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

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

* **Do I want to mention “Urban” somewhere???**
* **Do I want to mention “Areas where malaria is absent” somewhere???**

Charles Whittaker1\*, Peter Winskill1, Gina Cuomo-Dannenburg1, Patrick G.T. Walker1, Marianne Sinka2, Ashwani Kumar3, Azra Ghani1, Thomas Churcher1, Samir Bhatt1,4 & 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. However, this property of urban locations 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 establish 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 likely to have material consequences for both the nature of malaria establishment in the Horn of Africa, as well as the potential impact of currently available control interventions.

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 highlight the material consequences this variation has for the speed and dynamics of malaria establishment 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 previously published systematic reviews of literature relating to *Anopheles stephensi*27,32, and updates 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 (full details of keywords and search methodology available in **Supplementary Information**). 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 model to smooth these noisy mosquito count data, 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 . Given that mosquito population dynamics are typically characterised by repeating patterns occurring either seasonally or annually, a periodic kernel function was used to define the covariance between pairs of points, which depends on both the Euclidean distance in time between points as well as 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 . Fitting was undertaken with the probabilistic programming language STAN33.

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

Motivated by 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 Negative Binomial Gaussian Process fitting (that describes the dominant modality of annual or sub-annual temporal variation present in the data), the proportion of points in each smoothed time-series where the total catch was greater than 1.65x the mean (providing a measure of how peaked the time-series is), the distance of the first peak from January (providing an indication of timing), 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 (providing an indication of where in the year the region of highest catch numbers were), 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. We therefore 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 Seasonality and Peaks**

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 whether a given time-series was either “unimodal” (possessing a single distinct seasonal peak, as defined by ) or “multimodal” (lacking a distinct single seasonal peak, which typically translated to either possessing two distinct seasonal peaks or more perennial patterns of transmission – see **Supplementary Figure X** for the individual fitted profiles for each time-series), with these definitions defined per previous work34 based on the results of fitting and comparing 1 and 2 component Von-Mises distributions to each smoothed time-series**.** These models were fit 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 estimates in all instances. Further details on defining the outcome variable and the Random Forest modelling framework can be found in the **Supplementary Information**.

**Modelling Malaria Establishment and Transmission**

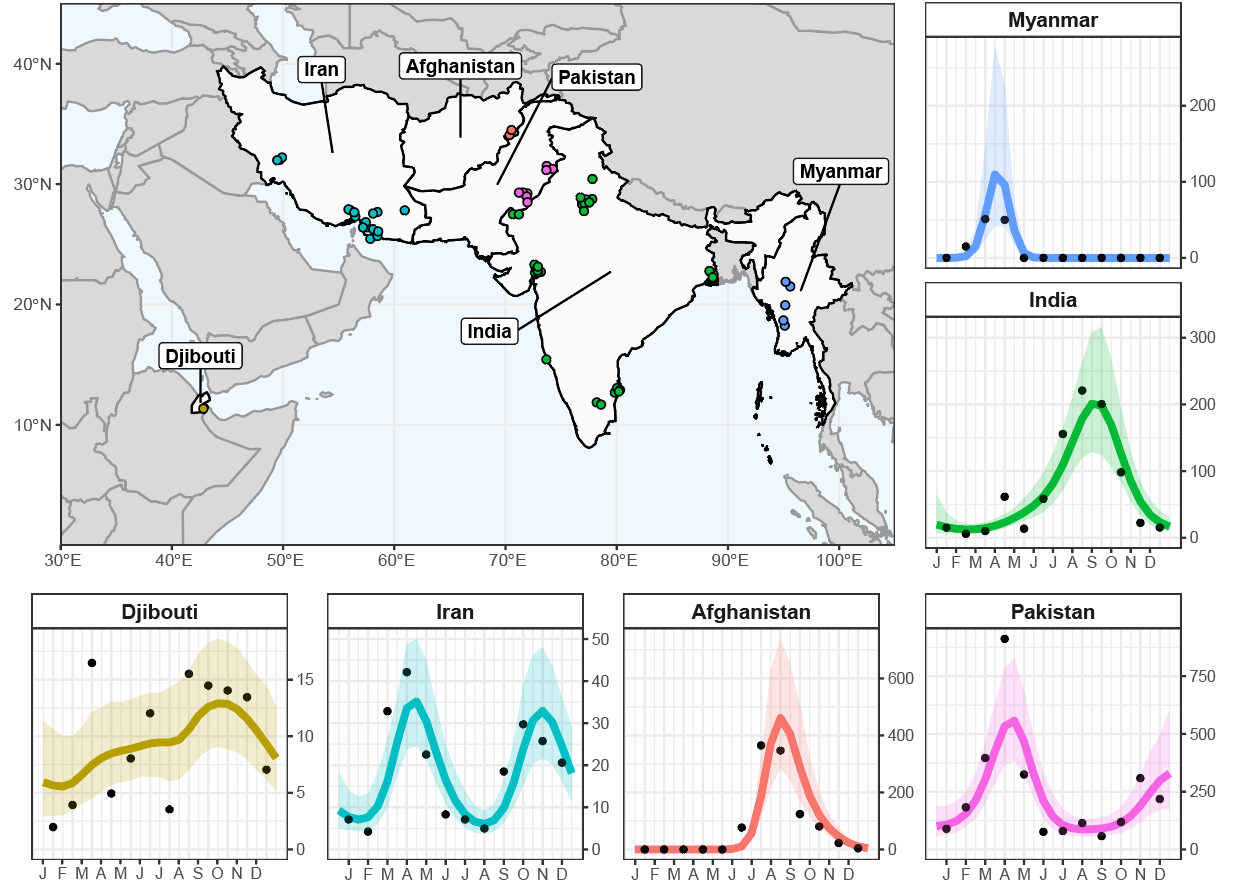
We integrated the temporal profiles of *Anopheles stephensi* abundance into a deterministic version of a well-established compartmental model of *Plasmodium falciparum* malaria transmission and disease39–41 to explore the implications of *Anopheles stephensi* establishment and seasonality on the dynamics of malaria transmission, with a particular focus on areas where malaria transmission is currently absent or only minimally present. 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. Full details of the model can be found in **Supplementary Information**. For the purposes of exploring the impact of *Anopheles stephensi*, we integrate the collated temporal profiles of mosquito abundance, and integrate these into this modelling framework, making a number of assumptions regarding 1) the rapidity with which *Anopheles stephensi* populations scale and establish in the Horn of Africa; and 2) the final average annual density level the *Anopheles stephensi* population reaches – sensitivity of our conclusions to these assumptions are presented in both the main text and **Supplementary Figures X** and **X**.

**Results (1000-1200 words)**

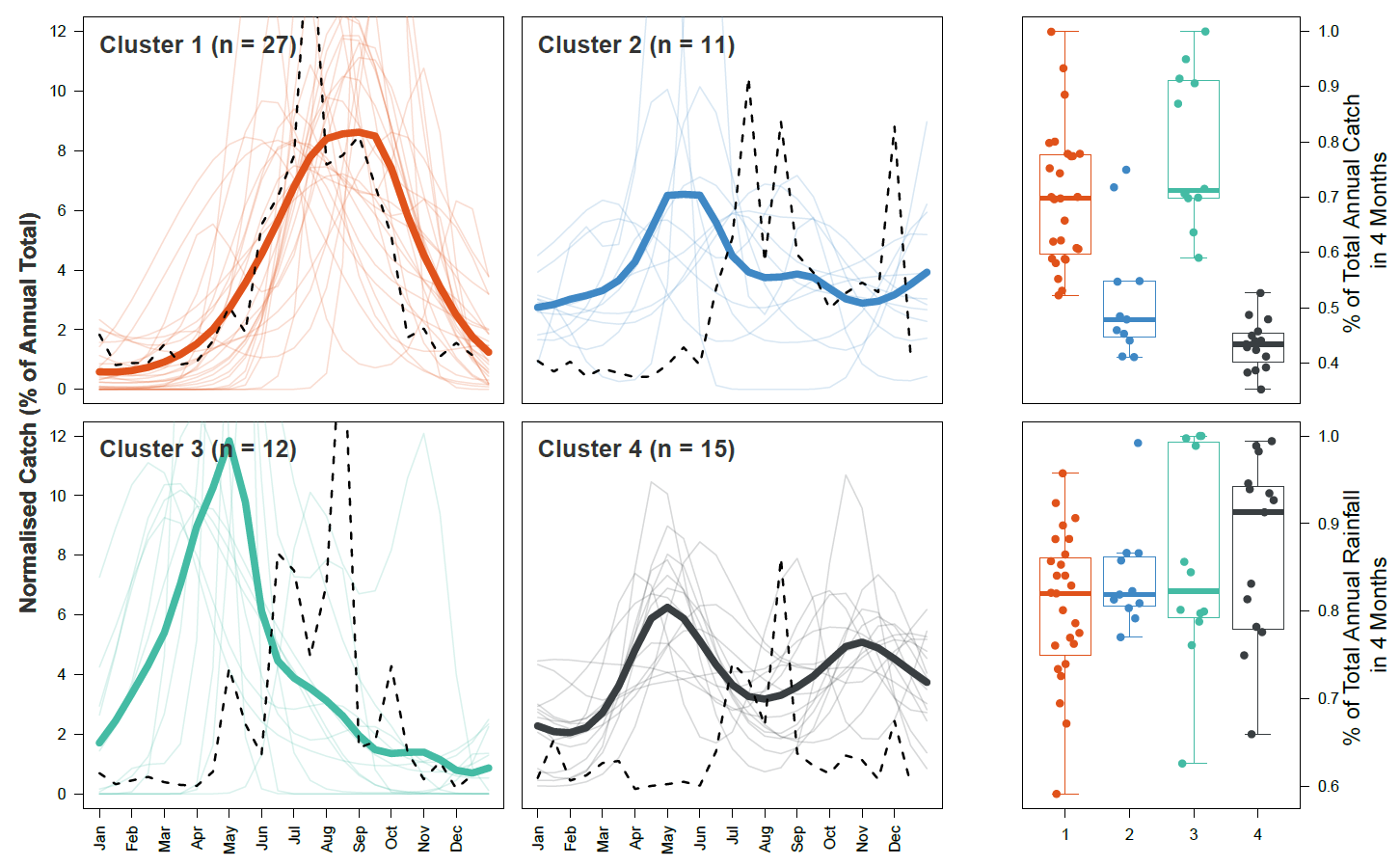
* **Literature Review Results & Description of Variation**
* **Characterisation, PCA and Clustering**
* **Urban/Rural Chi-Squared and Random Forest Modelling/Mapping**
* **Malaria Modelling and Implications for Establishment**

**Discussion (800-1000 words)**

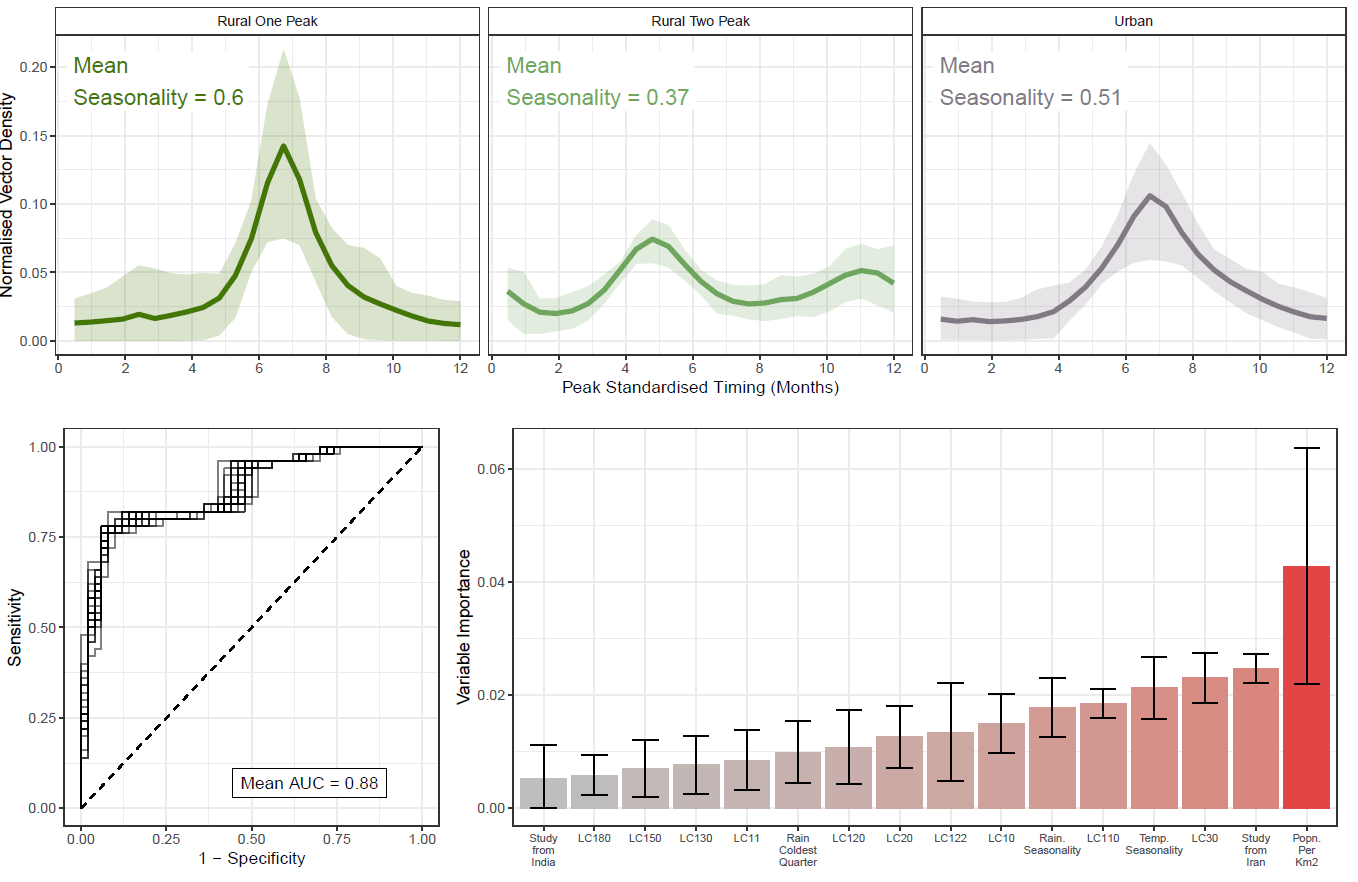
**Total (~3500-4000 words)**



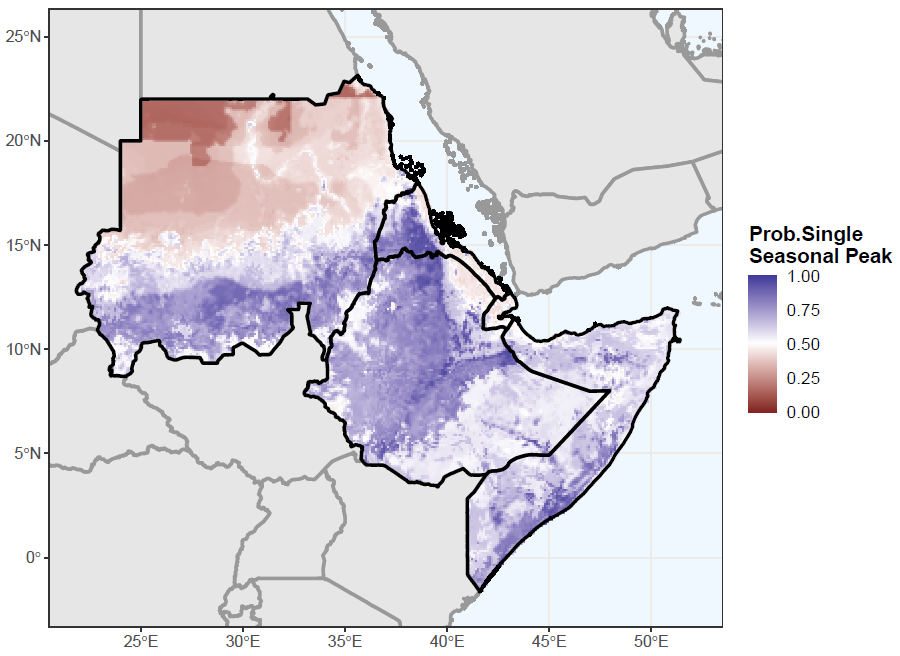
**Figure 1: Sources and Locations of *Anopheles stephensi* Time-Series Data and Examples for Each Country.**



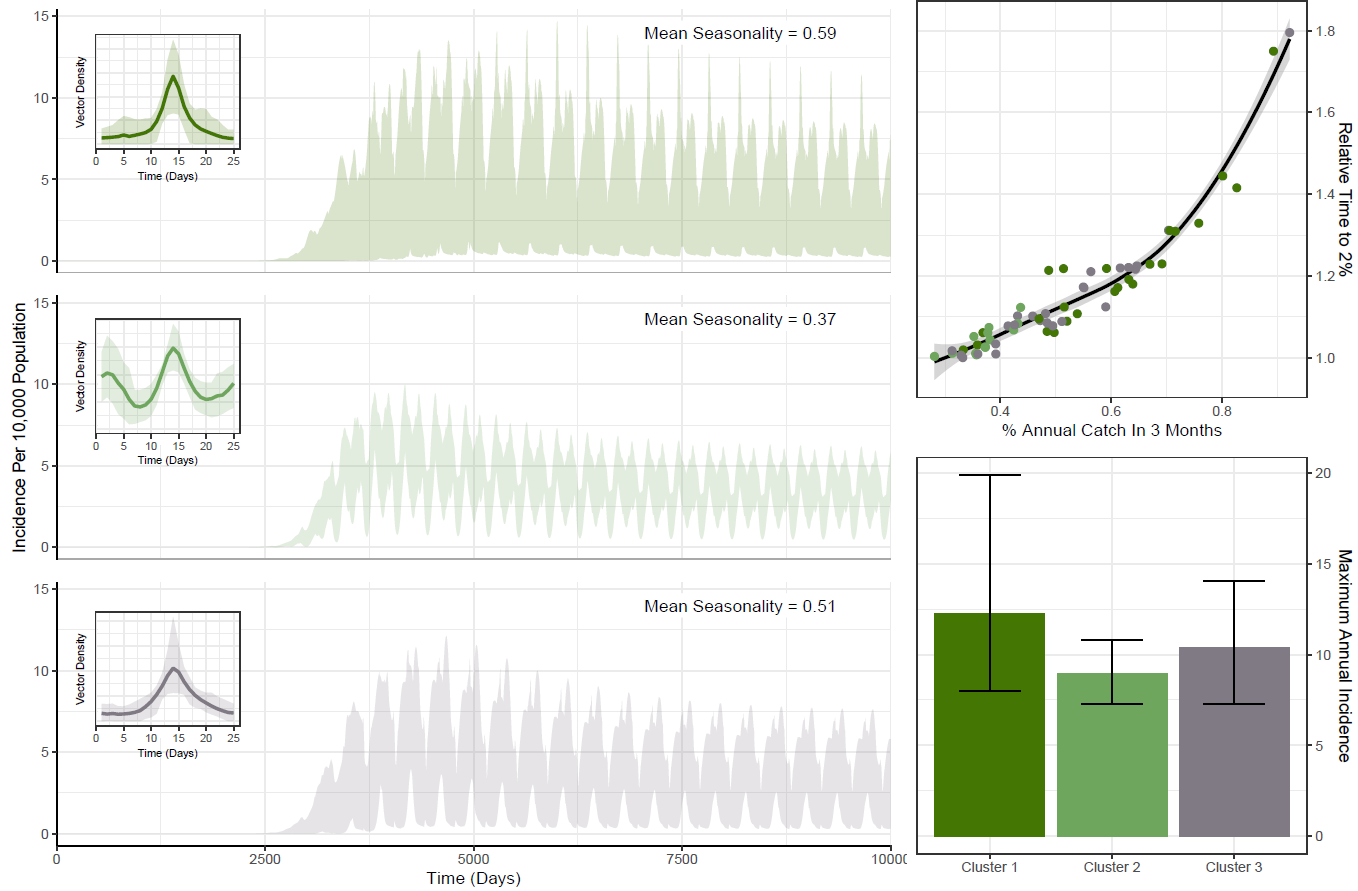
**Figure 2: Characterisation and Clustering to Identify Time-Series with Similar Temporal Properties.**



**Figure 3: The Ecological Factors Underpinning Variation in *Anopheles stephensi* Seasonality.**



**Figure 4: Predicting the Possible Seasonal Dynamics of *Anopheles stephensi* Across the Horn of Africa.**



**Figure 5: Potential Dynamics of Malaria Establishment Following *Anopheles stephensi* Importation In Settings Where Burden Is Currently Minimal.**

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