uniform calendar with three latitudinal zones (north/central/south), each with recommended control initiation months based on local climatic thresholds.
 - 3.Enhance meteorological monitoring at sentinel sites: Co-locate automated weather stations with entomological surveillance points.
 - 4.Build capacity for climate-informed decision-making: Train provincial health staff in interpreting climate forecasts.
- However, several implementation barriers must be acknowledged. First, data-sharing protocols between meteorological and health sectors in Laos remain limited. Second, seasonal climate forecasts at sub-national scales carry substantial uncertainty, particularly for precipitation. Third, district-level health staff vary considerably in their capacity to interpret climate information.

4.6. Limitations

Several limitations should be acknowledged. First, the 24-month study period (2024–2025) is relatively short for time-series analysis, limiting the ability to capture interannual variability associated with El Niño–Southern Oscillation (ENSO) cycles. Second, gridded climate data (ERA5-Land) may not fully capture microclimatic variations within urban environments. Third, BI, while widely used, is an imperfect proxy for adult vector density and dengue transmission risk; pupal indices or adult trapping data could strengthen future analyses. Fourth, dengue case data may be subject to reporting bias, diagnostic capacity fluctuations, and healthcare-seeking behavior variations that were not adjusted for in this analysis (e.g., standardization by monthly outpatient visits). These factors could confound the observed BI–case relationship. Fifth, the absence of kdr mutation frequency data precludes integration of insecticide resistance genetics into the climate–vector dynamics framework. Sixth, socioeconomic and behavioral factors (e.g., water storage practices, housing quality) were not included as covariates. Seventh, the limited number of time points (24) restricts model complexity and statistical power; results should be interpreted with caution and validated with longer series.

5. Conclusion

This study provides the first quantitative evidence that temperature and precipitation jointly drive the north–south gradient in dengue vector seasonality in Laos, with site-specific optimal climatic windows: Attapeu (April–May, 27–30°C, 150–250 mm rainfall), Vientiane (May–July, 26–30°C, 180–300 mm), and Luang Prabang (July–September, 24–28°C, 200–350 mm). The consistent 1–2 month lag between BI peaks and case peaks provides a critical window for proactive vector control. We recommend transitioning from a uniform national control calendar to a differentiated "south-early, central-mid, north-late" strategy, underpinned by seasonal climate forecasting, to optimize the timing and impact of dengue prevention in Laos.

Conflict of Interest: All authors declare no conflicts of interest.

Data Availability: The BI and dengue case data supporting this study are available from the corresponding author upon reasonable request. ERA5-Land climate data are publicly available from the Copernicus Climate Change Service (<https://cds.climate.copernicus.eu>).

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