**Seasonal Dynamics of an Emerging African Malaria Vector, *Anopheles stephensi*: Implications for Malaria Control**

**Population Dynamics and Seasonality of the Emerging African Malaria Vector *Anopheles stephensi*: Implications for Malaria Control**

Charles Whittaker1\*, Ellie Sherrard-Smith1, Peter Winskill1, Gina Cuomo-Dannenburg1, Patrick G.T. Walker1, Marianne Sinka2, Ashwani Kumar3, Azra Ghani1, Samir Bhatt1,4, Thomas Churcher1 & Arran Hamlet1

1MRC Centre for Global Infectious Disease Analysis & Abdul Latif Jameel Institute for Disease and Emergency Analytics, School of Public Health, Imperial College London, London, UK

2Department of Zoology, University of Oxford, Oxford, UK

3Vector Control Research Centre, Indira Nagar, Puducherry, India

4Section of Epidemiology, Department of Public Health, University of Copenhagen, Copenhagen, Denmark

**\*Corresponding Author:** Charles Whittaker, Department of Infectious Disease Epidemiology, School of Public Health, Imperial College, London W2 1PG, United Kingdom. Email: [charles.whittaker16@imperial.ac.uk](mailto:charles.whittaker16@imperial.ac.uk)

**Abstract**

**Reductions in malaria prevalence have been driven by extensive scale up of control interventions, but also increasing urbanisation, particularly given urban areas in SSA tend to have lower prevalence, underpinned by improved housing and reduced availability of larval habitats.**

**Urban malaria, underpinned by *Anopheles stephensi*, is immensely prevalent elsewhere. The past decade has seen reports of *Anopheles stephensi* across parts of the Horn of Africa, where it is thought to be underlying dramatic rises in malaria cases in Djibouti.**

**Significant uncertainty remains however, particularly with respect to the temporal and seasonal dynamics of the vector – the degree, extent and timing of seasonality in mosquito abundance underlies the temporal profile of malaria and disease risk and therefore has material consequences for 1) which malaria control interventions will be most effective at preventing its establishment/reducing disease burden and 2) the dynamics and speed of malaria establishment in the region.**

**Here we collate *Anopheles stephensi* longitudinal catch-data from \_\_\_ locations and \_\_\_ countries in order to better understand the population and seasonal dynamics of *Anopheles stephensi*, and further out understanding of how these dynamics might play out in the Horn of Africa.**

**Our analyses reveal pronounced variation in dynamics across locations in the extent of seasonality and timing of seasonal peaks observed for *Anopheles stephensi*, ranging from single, highly seasonal peaks, to bimodality and near-perennial patterns of annual abundance. Importantly, we discover systematic variation in seasonal dynamics between urban and rural settings, suggesting structural differences in how these environments facilitate vector abundance.**

**Integrating these seasonal profiles with a model of malaria transmission, our results reveal the degree of vector seasonality materially impacts the speed, scale up and dynamics of malaria establishment in settings where transmission is currently minimal or even absent, and thus more generally, highlights significantly uncertainty in our understanding of how *Anopheles stephensi* could influence the timing and dynamics of malaria establishment in settings across the Horn of Africa where malaria transmission is currently absent or minimal.**

**Our results underscore the significant threat this emerging, urban malaria vector poses to public health in this region of sub-Saharan Africa, as well as the continent more generally, and highlights the fundamental, urgent need for significant scale up on entomological monitoring across the region.**

**Keywords:** *Anopheles stephensi*; malaria ecology; urban malaria; population dynamics; seasonality; epidemiology.

**Introduction**

With an estimated 241 million cases and over 600,000 deaths across endemic countries in 20201, malaria represents one of the most significant infection diseases globally2. Burden of disease is concentrated in sub-Saharan Africa where an estimated 96% of malaria deaths in 2020 occurred – 80% of these in children under 53. This represents an almost 40% reduction in clinical disease since the year 2000, an achievement underpinned predominantly by significant scale-up of control interventions including insecticide-treated bednets4.

Alongside this significant expansion of control efforts, increasing urbanisation of Africa’s populace (rising from 31% to 43% between 1990 and 2018, with over 60% expected to live in urban areas by 20505) is also thought to have indirectly contributed to reductions in disease burden. Previous work has highlighted significantly lower annual Entomological Inoculation Rates (EIR) in urban compared to rural settings6,7, a finding thought to be underpinned by features factors including systematic differences in the quality of housing8,9, reduced availability and suitability of habitats for *Anopheline* breeding in urban settings10–12, better access to treatment13, and higher population densities leading to higher human to mosquito ratios (and reduced transmission)14. Whilst these trends are not always consistently identified (see e.g. surveys in Libreville, Gabon15 or Cotonou, Benin16 where prevalence of malaria is higher in urban areas than immediately surrounding locations; or previous work highlighting that *Anopheles gambiae s.s.* can adapt to breeding in polluted water characteristic of urban environment17), increasing urbanicity across sub-Saharan Africa is likely to complement planned scale-up of malaria control interventions aimed at achieving the targets outlined in the World Health Organization’s 2030 Global Technical Strategy for Malaria18.

This potential positive impact of increasing urbanization is contingent on urban settings across the continent remaining areas of comparatively low malaria transmission. This phenomenon is currently under threat by the invasion and establishment of the malaria vector *Anopheles stephensi*. Found throughout South Asia, *Anopheles stephensi* is a highly efficient urban vector capable of transmitting both *Plasmodium falciparum* and *Plasmodium vivax* parasites, with this efficiency thought to be underpinned by both an increased tolerance for breeding in polluted water sources25, and a superior ability to access and utilise the manmade hydrological habitats present in urban settings20,21. The species was first identified in sub-Saharan Africa in Djibouti City in 201219 and has since been recorded in both Ethiopia20,21 and Sudan22,23, with recent work highlighting likely suitability for the species across some of the continents largest population centres comprising over 100 million people24. Whilst causality has yet to be conclusively established, its emergence is thought to have contributed to the significant resurgence of malaria transmission in Djibouti (which experienced a 10-fold increase in cases between 2013 and 2019), highlighting the potential threat establishment of this vector poses to malaria control across the Horn of Africa28, as well as wider efforts across the continent29.

The situation in Djibouti, as well as recent modelling work suggesting *Anopheles* *stephensi*’s establishment in Ethiopia could lead to a 50% increase in malaria incidence28, highlights the significant public-health threat the vector poses. Despite this, substantial uncertainty remains how its future expansion and establishment might influence malaria in the region, particularly in the (predominantly urban) settings where the disease is currently largely absent. A key driver of this will be the seasonal patterns of abundance by *Anopheles stephensi*. Mosquito populations are highly temporally dynamic, often exhibiting substantial annual fluctuations in size that drive the temporal profile of disease risk. Understanding the factors underlying these dynamics is crucial given that the effectiveness of many malaria control interventions (such as seasonal malaria chemoprevention30 or indoor-residual spraying31) depends on the timing of their delivery relative to seasonal peaks in transmission. A better understanding of *Anopheles stephensi*’s seasonal dynamics is therefore likely to have material consequences for the effective control of malaria across the Horn of Africa.

Despite this relevance, substantial uncertainty remains regarding *Anopheles stephensi*’s seasonal dynamics; numerous studies carrying out longitudinal catches are present in in the literature, but these typically only focus on a single location, precluding systematic comparison and identification of generalisable patterns. Here we collate longitudinal mosquito catch data for *Anopheles stephensi* from 6 countries and 45 unique locations across South Asia, the Middle East and the Horn of Africa in order to better understand these dynamics, and the ecological factors underpinning them. Our results highlight pronounced variation in the extent and timing of seasonality, with distinct dynamics observed across rural and urban settings. Integrating these results with a model of malaria transmission highlights how this variation will influence the efficacy and impact of malaria control efforts in parts of the Horn of Africa where the disease is currently (or has previously been) largely absent and underscores the crucial, urgent need for rapid scaleup of entomological monitoring for this emerging urban vector across the region.

**Methods**

**Systematic Review of *Anopheles stephensi* Literature**

We collated references from published systematic reviews of literature relating to *Anopheles stephensi*27,32, and updated these previous searches (both conducted in 2017) by searching *Web of Science* and *PubMed* databases from January 2017 to September 2020 for further relevant references. We included all records containing temporally disaggregated adult mosquito catch data with monthly (or finer) temporal resolution spanning at least 10 months, that had not been conducted as part of vector control intervention trials, where sufficient information to geolocate the catch site to the administrative unit 2 level, and where a total of at least 25 *Anopheles stephensi* had been caught over the study period. Overall, a total of 36 references were collated containing 65 time-series from catch surveys carried out in distinct locations from across Afghanistan (n=2), Djibouti (n=1), India (n=32), Iran (n=17), Myanmar (n=5) and Pakistan (n=8). See **Supplementary Information** for further details.

**Gaussian Processing Fitting and Smoothing of Time Series Data**

Per previous work32, we fitted the following Gaussian Process-based model to smooth these noisy mosquito count time-series, using a Negative Binomial likelihood to account for overdispersion in the data:

where is a distribution over functions from a zero-mean Gaussian Process with covariance function , with the covariance between two timepoints and defined according to the kernel function . A periodic kernel function was used to capture the seasonally/annually repeating patterns often observed in mosquito populations, specified by the hyperparameters , and , where represents the period over which we would expect points to show similar dynamics (i.e. a period of twelve would imply we expect points separated by 12 months to be most similar), specifies the magnitude of the covariance, and  represents a lengthscale parameter further constraining the extent to which two values separated by a given time can co-vary. (x) are function evaluations at times , are the observed mosquito counts indexed by timepoint . Model fitting was carried out within a Bayesian framework, using the probabilistic programming language STAN33.

**Characterisation of Temporal Properties and Clustering of Similar Time-Series**

Based on previous work providing a framework to statistically characterise the empirical structure of time-series data34, we calculated a number of summary statistics for each smoothed time-series to characterise their temporal properties. These include the median of the period () from the gaussian process fitting, the relative entropy between the time-series and uniform distribution, the proportion of points in each smoothed time-series where the total catch was greater than 1.65x the mean, the difference in timing between the peak in vector density and the highest rainfall recorded in the location, the proportion of the total catch that was found in any 4 month period (in-keeping with previous definitions of malaria seasonality35), and then 3 features arising from fitting 1 and 2 component Von-Mises distributions (a continuous probability distribution representing the maximum entropy distribution for circular/repeating data) to the smoothed time-series. These 3 features were the mean of the 1 component Von-Mises distribution, the number of peaks (determined by comparing the quality of fit for 1 and 2 component Von-Mises distributions, roughly corresponding to asking whether assuming the time-series was unimodal or bimodal provided the best fit), and the weight (), specifying the comparative contributions of each component in the two-component fitting. See **Supplementary Information** for further details. From this, we obtain for each time-series a set of 7 real numbers providing a reduced representation of their temporal properties – to this, we then applied a Principal Components Analysis to identify a lower-dimensional representation of the structure present in the data amenable to visualisation and implemented k-means clustering to identify clusters of time-series with similar temporal properties.

**Random Forest Prediction of Cluster Membership**

For each of the 65 time-series, we extracted a suite of environmental variables derived from satellite data that together describe the ecological structure of the location the entomological survey was carried out in, including the *BioClimatic* variables (a suite of biological relevant covariates defined from monthly rainfall and temperature satellite data36) as well as measures of landcover and urbanicity amongst others (a complete list can be found in **Supplementary Table X**). These environmental variables were then used as predictors within a Random-Forest based classification framework aimed at predicting the cluster membership (i.e. the results of the k-means algorithm described above) of a given time-series – see **Supplementary Figure X** for the individual fitted profiles for each time-series)**.** These models were fitted using the software package *Ranger*37, implemented in the *tidymodels* framework for R38, with 6-fold cross-validation utilised to optimise hyperparameter combinations; presented results are based on averaging the results of 25 separate iterations of cross-validation and model fitting (to account for stochasticity in model fitting), and any predictions made using out-of-bag model estimates in all instances. There were significant imbalances in class size across the clusters considered, we carried out upsampling using the SMOTE (synthetic minority over-sampling technique39) algorithm (though results are also presented without upsampling). Further details on defining the outcome variable and the Random Forest modelling framework can be found in the **Supplementary Information**.

**Modelling *Anopheles stephensi*-Driven Malaria Dynamics and Control**

We integrated the temporal profiles of *Anopheles stephensi* abundance into a deterministic version of a well-established compartmental model of *Plasmodium falciparum* malaria transmission and disease40–42 to explore the implications of *Anopheles stephensi* establishment and seasonality on the dynamics of malaria transmission and control; with a focus on settings in the Horn of Africa where malaria is currently largely absent. In brief, the human population is split into either susceptible or infected individuals, with those infected either being asymptomatic, currently suffering from clinical disease, possessing a sub-microscopic infection, having been treated, or currently in a period of prophylaxis following treatment. Mosquito populations are divided according to different life-stages, with larvae, pupae and adults all simulated, and subdivided into either susceptible, exposed or infectious (after the extrinsic incubation period, EIP) classes. The model explicitly takes into account heterogeneity in transmission, age-dependent mosquito biting rates, and the acquisition of natural immunity following exposure. For the purposes of exploring the impact of *Anopheles stephensi*, we integrated the collated temporal profiles of mosquito abundance alongside estimates of *Anopheles stephensi* bionomics from the literature to explore the potential impact the vector could have on settings across the Horn of Africa where malaria is currently largely absent – specifically how variation in seasonality of the mosquito might influence the impact of indoor residual spraying (IRS), a key vector control intervention. Full details of the model can be found in **Supplementary Information**.

**Results**

**Extensive Diversity In Temporal Dynamics Across the Collated *Anopheles stephensi* Time-Series:** A total of 65 time-series from entomological studies across Afghanistan, Djibouti, India, Iran, Myanmar and Pakistan were identified (**Fig.1A)**. These noisy time-series were then smoothed using a negative binomial gaussian process-based framework (see **Fig.1B** for a single example time-series from each country, see **Supp Fig.1** for all individual time-series). Substantial variation in temporal dynamics was observed across the collated time-series in terms of the degree and timing of seasonality – this ranged from highly seasonal dynamics with a single, clear seasonal peak (e.g. as with Afghanistan example in **Fig.1B**) to more perennial patterns of abundance (see Pakistan example in **Fig.1B**) and even bimodal annual population dynamics with two peaks observed across the course of a single year (as with the Iran example in **Fig.1B**).

**Statistical Characterisation and Clustering of Temporal Properties Highlights Distinct Temporal Archetypes:** An array of summary statistics were calculated for each time series to characterise their temporal properties (see **Supplementary Methods *Time Series Characterisation and Analysis*** and **Supp Fig.2**). A reduced representation of this variation was then generated using principal components analysis (PCA) and the result then clustered using the k-means algorithm to cluster the time-series into discrete groups of time-series that all share similar temporal patterns. Our results highlight two distinct clusters of time-series, with each group characterised by distinct and different temporal patterns **(Fig. 2A)** and that differ in the extent and degree of their seasonality (here defined as the percentage of total vector density that occurs across any continuous 3-month period, **Fig. 2B**). Cluster 1 time-series typically had clear, single seasonal peaks and were more seasonal on average (57% of total vector density in a 3-month period) than Cluster 2 time-series, which had less seasonal (more perennial) patterns of annual abundance (average 36% vector density in any consecutive 3-month period), including time-series with two peaks across the course of a single year. These results were sensitive to our choice of the number of clusters the k-means algorithm was specified to identify – specifying 4 clusters instead of 2 resulted in further disaggregation of the 49 time-series in Cluster 1 into 3 separate clusters, each characterised by a single seasonal peak, but which differed in the timing of their seasonal peak relative to the timing of peaks in monthly rainfall (**Supp Fig 3**). Despite differing significantly in vector abundance seasonality **(Fig. 2C**, top panel, p<0.001**)**, there was no significant difference across the Cluster 1 and Cluster 2 time-series and their respective locations in terms of rainfall seasonality **(Fig. 2C**, bottom panel, p=0.59**)**. This suggests that the differences in vector seasonality across the two clusters was not being driven by differences in the timing and seasonality of rainfall across the surveyed locations.

**Random-Forest Modelling and Prediction of Seasonal Dynamics Highlights Urbanicity As a Key Driver:** In order to explore the ecological factors underpinning this observed variation in *Anopheles stephensi* seasonality, we fitted a random forest-based classification modelling framework to a suite of satellite-derived environmental covariates in order to predict cluster membership (either Cluster 1 or Cluster 2, as defined in **Fig 2)**. Due to the significant class size imbalance between Cluster 1 (n=49) and Cluster 2 (n=16), we up-sampled the Cluster 2 data using the smote algorithm in order to generate balanced class sizes. Model discriminative and predictive performance was good – across the 25 iterations of random forest model fitting carried out, the mean AUC was 0.89 (indicating good predictive performance, **Fig. 3A**) and on average, the model was able to correctly classify Cluster 1 time-series 83% of the time, and Cluster 2 time-series 85% of the time. Annual temperature seasonality and rainfall seasonality (both bioclimatic variables describing the extent of annual fluctuations in the two environmental quantities), population per square kilometre and a number of measures of landcover (specifically LC30 which corresponds to mosaic cropland/natural vegetation, and LC20 which corresponds to irrigated or post-flooding cropland) were all highly predictive of Cluster membership **(Fig. 3B)**. Areas with lower population density were more likely to belong to Cluster 2, as were areas with high values of LC20 (i.e. land predominantly occupied by irrigated or post-flooding cropland). By contrast, areas with high values of LC10 and LC30 (i.e. substantial fraction of land covered either rainfed cropland or a mosaic of cropland and natural vegetation) were more likely to belong to Cluster 1, as were areas in which rainfall was strongly seasonal (see **Supp Fig. 4** for the full array of partial dependence plots for all covariates used in the analysis).

Motivated by this strong association with population per square kilometre, we manually collated whether each time-series had been carried out in a rural or urban setting (as defined by the authors of each study) and examined the association between rurality/urbanicity and temporal dynamics **(Fig. 3C)**. There was a near-significant association with rurality/urbanicity and cluster membership (chi squared test, p=0.07) – of the 25 time-series from studies carried out in urban settings, a significant majority (n=22) were assigned to Cluster 1 (88%) and only 3 to Cluster 2 (12%). By contrast, time-series carried from rural locations were more evenly spread between the two clusters – of the 37 time-series from studies in rural settings, 24 were assigned to Cluster 1 (65%) and 13 assigned to Cluster 2 (35%).

These results are qualitatively robust to the up-sampling procedure that we applied. Model predictive performance and variable importance rankings were similar when no up-sampling was applied to the dataset (mean AUC of 0.81, still indicating good predictive performance, see **Supp Fig. 5** for the ROC and variable importance plots), though average predictive accuracy on Cluster 2 (50%) was significantly lower than predictive accuracy on Cluster 1 time-series (94%). Model performance and relative ranking variable importance ordering was similarly retained when fitting the model and explicitly holding out a small subset of the data to evaluate model performance (n=7 time-series, see **Supp Fig. 6** for both results with and without upsampling). Overall study catch size was highly variable between the studies **(Supp Fig 7)**, and whilst the median catch size across the two clusters did not differ (Moody’s Median Test, p=0.47, median Cluster 1 = 292, interquartile range Cluster 1 = 116-793, median Cluster 2 = 235, interquartile range Cluster 2 = 112-434), the mean catch size did differ (t-test, p=0.025, mean Cluster 1 = 876, mean Cluster 2 = 364). We therefore carried out an additional sensitivity analysis including study average monthly catch as a covariate in the random forest model (**Supp Fig 8)** – predictive performance and relative rankings of variable importance remained similar despite this addition. The ability of the model to predict seasonality (as measured by the percentage of total vector catch in any 3-month period) was more modest, although estimates were positively correlated (r = 0.43, see **Supp Fig. 9**), with various measures of landcover including LC10, LC20 and LC30, as well as temperature and rainfall seasonality all strongly associated predictors of seasonality, as in the classification modelling.

**Exploring Possible Seasonal Dynamics and Surveillance of *Anopheles stephensi* Across the Horn of Africa:** We next collated the same satellite-derived environmental covariates described above for countries across the Horn of Africa where *Anopheles stephensi* has been found. These covariates were then inputted into the trained random forest classification model to predict potential temporal dynamics (as denoted by Cluster membership) of *Anopheles stephensi* across the Horn of Africa region **(Fig. 4A)**. Our results highlight distinct geographical areas across the region considered more likely by the model to belong to Cluster 1 (more seasonal, **Fig. 4B**) and Cluster 2 (less seasonal), as well as substantial areas of significant uncertainty. We next asked what consequences this uncertainty about the degree and timing of *Anopheles stephensi* seasonality might have on entomological surveillance of the vector across the region. Specifically, what the probability of missing *Anopheles stephensi* in entomological catches might be as a function of the number of consecutive months sampled (with start month selected randomly i.e., assuming no knowledge of *Anopheles stephensi’s* temporal dynamics). Our results highlight that in instances where sites are sampled for a limited number of months, there is a significant risk of missing *Anopheles stephensi* – even at 5 months of consecutive entomological sampling ranging from X-Y% (mean Z%) for Cluster 1 time-series and A-C% (mean Y%) for Cluster 2 time-series, with the exact value dependent on the specific time-series (and *Anopheles stephensi* temporal profile) being considered **(Fig. 4C)**. Our results highlight that in the absence of a detailed understanding of *Anopheles stephensi*’s anticipated temporal dynamics, sampling for a limited number of months poses a significant risk of missing an already present *Anopheles stephensi*, and erroneously concluding it is absent.

**Modelling the Impact of *Anopheles stephensi* Seasonality On the Impact of Vector Control Measures:** We next adapted an existing model of malaria transmission (see ***Supplementary Information*** for further details) to directly incorporate the different temporal profiles of *Anopheles stephensi* abundance that had been collated, and used the model to explore how variation in *Anopheles stephensi* temporal dynamics influences the impact of the indoor residual spraying (IRS), a key malaria control intervention. We explore three different IRS compounds (each of which is commonly used throughout the Horn of Africa), with a range of different functional half-lives (with respect to mosquito killing potential) following spraying **(Fig. 5A)** and model the impact of a single annual round of IRS. Across each of the 65 time-series assessed, pirimiphos methyl consistently had the highest impact, reducing annual malaria incidence by 47.6% in the 12 months following spraying on average, compared to 43.9% and 28.9% on average for clothiandin and bendiocarb respectively **(Fig. 5B)**. The overall magnitude of reduction in malaria burden was highly dependent on the degree of seasonality however, with IRS having the most impact in highly seasonal settings, and the least impact in settings where the degree of seasonality was minimal **(Fig. 5C** and **Fig. 5D)** – the influence of seasonality on impact was largest for bendiocarb (the insecticide with the shortest half-life), with reduction in malaria burden ranging from only 21.7% in the lowest seasonality setting, through to as high as 37.0% in the most seasonal setting, a 1.7 fold difference. Important to note however is that achieving this impact is dependent on optimal timing of intervention delivery relative to any seasonal peaks, which in turn requires sufficient understanding of the vector’s population dynamics and when peaks in vector density are likely to occur.

**Discussion (800-1000 words)**

**Total (~3500-4500 words)**

**Data and Code Availability**

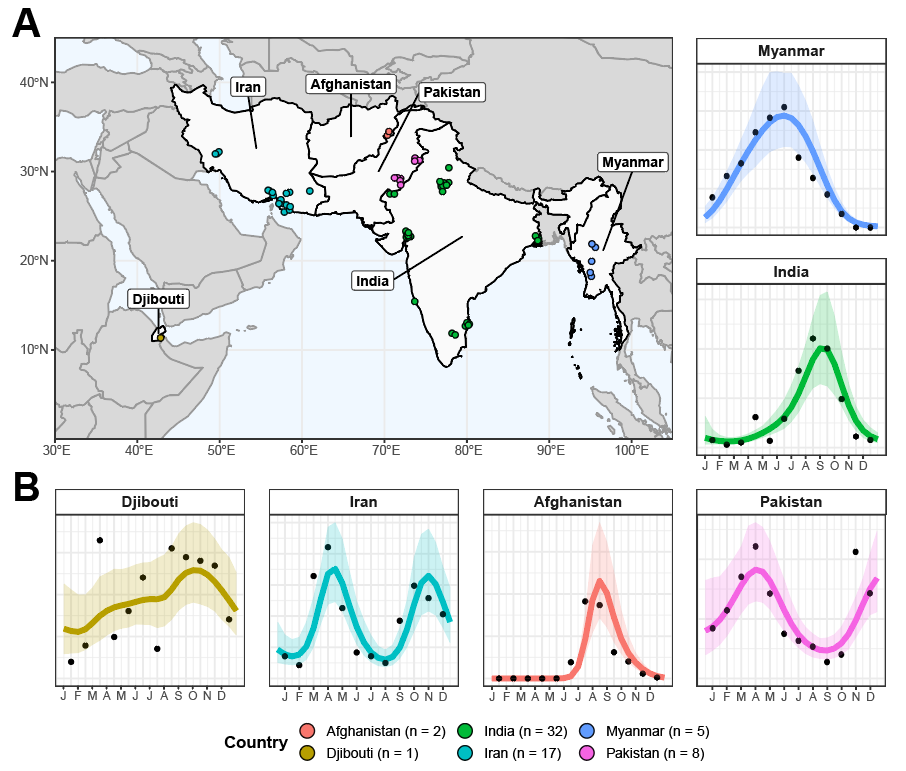
All data collated as part of this study and the code required to reproduce these analyses can be found at the following link: <https://github.com/cwhittaker1000/stephenseasonality>. A copy of the data is also available with the manuscript supplementary information. A static, archived version of the repository can be found via the following DOI: \_\_\_\_\_\_\_ and associated GitHub Release: \_\_\_\_\_\_\_\_.

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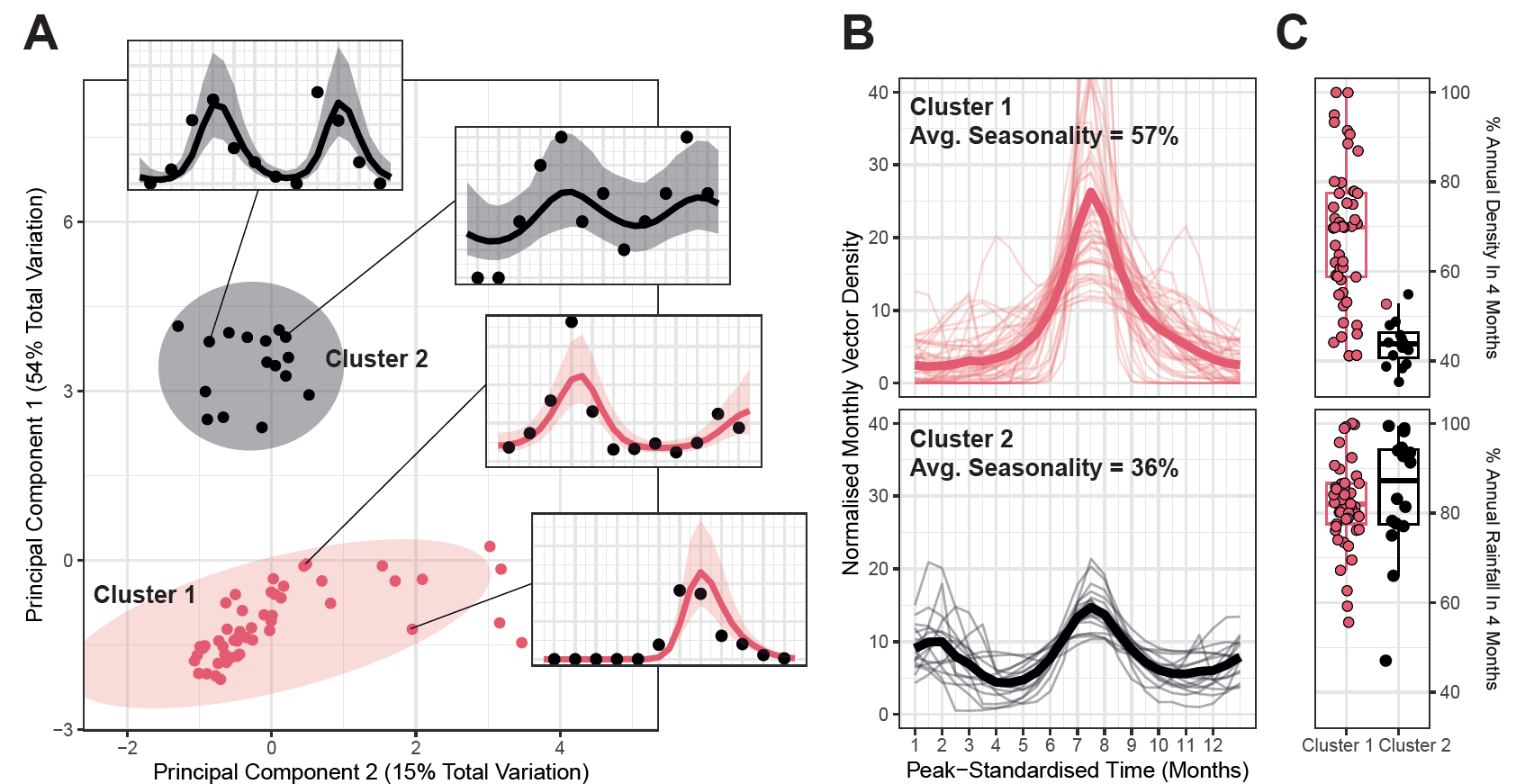
C.W. is supported by a Medical Research Council Doctoral Training Partnership PhD Studentship. SB & AG both acknowledge grant support from the Bill and Melinda Gates Foundation. *-more here-*

**Author Contributions**

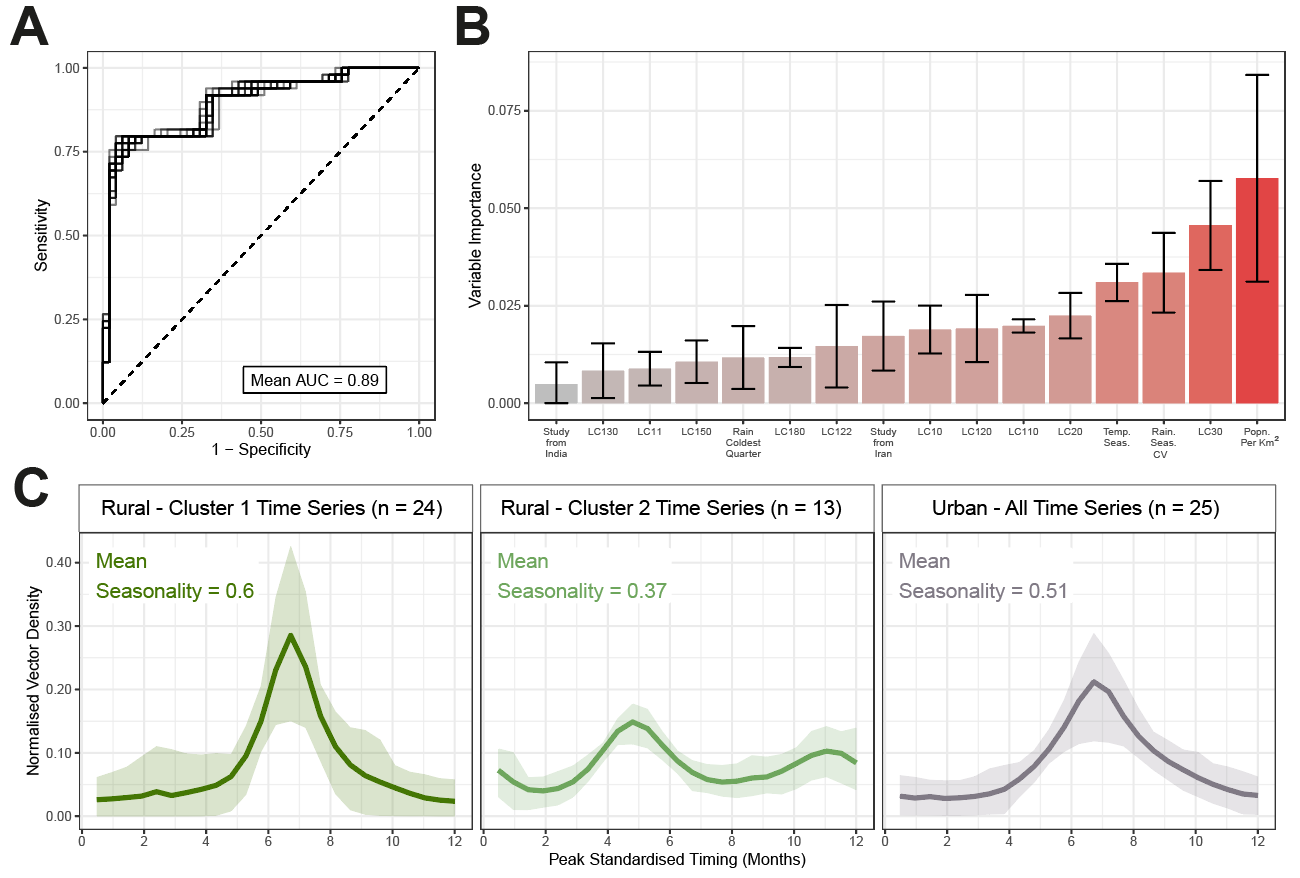
CW and AH conceived the study. TC, ESS and SB contributed to the design of the study. CW carried out the systematic review. CW and SB developed the underlying statistical framework, with input on the analyses from AH, AG, TC, GCD, PGTW, PW and ESS. CW wrote the first draft manuscript, with all authors providing feedback and suggestions during manuscript drafting. All authors approved the final version of the manuscript.



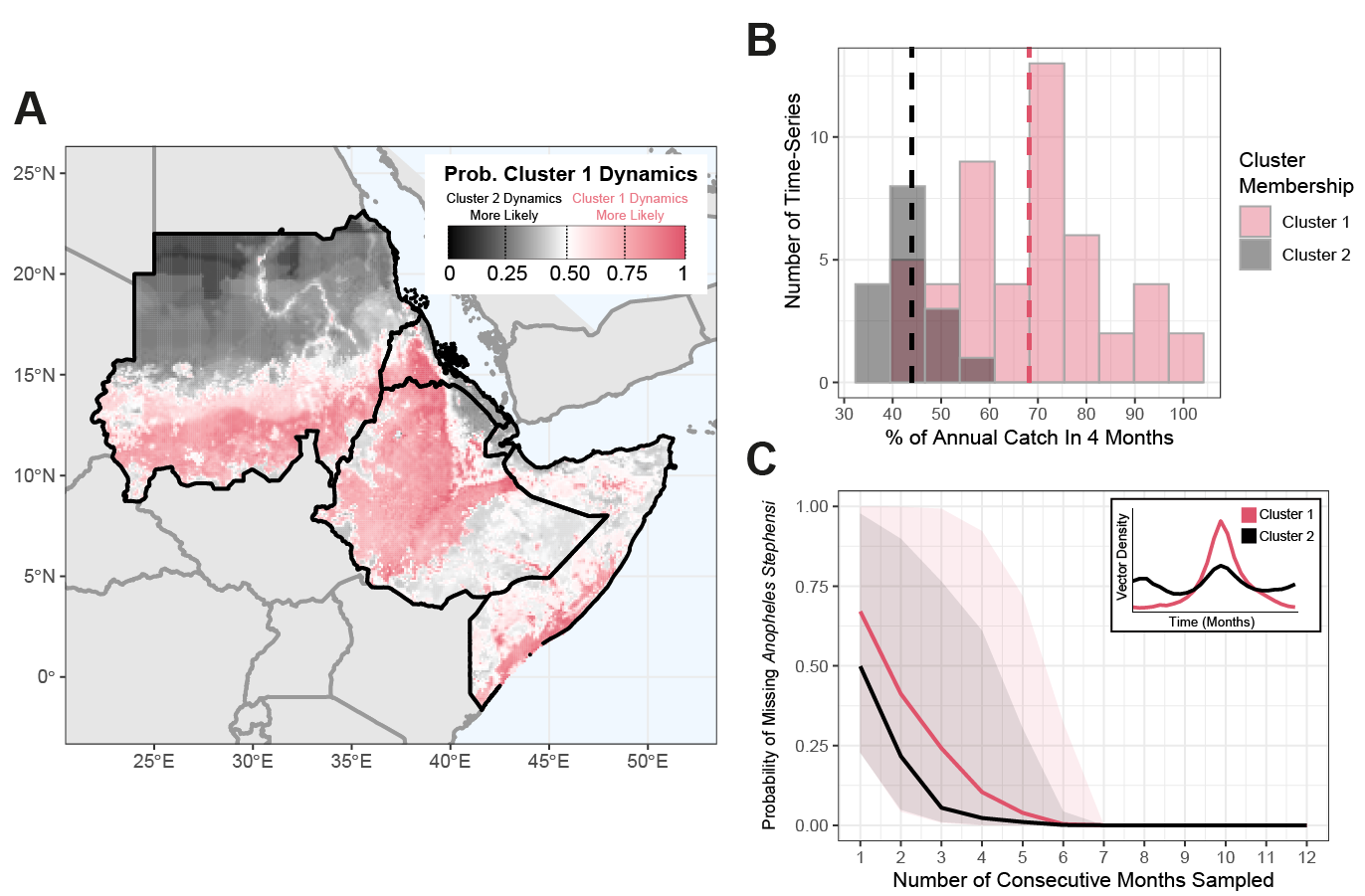
**Figure 1: Sources and Locations of *Anopheles stephensi* Time-Series Data and Examples for Each Country. (A)** Map of the geographical range over which collated time-series had been carried out in, with countries where studies had been carried out in highlighted in light grey, and the locations of individual studies indicated by points, coloured according to the country they were carried out in (Afghanistan = red, Djibouti = yellow, India = green, Iran = turquoise, Myanmar = blue and Pakistan = pink). **(B)** Example *Anopheles stephensi* time-series from each country, with the empirical monthly mosquito catch (black points) and fitted gaussian process curves (mean = coloured line, ribbon = 95% Bayesian Credible Interval) for each, coloured according to country. The x-axis indicates the month of sampling, the y-axis normalised annual vector density (i.e. arbitrary units).



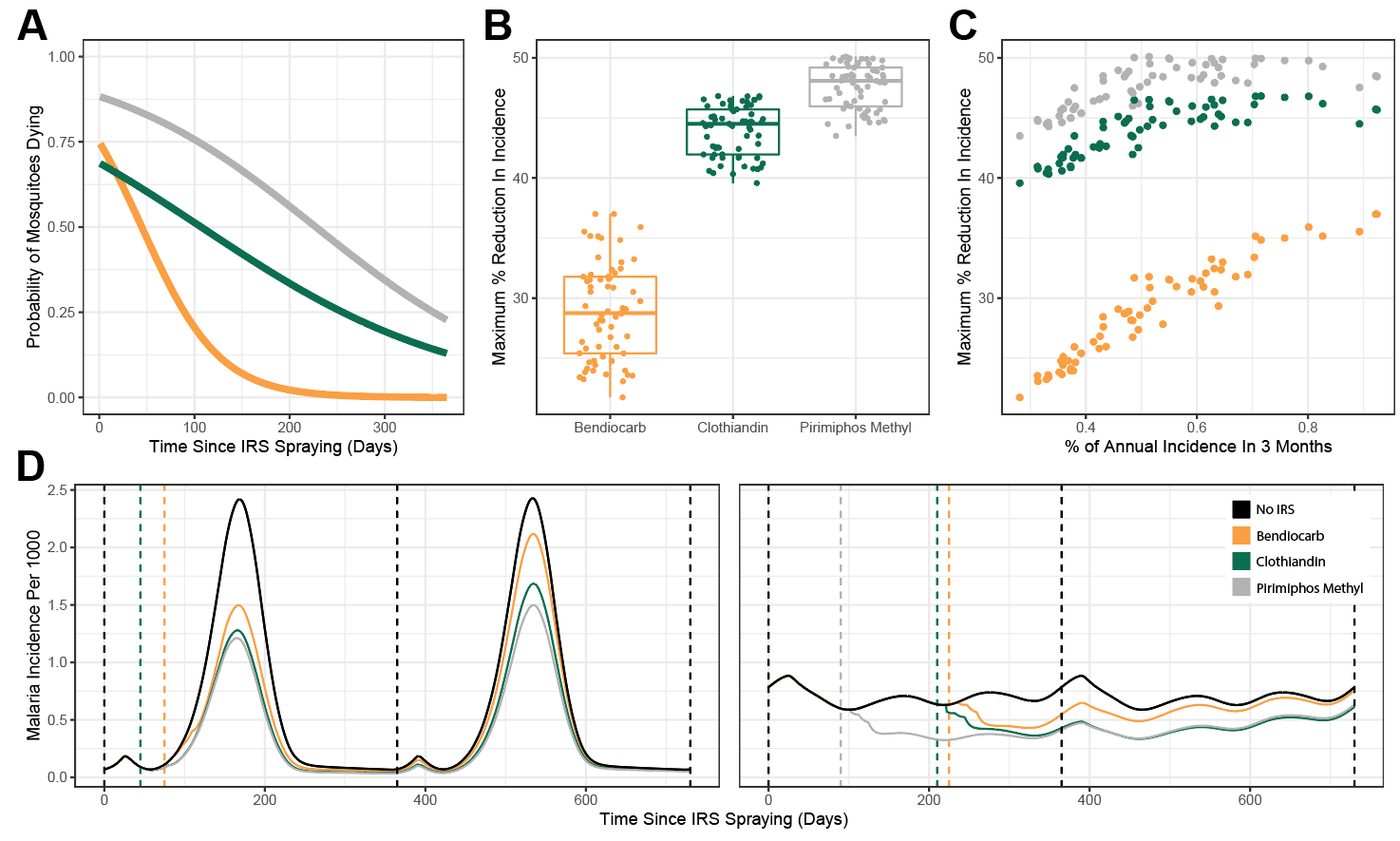
**Figure 2: Characterisation and Clustering to Identify Time-Series with Similar Temporal Properties. (A)** Results of principal components analysis (PCA) and k-means clustering for 2 clusters. Points indicate individual time-series, with point colour indicating cluster membership. Ellipsoids demarcate the 75th quantile of the density associated with each cluster. Principal components 1 and 2 are plotted, together explaining 69% of the total variation in temporal properties across the time-series. **(B)** Time-series belonging to each cluster. Pale lines represent individual time-series, brighter line the mean of all the time-series belonging to that cluster – in all cases vector density is normalised to sum to 1 over the course of the year, and time-standardised so that the highest vector density for each time-series is arbitrarily set to occur at month 7. **(C)** Boxplots of the percentage of annual total mosquito catch (top) and annual total rainfall (bottom) for each time-series. Rainfall data comes from the *CHIRPS* dataset37 and is specific to study location and time-period. Each point indicates an individual time-series.



**Figure 3: Random Forest Prediction of Temporal Cluster Membership.** A random forest-based classification modelling framework was used to predict membership of the temporal cluster (either Cluster 1 or Cluster 2, as defined in **Fig 2)** and explore the ecological factors underpinning variation in *Anopheles stephensi* seasonality. **(A)** Receiver-operator curve (ROC) for each of the 25 individual iterations of random forest model fitting carried out, with results for each displayed as grey lines. The mean AUC across these 25 iterations was 0.89. **(B)** Variable importance plot for the covariates included in the random forest model – bar height indicates the mean variable importance across the 25 individual iterations of random forest fitting, with error bars representing the 95% confidence interval. **(C)** Collated *Anopheles stephensi* time-series, disaggregated according to urbanicity and cluster membership. Cluster 1 and Cluster 2 time-series from rural locations are plotted separately; all time series for time-series carried out in urban locations (22 belonging to Cluster 1 and only 3 belonging to Cluster 2) are plotted together. Coloured line indicates the mean and ribbon indicates the 90% range spanned by the group of time series belonging to each displayed grouping.



**Figure 4: Predicting the Possible Seasonal Dynamics of *Anopheles stephensi* Across the Horn of Africa. (A)** Environmental covariates were collated across countries in the Horn of Africa where *Anopheles stephensi* has been found, and the fitted random forest classification model from **Fig 3** used to predict potential temporal dynamics. Map shows the probability of temporal dynamics belonging to Cluster 1, with pink corresponding to Cluster 1 dynamics being more likely than Cluster 2, black indicating Cluster 2 dynamics are more likely than those for Cluster 1, and white indicating both are equally likely. **(B)** Histogram of the percentage of total annual catch in any continuous 4-month period for each time-series, coloured according to the cluster the time-series belong to. **(C)** The probability of missing *Anopheles stephensi* in an entomological catch survey (y-axis) as a function of the number of consecutive months randomly sampled, assuming the start-month is picked at random. Coloured lines are the mean results across all time-series belonging to each cluster, with the shaded area indicating the range spanned by all time-series belonging to each cluster.



**Figure 5: Modelling the Public-Health Impact of Indoor Residual Spraying (IRS) and How This Is Impacted by *Anopheles stephensi* Seasonality. (A)** Probability of mosquitoes dying upon exposure to each IRS compound – yellow indicates bendiocarb, green indicates clothiandin and grey indicates pirimiphos methyl. **(B)** Percentage reduction in annual incidence (with optimal timing of IRS delivery), for each of the IRS compounds considered. Individual points correspond to specific time-series. **(C)** The relationship between percentage reduction in annual malaria incidences and the overall seasonality of malaria incidence in the setting (as modelled and implied by each *Anopheles stephensi* temporal profile). **(D)** Malaria incidence modelling results for a highly seasonal (left) and less seasonal (right) setting. Black lines indicate endemic dynamics in the absence of any IRS, coloured solid lines indicate incidence following a single IRS round (with timing of the round indicated by the coloured dashed lines).

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