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PMI Action Plan to respond to the threat of *Anopheles stephensi* in Africa

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To further bridge local capacity for epidemiological and entomological surveillance, the CDC FETP can be engaged and entomologists recruited for the program. The FETP trains field epidemiologists to detect and respond to public health threats, expanding the global health workforce and enhancing public health capacity in coordination with Ministries of Health. In Ethiopia, An. stephensi training modules have been included in the malaria FETP track and a subset of residents are working on An. stephensi research projects. PMI countries where An. stephensi has been detected can engage with FETP to address critical surveillance, research, and implementation needs. 22

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EXECUTIVE SUMMARY

Anopheles stephensi was first detected on the African continent in 2012 in Djibouti. Since then, it has been detected in Ethiopia, Somalia, Sudan, Nigeria, and Kenya and appears to continue to spread. In 2016, *An. stephensi* was first detected in Ethiopia, and it has now been detected throughout the country and to the borders with Sudan and Kenya. PMI has supported much of the surveillance and research to understand invasive *An. stephensi* behavior and bionomics through entomological monitoring in Ethiopia in collaboration with Baylor University, World Health Organization (WHO), Pan African Mosquito Control Association (PAMCA), Armauer Hansen Research Institute (AHRI) and other partners.

While the full impact of this spread is not yet understood, *An stephensi* has been shown to be a competent malaria vector in its endemic and invasive range. A 36-fold increase in malaria was reported in Djibouti following its initial detection. Modeling has predicted an additional 126 million people are at risk of malaria if *An. stephensi* spreads across Africa. Another model estimated that malaria cases in Ethiopia could increase by 50% if *An. stephensi* spreads to suitable areas. However, these studies noted considerable uncertainty in the estimates which make sound decisions more difficult. Action is needed to understand the extent of the distribution and spread of *An. stephensi*, quantify its impact on malaria transmission, and evaluate and implement appropriate control measures.

A PMI *Anopheles stephensi* Task Force was created in 2021 to develop an action plan to address this global health threat and provide guidance on activities that can be used for a coordinated PMI response to *An. stephensi.* In this action plan for PMI partner countries in Africa, PMI describes **two scenarios (*An. stephensi* present and high risk of invasion) and the accompanying guidance** on how to conduct surveillance and control measures to mitigate impacts that might result from establishment of this vector, in alignment with the five strategic focus areas of the 2021–2026 PMI Strategy.

When dealing with invasive species in general, there are three main responses: *elimination* of the invasive species from the area of interest, *containment* of the species within a defined area, and *mitigation* of the harmful effects of the invasive species. With the limited information available at this time on the extent of the distribution of *An. stephensi* in Africa and the most effective vector monitoring and control tools for the species, **the PMI approach at this time focuses on mitigation of the harmful effects of *An. stephensi* utilizing enhanced vector and disease surveillance, coordinated intervention implementation, and close monitoring.** This approach will not halt the expansion of *An. stephensi*; therefore specific knowledge needs must be met to continually update this approach.

Despite the emphasis on mitigation, the response to *An. stephensi* is not limited to entomological monitoring and vector control. A mitigation approach means that malaria and health systems will need to deal with malaria transmitted by a mosquito that is well adapted to urban settings and dry seasons, a non-traditional setting for malaria control in Africa. As such, National Malaria Programs (NMPs) may need to consider ways to prevent malaria outbreaks in urban settings where people may have less malaria immunity, less utilization and adherence to malaria interventions, but more political influence. Protecting urban populations without reducing services to rural and / or existing high malaria burden areas will be an important challenge in achieving PMI's strategic objectives.

PMI has played an important role in developing the response to *An. stephensi* in cooperation with NMPs, WHO, Rollback Malaria (RBM), PAMCA, African Leaders Malaria Alliance (ALMA), and other partners in alignment with the WHO initiative to stop the spread of *An. stephensi*. Developing methods for enhanced surveillance for early detection and mitigation will require continued global coordination and collaboration to determine appropriate *An. stephensi* strategies.

BACKGROUND

Spread of *Anopheles stephensi* in Africa

Anopheles stephensi is a primary vector of malaria in South Asia and the Arabian Peninsula and is responsible for both urban and rural transmission of *Plasmodium vivax* and *P. falciparum* in these locations.

Anopheles stephensi was first detected in Africa in Djibouti in September 2012 (Faulde et al. 2014). These collections were made just prior to a malaria outbreak in Djibouti in January–April 2013, and a second increase in malaria cases in early 2014. In late 2016, *An. stephensi* was collected in Kebridehar, Somali Region, Ethiopia and species identification was confirmed by genetic sequencing (Carter et al. 2017). It has since been found primarily throughout eastern and parts of central Ethiopia (Balkew et al. 2021). *Anopheles stephensi* has been detected in several locations in Sudan, including Khartoum (Ahmed et al. 2021) and as far west as North Darfur (Abubakr et al., submitted). It has also been found in several locations in Somalia (Somaliland, Puntland), from collections made in 2020 from Gombe State, Nigeria (WHO Threat Map), and most recently in 2022 from Marsabit, Kenya (Ochomo et al. 2023). The current known distribution of *An. stephensi* in Africa is shown in Figure 1 and negative sites have now been added to the WHO Threat Map to help visualize expansion over time.

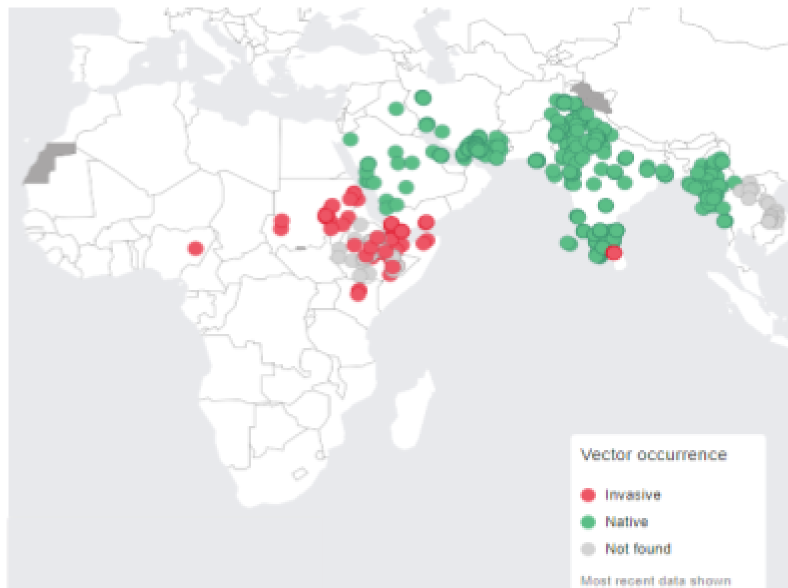


Figure 1. Sites where *An. stephensi* has been detected in Africa since 2012 (as of October 14, 2022). Red dots indicate locations where *An. stephensi* is invasive and green dots indicate detections within its native range, grey dots indicate surveillance and no detection.

There is no current information available to describe how *An. stephensi* is entering and moving within countries, although it is hypothesized that the vectors are entering through sea ports and traveling along major transport/commerce routes. The initial detections in Djibouti, Sudan, and Somalia were all in or near sea ports. The sites of initial *An. stephensi* detection in Ethiopia were along a major transport route with Djibouti. Ethiopian *An. stephensi* population genetics suggest multiple introductions into Ethiopia along two major transport routes (Carter et al. 2021); one from the northeast and one from the southeast, perhaps through livestock trade routes.

In September 2022, WHO released an initiative to stop the spread of *An. stephensi*. This initiative recommends a five prong approach that aligns with this action plan. The five aims within the initiative are to:

Increase of malaria cases in Djibouti and dry season outbreak in Ethiopia

Malaria cases in Djibouti increased dramatically after 2012 (Figure 2), coinciding with the suspected arrival of *An. stephensi*. This trend has continued, but further epidemiological investigations are needed to determine the extent to which the increase in cases is attributable to *An. stephensi.*

In February 2022 an outbreak of malaria was reported during the dry season in Dire Dawa, Ethiopia, one of the largest urban centers in the country which continued on for several months. An epidemiological case-control and entomological investigation led by AHRI linked the outbreak to *An. stephensi* with larval habitats reported nearly ~100% of *Anopheles* collected throughout this period were *An. stephensi* and *P. falciparum* and *P. vivax* sporozoite positive. The case control study revealed that most infections were detected in index households (28.5%) when compared to control households (9.1%), with an odds ratio of 3.8 (Tadesse et al. 2022).

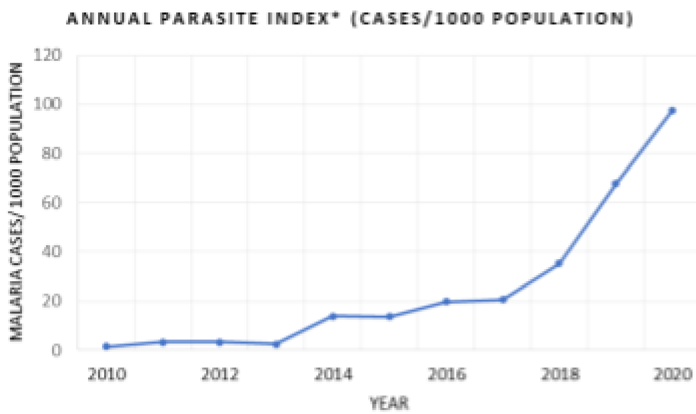


Figure 2: Annual parasite index* (cases/1000 population) from Djibouti 2010–2019 from World Malaria Report (WHO 2020). Note that *An. stephensi* was first detected in Djibouti in 2012.
*assumes that all cases were new cases

Investigation of *An. stephensi* behavior and bionomics in Africa

PMI has supported much of the surveillance conducted to understand *An. stephensi* behavior and bionomics in Africa, as most of that work comes from Ethiopia, which is a PMI partner country. These activities have been conducted as part of entomological monitoring in Ethiopia by PMI VectorLink and have been enriched through collaboration with partners including Baylor University, WHO, PAMCA, AHRI in Ethiopia and others. Investigations by PMI in Ethiopia in 2020 also found *An. stephensi* to be present in rural villages within 20km of urban sites where it is established, indicating that *An. stephensi* is not limited to urban settings (Balkew er al. 2021). The extent of spread across Africa is unknown, but many PMI partner countries have increased entomological surveillance efforts.

Figure 3: Examples of larval habitats that *An. stephensi* is typically found in. Water tankers and concrete cisterns are habitats where *An. stephensi* is often detected in Ethiopia.

Larval sites for *An. stephensi* in the Horn of Africa have been broadly similar to those reported for *An. stephensi* in Asia. These include concrete water cisterns (birkets, birkas), tires, water tanks, steel drums, plastic sheet water storage and buckets (Faulde et al. 2014; Balkew et al. 2020; Balkew et al. 2021). In Djibouti and in Ethiopia, *Aedes aegypti* larvae have been found in the same larval habitats where *An. stephensi* larvae were collected (Balkew et al. 2021; Tadesse et al. 2021).

Finding a reliable method for collecting adult *An. stephensi* has been a challenge. Standard methods such as pyrethrum spray catch, human landing collections, and CDC light traps have not consistently collected *An. stephensi.* Adult *Ae. aegypti* traps also do not seem to work well in capturing adult *An. stephensi*. Aspiration from animal shelters does seem to collect higher numbers of *An. stephensi*, which is consistent with some findings from *An. stephensi*'s native range; however, more work to determine an appropriate, standardized adult collection method would assist in vector control evaluations.

Importantly, it appears that the host-preference of *An. stephensi* may vary with the availability of hosts (Basseri et al. 2010). Analyses of blood fed *An. stephensi* in Ethiopia have shown that most blood meals are from animals (particularly goats and cattle) (Balkew et al. 2021). This is broadly similar to findings in Asia (Thomas et al. 2017). The apparent preference for animal feeding would seem to indicate that *An. stephensi* would not be an efficient vector of malaria. However, higher proportions of Ethiopian *An. stephensi* in a laboratory setting fed *P. vivax*-infective bloodmeals developed sporozoites than did *An. arabiensis* fed on the same blood, indicating that they have the potential to be efficient malaria vectors (Tadesse et al. 2021; Balkew et al. 2021). The importance of *An. stephensi* as a vector may not lie in its attraction to humans, but rather its physiological ability to transmit malaria parasites and its opportunistic nature in selecting a host for blood meals.

Insecticide resistance has been monitored in *An. stephensi* in Ethiopia since 2018 (Balkew et al. 2021). Resistance to pyrethroids and carbamates has been consistently reported, with few exceptions. Organophosphate resistance (pirimiphos-methyl) has been found in some sites but not others. The addition of the synergist piperonyl butoxide (PBO) to pyrethroids in bioassays has consistently resulted in improvements of mortality in *An. stephensi*, often fully restoring susceptibility.

Lessons on *An. stephensi* control from Asia

While the malaria threat that *An. stephensi* poses to Africa is a more recent development, the transmission of malaria by *An. stephensi* is not new. Furthermore, malaria control in Asia has been working for more than 100 years on how to best control malaria transmitted by *An. stephensi*. The primary trials evaluating the impact of vector control on *An. stephensi* are noted below. Further lessons can be learned through ongoing engagements with the Indian, Sri Lankan, Pakistani, and Iranian malaria control programs.

Insecticide treated nets

Insecticide-treated nets (ITNs) have been used in areas where *An. stephensi* are primary malaria vectors. However, there are only limited epidemiological trials evaluating the impact of ITNs on malaria transmitted by *An. stephensi* (Pryce et al. 2018), and limited information on biting behaviors and biting times in its native and invasive ranges. The only study on *An. stephensi* cited in the ITN review conducted by Pryce et al. was conducted in Pakistan in refugee camps (Rowland et al. 1996), which found that permethrin-treated net users had 0.58 (95% CI: 0.49–0.68) and 0.39 (95% CI: 0.29–0.53) times the risk of *P. vivax* and *P. falciparum* malaria, respectively, significantly lower than to non-users.

Other insecticide-treated materials

Although high levels of pyrethroid resistance are being detected in *An. stephensi* in the Horn of Africa now, historically, a number of studies examined materials treated with pyrethroids to prevent human-vector contact. For example, in 1994, treatment of window and door curtains with deltamethrin (100mg/m^2) resulted in 93.1% reduction in *An. stephensi* and 95.4% reduction in malaria cases (Ansari & Razdan 2001). In refugee camps, the treatment of tents, tarpaulins, chaddars, and top sheets also showed positive effects (Hewitt et al. 1995; Bouma et al. 1996; Rowland et al. 1999; Graham et al. 2002; Graham et al. 2002).

Indoor residual spraying

Indoor residual spraying (IRS) targeting *An. stephensi* has been conducted widely in Asia. However, there was only one randomized control trial included in a Cochrane review of IRS (Pluess et al. 2010). This was a community-randomized trial of IRS with alpha-cypermethrin, both wettable powder (WP) and suspension concentrate (SC) formulations. Spray campaigns had high coverage (96%). The WP formulation of alpha-cypermethrin resulted in a 95% decrease in *P. falciparum* malaria incidence and 80% decrease for *P. vivax* malaria incidence. The SC formulation had similar results . *Anopheles stephensi* populations were 68% reduced in IRS areas compared to unsprayed areas over the 7-month spray monitoring period.

Larval source management

Larval source management comprises environmental modification (permanent change to environment, e.g., drainage), environmental manipulation (recurrent activity, e.g., flushing of streams, source reduction), larviciding, and/or biological control (i.e., introduction of natural predators such as larvivorous fish). Limited evidence is available on the effect of larval source management on *Anopheles* (Tusting et al. 2013). Application of pirimiphos-methyl at 12.5g a.i./ha in Bhiwani (Haryana State, India) resulted in significant decreases in *Anopheles* larval indices, rates of positive thick films, and malaria positivity rates in infants and children 1–5-year-olds. In Goa, the combination of two larval control measures (weekly treatments of *Bacillus thuringiensis* var. *israelensis* and distribution of the larvivorous fish *Aplocheilus blocki*) resulted in significant decreases in malaria cases (Kumar et al. 1998).

Insecticide-treated cattle

After initial work to determine whether treatment of cattle with a pyrethroid might have short-term effectiveness against mosquitoes (Hewitt & Rowland 1999), a cross-over trial was conducted in Pakistan to evaluate its impact on malaria (Rowland et al. 2001). This study found decreases in *P. falciparum* (56%) and *P. vivax* (31%) incidence, as well as reductions in *An. stephensi* densities and parity.

Modeling the impact of *An. stephensi* and control interventions in Africa

Modeling is a useful tool to provide estimates of what could happen in the future, based on informed assumptions. Recent models have estimated the spread of *An. stephensi* in Africa as well as estimated the impact that spread may have on malaria cases. Additionally, models have been used to estimate the impact of existing tools to control malaria transmitted by *An. stephensi*.

A niche model was produced to estimate the possible spread of *An. stephensi* on the African continent (Sinka et al. 2020). This model found a heterogenous spread of *An. stephensi* with suitability in many major African cities, shown in light blue circles (Figure 3). High risk areas are shown in red. The authors estimated that the establishment of *An. stephensi* in all suitable areas across Africa would increase the number of people at risk of malaria by over 126 million people.

Due to initial detections in seaports in Djibouti, Sudan, and Somalia, a follow up study (Ahn et al. 2021, pre-print) examined the movement of goods though maritime trade and connectivity in 2011 (pre-*An. stephensi* detection in Africa), 2016, and 2020 between *An. stephensi* endemic countries and all coastal countries in Africa to determine the likelihood of *An. stephensi* introduction. Maritime connectivity to *An. stephensi* endemic countries in 2011 identified Sudan and Djibouti as the two countries in Africa with the greatest likelihood of *An. stephensi* maritime introduction, and indeed these were the first two coastal countries where the species was detected. When maritime data were combined with the habitat suitability data mentioned above on likelihood of establishment, a ranking of countries based on likelihood of invasion (introduction and establishment) was developed. A network model was also developed highlighting intracontinental connectivity between African coastal nations that may further facilitate introduction if *An. stephensi* appears in a new location.

PMI supported the development of a model to estimate the potential impact of *An. stephensi* on malaria cases in Ethiopia (Hamlet et al., submitted). This model used the Sinka et al. 2020 model to estimate *An. stephensi* spread in Ethiopia. Assuming *An. stephensi* spread to all of the projected locations, an increase of 50% (95% CI: 14–90) in malaria cases might be expected in Ethiopia. The authors examined the potential impact of vector control interventions, including ITNs (including PBO nets), IRS, and larviciding and found that in most cases, a single intervention would not return malaria case numbers to pre-*An. stephensi* levels and concluded that a combination of vector control interventions would be needed to reduce malaria after the introduction of *An. stephensi*. Insecticide resistance data from Ethiopia shows widespread resistance to pyrethroids, carbamates, and organophosphates but partial recovery of susceptibility to pyrethroids following exposure to PBO ([Balkew et al. 2021](); PMI VectorLink Ethiopia 2022). The use of PBO and new types of nets may be beneficial in controlling *An. stephensi*.

Climate change, seasonality, and **An. stephensi**

Based on thermal thresholds, because of *An. stephensi*'s ability to adapt to more extreme climatic conditions, a larger proportion of Africa is suitable for malaria transmission by *An. stephensi* than by current primary malaria vector *An. gambiae*. This means that even in rural areas *P. falciparum* transmission could occur 12 months of the year by *An. stephensi* as the species becomes more established in Africa ([Villena et al. 2022]()), presenting an additional potential threat to malaria control in Africa. A more [recent pre-print (Ryan et al. 2022)]() used global climate forecasts, population predictions, and temperature thresholds to predict where and how malaria disease risks will change in the future, noting that by thriving in urban and arid cities the actual extent of malaria risk will not just depend on temperature, but also on the spread of *An. stephensi*, that **up to one-third of the world's population** lives in areas of potential risk. Therefore it is crucial to identify where *An. stephensi* invasion is possible and to enhance global surveillance efforts for container thriving species like *An. stephensi* which would alter existing malaria climate predictions and provide opportunities for poleward expansion.

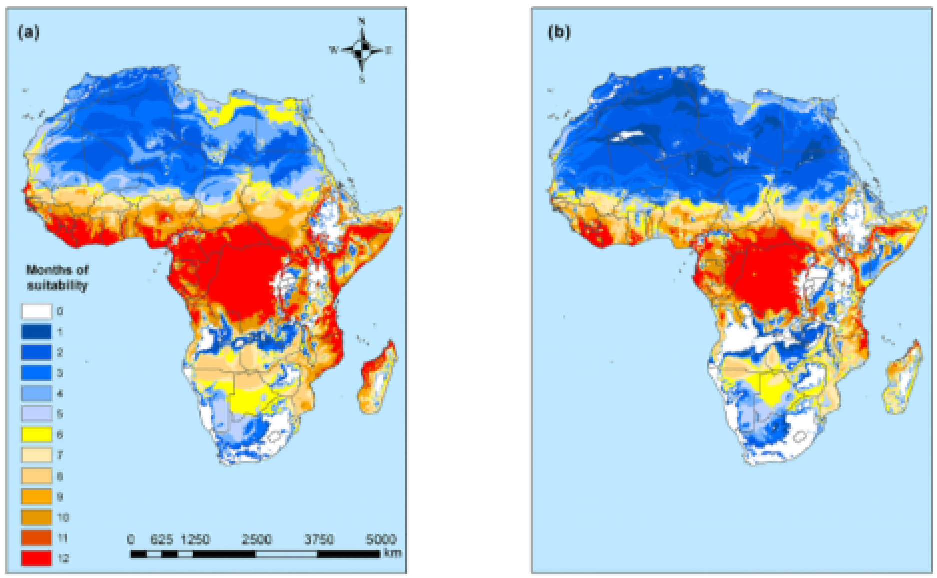


Figure 4: The number of months per year that locations are predicted to be suitable for the transmission of *Plasmodium falciparum* by *Anopheles stephensi* (left panel) and *Anopheles gambiae* (right panel) mosquitoes in Africa (Villena et al. 2022).

In Djibouti and Ethiopia, entomological surveillance data have shown *An. stephensi* populations to persist year-round (PMI VectorLink 2021), including throughout dry periods, whereas endemic vector populations are more seasonal. In 2022, an outbreak of malaria occurred in the major urban area of Dire Dawa, Ethiopia during the dry season and this was linked to *An. stephensi* which made up nearly ~100% of the *Anopheles* collected throughout this period (Tadesse et al. 2022).

A recent modeling study also shows that *An. stephensi* persists throughout dry periods unlike endemic malaria vectors, so seasonally targeted malaria interventions (such as IRS or SMC) may not have the same impact as with endemic vector transmission.

THE APPROACH

When dealing with an invasive species, there are generally three main approaches. The first is *elimination* of the species from the area of interest. The second is *containment* of the species within a designated area to prevent further expansion. The third approach accepts that sufficient information and resources are not available for the species to be eliminated or contained, and that the damage caused by the species should be *mitigated* as much as possible.

There are challenges inherent in both elimination and containment approaches due to limitations in what is known about routes of introduction, the current distribution of *An. stephensi* and the fact that the species is now well established in several countries. However, these challenges do not mean that either strategy is impossible. *Anopheles arabiensis* was spread across 54,000km^2 in Brazil, and was successfully eliminated in 1940, ten years after its first discovery. Similarly, *An. labranchiae* was nearly eliminated from Sardinia (24,000km^2) by 1951, five years after its discovery. While the area of *An. stephensi* invasion in Africa may be as wide as 1,000,000km^2, the actual area invaded could also be considerably smaller. Therefore, while the current situation does not seem to lend itself to a massive redistribution of existing funds to eliminate or contain *An. stephensi*, there must be immediate investments in enhanced surveillance, monitoring and control, with a periodic reassessment of the strategy. Increased funding would allow affected countries and the global community to apply appropriate measures (whatever these may be) more quickly.

With limited information available on the extent of the distribution of *An. stephensi* in Africa and which vector monitoring and control tools for the species are most effective, the **PMI approach at this time is mitigation of *An. stephensi* utilizing enhanced vector and disease surveillance, coordinated intervention implementation, and close monitoring**. This approach will not halt the continued expansion of *An. stephensi* in Africa, so periodic revision of this approach will occur as additional information becomes available.

The spread of *An. stephensi* may result in costs associated with increased malaria transmission by *An. stephensi* and health systems being burdened. Even modest increases in urban malaria transmission may result in the redistribution of malaria funding which might have disproportionately negative impacts on those furthest from urban centers.

Goals for approach modification

The chosen PMI approach at this time is mitigation; however, the possibility of containment or elimination should not be excluded. To determine whether containment or elimination should be pursued, the task force identified what information is needed to make a decision about alternate approaches (Annex 5). To view a summary of global studies on *An. stephensi*, teams can visit the MESA malaria page. Country teams and partners are encouraged to add activities to this page to ensure that knowledge gaps can be addressed and duplication of efforts can be minimized for broader coverage.

Timeline for periodic revision of the action plan

Regular and consistent revision is necessary to update this action plan (and possibly the suggested approach) with the latest findings and evidence-based recommendations. This will remain a living document to be updated as new information on *An. stephensi* is made available. Additionally, the Task Force will meet with the PMI Front Office quarterly, and as a group monthly to review and potentially revise the document, with special consideration for the following: coordination with relevant national and international stakeholders, new research findings, additional supporting materials and protocols to be added to the Annex, and updated detections of *An. stephensi*.

A STRATIFIED STRATEGY FOR COUNTRIES

Present	At risk	
Ethiopia (2016)	Angola	Rwanda
Nigeria* (2020)	Benin*	Madagascar
Kenya (2022)	Burkina Faso*	Senegal*
	Cameroon	Sierra Leone*
	Cote d'Ivoire*	Tanzania
	DRC	Uganda
	Ghana*	Zambia
	Guinea*	Zimbabwe
	Liberia*	
	Malawi	
	Mali*	
	Mozambique	
	Niger*	

Table 1. PMI countries in Africa by invasion scenario. For countries where *An. stephensi* is present, year of detection is denoted in parentheses.
*Countries which have potential infrastructure for urban vector surveillance through the West African *Aedes* Surveillance Network (WAASuN)

The strategy for responding to *An. stephensi* will differ by country and by status of invasion. For the purposes of this document, PMI countries are divided into two scenarios: **1.** *An. stephensi* present, **2.** At risk of *An. stephensi* invasion, which includes all other PMI partner countries. Countries with an asterisk (*) are those which have existing potential infrastructure for urban *An. stephensi* vector surveillance through the West African *Aedes* Surveillance Network (WAASuN), although the level of arboviral disease surveillance activity in these countries is highly variable. A few factors should be considered when conveying risk of invasion to NMPs and stakeholder groups including: shared borders with countries where *An. stephensi* is present, location of known detections, likelihood of introduction based on the volume and movement of goods through maritime trade between each country and countries with established *An. stephensi* populations (Ahn et al. pre-print), and likelihood of *An. stephensi* population establishment based on habitat suitability modeling (Sinka et al. 2020). These indices were combined to suggest a prioritization ranking of likelihood of both introduction and establishment in PMI countries. For more detailed information on habitat suitability rankings in specific cities within PMI partner countries, see Table S3 [here](#).

Actions to consider for all PMI partner countries

The following activities are recommended for all countries if resources are available to support them. However, given potential resource limitations these activities have been listed below in order of priority.

Suggested actions requiring <u>little or no additional resources</u>:

- Identify existing *Aedes* surveillance groups to determine whether *Anopheles* are collected in larval surveys and if so, coordinate to examine collected *Anopheles* to determine identification.

- Send DNA from mosquitoes that did not amplify during *An. gambiae* sl or *An. funestus* sl species identification PCR for molecular testing and/or sequencing. For a list of potential testing facilities in country, coordinate with your VMCT Entomology and Operational Leads and Laboratory Teamlet. Molecular testing and sequencing in country is highly encouraged and working with stakeholders to ensure that sequencing capacity is being established is critical (see Annex 1 for molecular methods). CDC Entomology Branch can provide sequencing confirmation in situations where laboratory infrastructure is still being developed, or if parallel confirmation is requested.
- Ensure entomologists are familiar with distinguishing characteristics between *An. stephensi* and other vectors like *An. gambiae* sl. (below).

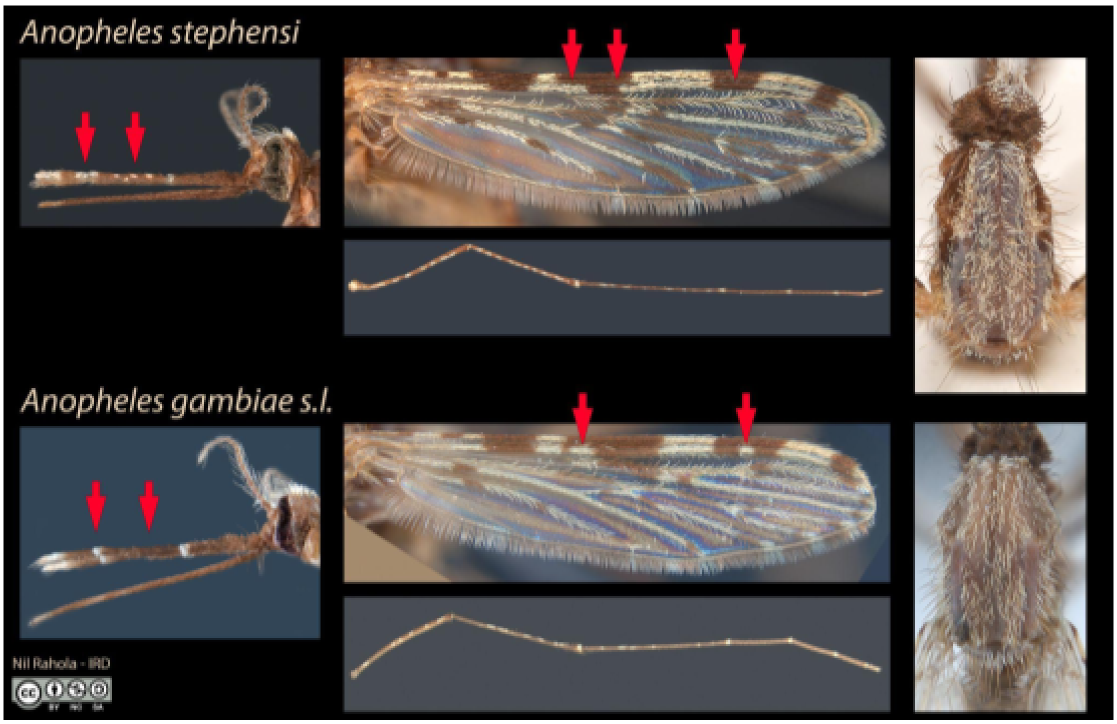


Figure 5: Morphological characteristics can be used to distinguish *An. stephensi* from *An. gambiae*. The photos above highlight these key features on the wings and palps that should be examined in suspect specimens. Credit: IRD 2022.

Suggested actions requiring <u>some additional resources</u>:

- Establish larval surveys in locations with high likelihood of *An. stephensi* establishment (see Annex 11 : country specific maps) or in dry/sea ports
  - Adult *Anopheles* collections should also be considered in these locations using Prokopack backpack aspirators around livestock dwellings. While this has shown to be effective in Ethiopia, revisions to this approach may be made as new information is made available.
- WHO reporting of *An. stephensi* through the Malaria Threat Map now includes negative sites. If larval surveys are conducted specifically for *An. stephensi* and no *An. stephensi* is found, **the GPS coordinates of these negative detections should still be reported to WHO using the reporting form (also in Annex 3). This will allow for documentation of spread into new locations.**

Urban and port larval surveillance: *Anopheles stephensi* often shares larval habitats with other mosquito vectors of public health concern, primarily *Aedes aegypti.* Therefore, identification of urban vector surveillance and control activities, specifically for *Aedes* species, should be conducted. In locations where no urban vector surveillance is conducted, it should be initiated and community-based mosquito collector programs (Annex 6) should be considered in coordination with NMPs and arboviral or other vector-borne disease programs. Pinned reference specimens of *An. stephensi* can be requested from CDC for training (see Annex 2 for taxonomic keys for morphological identification in French and English). This activity will identify programs which can be coordinated with for early detection and rapid response to *An. stephensi*, and would strengthen local and national public health entomology networks to build resilience and sustainability against urban malaria and invasive mosquito vectors.

Detection reporting: All detections of *An. stephensi* regardless of country scenario should be reported **immediately** to the Ministry of Health, WHO, and PMI simultaneously and added to the WHO malaria threat map through the reporting form (see Annex 3 for reporting information). Delayed reporting of detections would hinder opportunities for revision to the strategy and identification of revised countries at high risk of invasion and delay response, allowing for continued expansion of the vector and its impacts on malaria. Reporting immediately is public health best practice (Kolaczinski et al. 2021). Early detection and rapid response is necessary to address the threat of *An. stephensi* in Africa. To improve detection and reporting, training and education about *An. stephensi* identification and health risks should be provided to entomologists and public health officials who may work with mosquito surveillance activities.

Urban malaria monitoring: Stratification of malaria data in urban and peri-urban environments to detect unexpected increases in malaria cases is recommended. In particular, monitoring general malaria trends for unusual dry season patterns in areas with typically seasonal transmission may be beneficial. While there may be many causes for increases in urban malaria, support for strengthening national efforts to monitor urban malaria could be used to determine thresholds of concern, and under scenarios with high risk of invasion, *An. stephensi* could be considered as a potential cause. For more information on how to respond to urban malaria, in 2022, WHO released a Global framework for the response to urban malaria.

Partner coordination: To support NMPs, coordination among PMI implementing partners from different technical groups will benefit by integrating efforts for a comprehensive response if *An. stephensi* is detected. Through common implementing partners, initiating coordination and collaboration across borders with neighboring countries may also be possible using existing mechanisms.

Multisectoral coordination: In urban areas, engaging in malaria prevention activities should also involve coordination with local governments and multi-sectoral communication. Urbanization and urban expansion may create *An. stephensi* larval habitats or provide opportunities for vector expansion. Engaging with city planners and municipalities to discuss appropriate drainage and prevention of flooding can reduce urban habitats for malaria vectors including *An. stephensi.* Similarly, coordination with transportation and commerce sectors for surveillance in ports (air, sea, land cargo) that align with suitable *An. stephensi* habitats may be beneficial for early detection of *An. stephensi.* Port authorities likely already have mechanisms in place to combat the introduction of invasive species. Familiarity with these mechanisms could accelerate the process of early detection and prevention of *An. stephensi* introduction.

Coordination with WASH programs may be particularly beneficial as water storage conditions may create larval habitats for both *An. stephensi* and *Aedes* vectors and familiarity with WASH recommendations to ensure consideration for vector habitat production could be considered.

Larvicide registration and environmental compliance: PMI approves of larviciding with close monitoring for *An. stephensi* without OR/PE approval, as long as proven interventions are used and national registration of WHO PQ-listed larvicides and environmental compliance is obtained. In some situations this process may be lengthy and *An. stephensi* populations could further expand while waiting for appropriate environmental approvals. To allow for rapid implementation as soon as *An. stephensi* is detected, an assessment of what larvicide registrations and environmental compliance approvals are in place or necessary for larviciding implementation should be conducted.

Develop a national response plan: Regardless of scenario, working closely with the NMP to develop a plan to respond to *An. stephensi* is encouraged so activities can be implemented swiftly. A national task force or working group with representatives from different sectors may help facilitate the development of this plan. Coordination and collaboration across borders and with other PMI country teams is encouraged. This PMI action plan can be used to support that process and for examples of activities to consider a table has been developed (Annex 9) showing where this action plan aligns with, the WHO Global Framework for the response to malaria in urban areas, the Ethiopian national action plan to eliminate *An. stephensi*, and activities implemented by the NMP in India where *An. stephensi* is endemic.

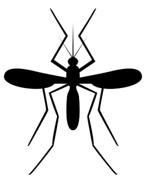
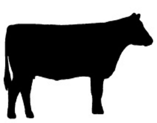
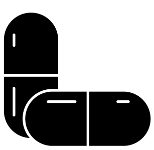
		Scenario 1: ***An. stephensi* present**	**Scenario 2:** **At risk of invasion**
	**Surveillance**	• Conduct larval surveillance as part of any malaria outbreak investigation • Entomological monitoring (larval surveys, Prokopack aspiration) in high risk sites	• Training on *An. stephensi* identification • Investigate *Anopheles* that do not amplify in species ID PCR ○ Confirm positives with sequencing • Entomological surveys in ports and high risk sites
	**Vector Control**	• Implement appropriate *An. stephensi* vector control (i.e.- LSM)	• Plan and prepare for *An. stephensi* vector control by registering insecticides needed
	**Social Behavior Change**	• Determine appropriate SBC activities using PMI SBC guidance document linked here	
	**Multisectoral Coordination**	• Liaise with veterinary, population mobility, transport, commerce, agriculture, and education partners	• Plan for discussions with veterinary, population mobility, transport, commerce, agriculture, and education partners
	**Case Management**	• Ensure sufficient malaria commodities are present in areas with *An. stephensi*	• Plan and prepare for potential shifts in commodity needs if *An. stephensi* is detected
	**Community Health**	• Community-based entomological surveillance • Community-based larviciding • Field Epidemiology Training Program	• Community-based entomological surveillance • Plan and prepare for community-based larviciding • Field Epidemiology Training Program

Figure 6: Recommended activities to pursue under different technical areas for the two scenarios described in the action plan. Specific guidance, reference, or protocol documents are available for several of these activities and are **linked** above where relevant.

SCENARIO 1: Countries where *An. stephensi* is present

Several of the countries in Africa where *An. stephensi* is already present have developed or are in the process of developing national strategies or action plans to deal with this challenge. PMI should support NMPs to develop these strategies and coordinate activities and targets. For example, Ethiopia is developing a national strategy to eliminate *An. stephensi* by 2026.

For the purposes of this document, any detection of *An. stephensi* would move a country to this scenario.

Surveillance, monitoring, and evaluation

Surveillance, monitoring, and evaluation (SME) will play a fundamental role in the development of a response to *An. stephensi*, particularly when *An. stephensi* is present in part of the country but not yet throughout the country, or when a country shares a border with one where *An. stephensi* is present. The recommended coordinated vector and disease SME approach will vary by country based on the epidemiological context and strength of the surveillance system and public health entomology capacity. Routine monitoring and analysis of malaria incidence is a core component of PMI efforts across settings and it is also a central component of early detection of potential *An. stephensi* in a given area. Unlike many other malaria vectors, *An. stephensi* abundance does not seem to follow seasonal rainfall patterns, and may thrive during dry periods may thrive due to use of water storage containers and wells as larval habitats. Closely monitoring malaria during "dry" periods may be beneficial as identification of less seasonal malaria transmission patterns and / or increases in urban malaria may indicate the presence of the *An. stephensi* vector. It may be challenging to use the available routine data to detect real-time changes in malaria due the cadence of case reporting (i.e., monthly) or issues of data quality, but these HMIS data can still provide important signals that additional investigation, including entomological surveillance, is warranted.

Identification of these anomalous trends in malaria cases as defined by existing country protocols (e.g. based on cut-offs used for epidemic or outbreak detection) should be followed up by an investigation that includes complementary entomological analysis or data collection. Such an investigation triggered by HMIS data may need to include larval surveillance (see Annex 4 for protocol and data collection form) if other factors are not clearly identifiable as the cause of rising malaria cases. To determine the extent of *An. stephensi* distribution, both positive and negative sites and corresponding GPS coordinates should be recorded for *An. stephensi*. This investigation can utilize existing PMI tools for investigating anomalous trends in malaria transmission which have been utilized in Guinea and Mozambique. Potential resources for assisting NMPs with investigations include SME implementing partners (PMI Measure Malaria or other bilateral partners) and in-country training programs such as Field Epidemiology Training Program (FETP) fellows.

Mobility of humans, animals, and goods

Population genetics of *An. stephensi* in Ethiopia have shown potential associations with livestock trade routes. Geospatial mapping of the movement of humans, goods, and livestock could be used to determine critical points of potential introduction of *An. stephensi* which could be targeted for surveillance and control. Mapping human movement patterns may also be beneficial for malaria elimination strategies. Rapidly deployable toolkits, such as the population connectivity across borders (PopCAB) mixed methods approach have been used in outbreak responses to map the movement of human populations for spatially targeted surveillance and control interventions (Annex 8). This toolkit has also been used in Uganda to characterize patterns of animal mobility through multi-sectoral community engagement. Countries with *An. stephensi* detections may consider geospatial mapping exercises to identify critical pinch points of mobility, where human/animal/goods movement is constrained and highly connected and where focal surveillance and targeted interventions are likely to have high impact.

Vector control

Vector control will play a major role in the mitigation of invasive *An. stephensi*. However, particularly in urban settings, vector control may need to adapt to the existing conditions. Urban populations may not be used to using ITNs and the acceptability of IRS may be too low for impact.

The model that evaluated the possible impact of different vector control interventions in Ethiopia found that PBO nets plus LSM would have the biggest impact in areas of low malaria prevalence (<0.1%) ([Hamlet et al., 2022](#)). However, neither of these interventions on their own would be sufficient to reduce malaria levels to pre-*An. stephensi* levels. When used in combination, PBO nets plus LSM had the lowest cost per case averted. At higher malaria prevalence levels (12%), PBO nets alone were estimated

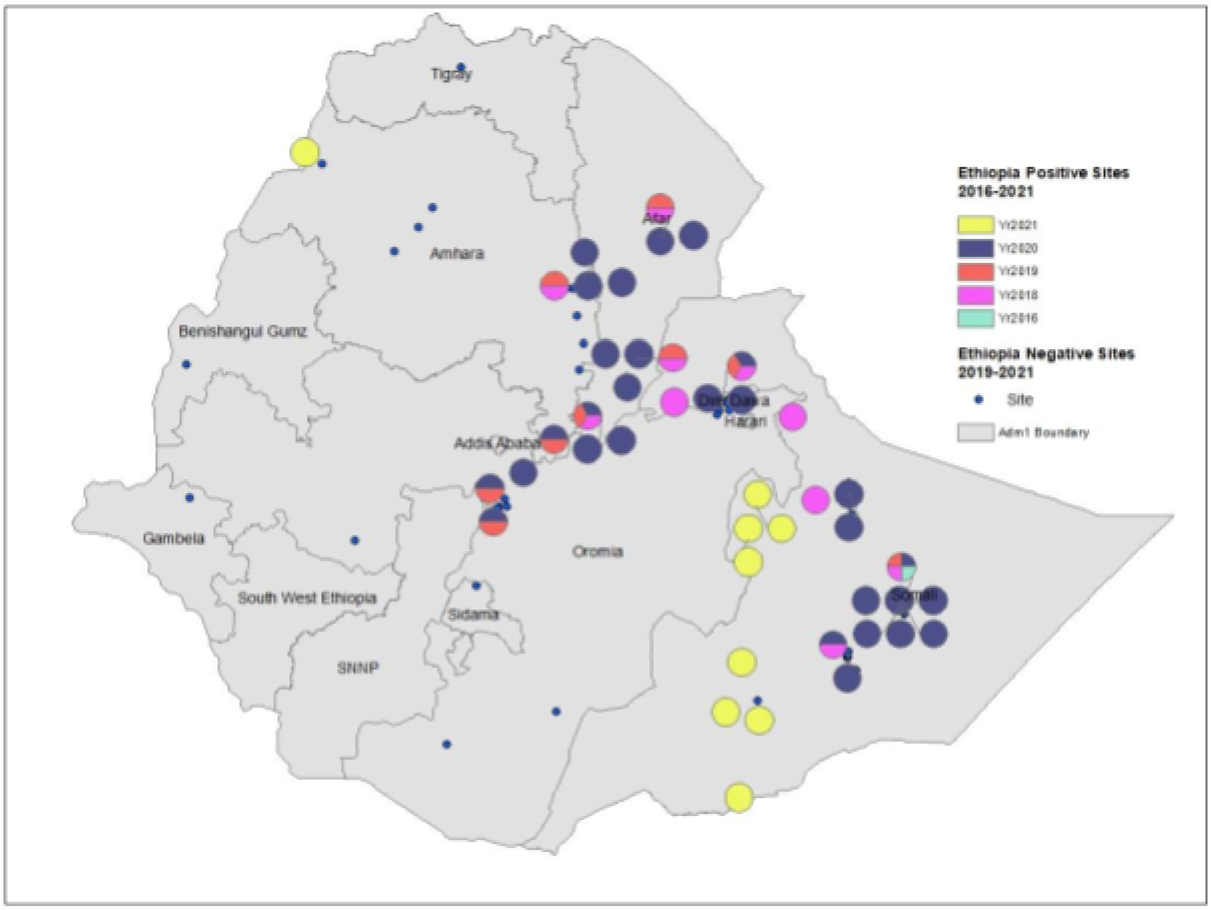


Figure 6: Locations where *An. stephensi* has been detected in Ethiopia from 2016-2021. In countries where *An. stephensi* has been detected, entomological surveillance can be used to identify the extent of distribution. Positive and negative sites should be documented and negative sites should be revisited to determine how *An. stephensi* is spreading and vector control should be implemented in locations where populations are established.

to be sufficient to bring incidence to pre-*An. stephensi* levels. However, it is important to note that the modeling exercise did not use actual data on how *An. stephensi* respond to these particular vector control interventions, highlighting the need for research to generate empirical data that would make the models much more accurate.

While PMI has experience in distributing ITNs and conducting IRS, LSM has not yet been conducted with PMI funding. Generally, the WHO recommends LSM in areas where larval sites are few, fixed, and findable (WHO, 2021). While there are no quantitative criteria for these descriptors, there have been successes in reducing malaria cases through community based urban LSM in Tanzania (Maheu-Giroux and Castro, 2013) and urban LSM in Cameroon (Antonio-Nkondjio et al., 2021). For *An. stephensi* there have also been successes in urban LSM in India (see above). PMI has authorized the use of LSM for control of *An. stephensi* and intends to gain experience in the best practices and impact of this intervention through direct implementation and monitoring in Ethiopia, although program evaluation/operational research activities should be planned as well. An estimated budget for larviciding in Ethiopia is provided in Annex 12.

In countries where *An. stephensi* is established, vector control through new ITNs and LSM can be conducted either as a responsive measure after detection of malaria in urban areas or as a preventative measure if deemed necessary and if funding allows. The recommendation of PBO (and new types of nets) nets and LSM is provisional, and countries may wish to pilot alternative methods of vector control including water storage coverings (see Knowledge Needs below). These recommendations will also be updated as further understanding of vector control of *An. stephensi* is generated. Insecticide resistance monitoring should remain rigorous with spatiotemporal considerations and *An. stephensi* adults and larvae from all sites where populations are detected should be tested to determine susceptibility to different insecticide classes. Country teams should work with implementing partners to determine the budget implications of this activity.

Once detection of *An. stephensi* is confirmed in a country, expanding vector surveillance along all major commerce and livestock transport routes, particularly those within suitable *An. stephensi* habitats (Sinka et al. 2020, and Annex 11) reaching borders with neighboring countries is recommended. Rather than conducting surveillance and then returning at a later time to positive sites to implement LSM, it is recommended that LSM occur **in parallel** with *An. stephensi* surveillance such that larvicide is applied immediately upon detection to prevent further expansion. The duration needed for LSM is unclear at this time, but countries should use this intervention as long as *An. stephensi* is being detected.

Social and behavior change

Social and behavior change (SBC) is an important component in guiding the development of a response to *An. stephensi* and in ensuring that control measures are acceptable to and adopted by the community. Particularly in urban settings with a low burden of malaria, populations may not be used to vector control interventions such as ITNs, IRS, or LSM or with the notion of rapidly seeking care for fevers. ITN use may require sensitization to ensure that people not only have access to nets, but that they are using them. IRS may require sensitization to ensure that people understand the purpose, risks, and benefits of spraying. In urban areas, IRS and ITN use and refusals may differ from these interventions in rural regions, SBC activities may be used to reinforce uptake of these critical vector control interventions and appropriate utilization of health services in urban areas.

Social and behavior change activities

Once a response to *An. stephensi* has been identified, SBC activities should be incorporated within that strategy to promote the interventions and associated individual, household, and community level behaviors that support the uptake and maintenance of interventions to combat invasive *An. stephensi*. Cross-cutting considerations for SBC strategies to address malaria transmitted by *An. stephensi* include:

1. Ensure target behaviors are feasible in terms of time, skills, and resources
2. Ensure SBC approaches meet the needs of target population(s) by considering
    a. Levels of literacy
    b. Local languages and cultural appropriateness
    c. Appropriate channels, including informal communication channels, to reach higher risk and mobile populations, e.g., construction workers, miners, agricultural and daily or seasonal workers or those who frequently move between rural to peri-urban and urban areas. Contextually relevant higher risk groups should be identified in the target area and channels tailored accordingly.
3. Identify and engage contextually relevant community leaders and community-based civil society organizations early in the process. This may include spiritual leaders, faith-based organizations, trade unions, rotary groups, scout groups, and others
4. Promote multi inter-sectoral collaborations such as municipal, transportation/commerce, education, and employer-based programs to increase engagement and promote target behaviors
5. Promote collaborations across malaria partners for a comprehensive malaria response (e.g., service delivery, SBC, vector control)
6. Tailor SBC activities and message framing to communicate in accordance with the level of *An. stephensi* risk

A prioritization activity was led by SBC implementing partners, in collaboration with the PMI SBC and VMCT, to develop a living SBC guidance document for *An. stephensi.* This guidance is available here. As a program defines their response to *An. stephensi,* and identifies specific interventions of interest, this document can be used to provide considerations for implementing the SBC aspects of those interventions to maximize effectiveness. Ideally, the information contained in this guidance can be incorporated into SBC aspects of malaria strategies and ongoing program activities. This guidance should be paired with local data to ensure SBC is tailored effectively.

Multisectoral coordination – One Health

The possibility of the integration of "One Health" measures is important for *An. stephensi* control, with particular emphasis on its capacity to thrive in urban settings and its preference for animal feeding.

Many countries have developed One Health strategies or mechanisms to facilitate multisectoral coordination with the Ministry of Transportation, Ministry of Education, Ministry of Environment, National Veterinary Services, Ministry of Agriculture and other governmental and non-governmental groups for collective action. Coordinating with these groups and using existing mechanisms for multisectoral coordination to address *An. stephensi* and urban malaria may fit in well with existing infrastructure.

Ethiopian *An. stephensi* often feed on animals (particularly goats and cattle) where they are present, and in the absence of animals, *An. stephensi* readily feeds on humans (Balkew et al. 2021). Estimates of human to livestock ratios, densities, and proximities may be beneficial in interpreting feeding plasticity and transmission dynamics.

Endectocides such as ivermectin are often used for the control of ecto- and endo-parasites and recent literature has shown increased mortality in some mosquito species which feed on blood from endectocide-treated animals. Laboratory evidence has shown increased mortality in *An. stephensi* (85%) fed on blood from endectocide treated animals (Dreyer et al. 2018). Therefore, the use of insecticides or endectocides on livestock may result in control of *An. stephensi* and other arthropods (flies, ticks, etc), resulting in higher milk or meat yields. However, it should be noted that some of the endectocides have withdrawal periods that must be respected before slaughter.

Regulatory needs

There may be important regulatory requirements for countries to use certain insecticides that are not yet registered in country and these should be identified as soon as possible. If insecticide-based activities are to be USAID-funded, they will need to be 22CFR216 compliant with the proper environmental compliance documents in place prior to any implementation.

Regulations for the covering of water storage containers may also be considered as this has been implemented in other countries where *An. stephensi* is endemic. Removal of standing water from construction sites has also been implemented in Singapore to keep construction sites free of mosquito larvae and could be considered.

Case management

An increase in urban malaria driven by *An. stephensi* would have important implications for malaria case management. Malaria case management has frequently been shown to be poorer in areas with lower malaria prevalence for a variety of reasons, including lack of malaria commodities and provider knowledge and practices such as failure to test all fevers for malaria (especially in the private sector which is more common in urban areas). Robust surveillance is required to detect increases in malaria transmission that in turn should be responded to with increased supplies of malaria case management commodities. Additionally, training and supervision of community and facility providers may be required to reinforce malaria case management capabilities in urban areas or areas that previously had lower malaria transmission. In addition, *An. stephensi* presence often overlaps with *P. vivax* endemicity which requires programs to incorporate the prescription of and patient adherence to primaquine radical cure.

Drug-based interventions

There is very little data on the potential impact of drug-based interventions for prevention of malaria derived from *An. stephensi.* There are ongoing studies on the effect of ivermectin for human malaria prevention and the drug has previously been shown to kill *An. stephensi*, but there are not currently sufficient data for its deployment.

Community health and engagement

Community based larval surveillance and control may be used as a cost-effective strategy for sustainable and resilient *An. stephensi* control. Materials including larval surveillance protocols for training and data collection sheets have been developed and implemented in Ethiopia with success (see Annex 7 for protocol and data collection form). This model may also be leveraged for community-based larvicide implementation. Additionally, an LSM guidance document is being developed by PMI VectorLink and will be made available once complete. In countries where *An. stephensi* has been detected, this approach can be used to expand and strengthen entomological surveillance networks.

In urban areas where community health workers may use smartphones, additional tools may be used to enhance *An. stephensi* surveillance. For example, the NASA Globe Observer app Mosquito Habitat Mapper tool, which was developed for community-based geolocation and mitigation of *Aedes* larval habitats could be used for *An. stephensi* larval habitat identification for targeted larviciding in real-time. While additional research is necessary, this tool has been used in three urban areas in Senegal to map mosquito larval habitats (Low et al. 2021).

Countries where *An. stephensi* is established could act as regional training hubs by reserving a small number of spaces in existing entomological training sessions (surveillance, laboratory methods, control activities) for entomologists from countries with immediate threat of *An. stephensi* introduction. This cross-border coordination and collaboration could increase regional preparedness for response.

To further bridge local capacity for epidemiological and entomological surveillance, the CDC FETP can be engaged and entomologists recruited for the program. The FETP trains field epidemiologists to detect and respond to public health threats, expanding the global health workforce and enhancing public health capacity in coordination with Ministries of Health. In Ethiopia, *An. stephensi* training modules have been included in the malaria FETP track and a subset of residents are working on *An. stephensi* research projects. PMI countries where *An. stephensi* has been detected can engage with FETP to address critical surveillance, research, and implementation needs.

SCENARIO 2: Countries at risk of *An. stephensi* invasion

Currently, Ethiopia, Nigeria, and Kenya are the PMI partner countries with confirmed presence of *An. stephensi*. Neighboring countries, those with high influx of trade traffic through major ports, and those with suitable habitats for population establishment should be considered high risk for *An. stephensi* invasion (Sinka et al. 2020). Specifically, using data available at this time from the three criteria above, all PMI partner countries may be considered at risk for the introduction and establishment of this vector. All countries in Africa are considered at risk of invasion and are encouraged to perform the activities described below and mobilize *An. stephensi* efforts.

With initial detections of *An. stephensi* in ports and along major transportation routes, countries at high risk of *An. stephensi* invasion should identify major cargo ports (air, sea, land), in particular those found within habitats identified as suitable for *An. stephensi* population establishment based on previous modeling work (Sinka et al. 2020). While the habitat suitability model is not without uncertainty and should be considered with caution, detailed habitat suitability maps for the high risk countries listed above can be found in Annex 11 to guide surveillance and monitoring strategies based on likely species distribution

Surveillance, monitoring, and evaluation

In the Horn of Africa where *An. stephensi* populations are well established, there are not always clear increases in urban malaria, or these increases have taken several years to appear. For example, in Djibouti although *An. stephensi* was detected in 2012 and there was an upward trend in malaria cases, significant increases were not seen until 2018. In Ethiopia, *An. stephensi* was first detected in 2016, but significant increases in urban malaria have not yet been reported. However, *An. stephensi* adults have been captured harboring infective sporozoites for both *P. falciparum* and *P. vivax* in Ethiopia. In high risk countries, close monitoring of malaria cases in urban and peri-urban environments, particularly those with major transport hubs and sea or land cargo ports, is recommended to monitor the potential impact of *An. stephensi*. It is suggested that countries continue to monitor malaria trends, and when an unexpected increase in cases is detected (however this may be defined in country), *An. stephensi* should be considered as a potential cause and larval surveillance for *An. stephensi* should be conducted as follow-up. Epidemiological/entomological investigations have been used by NMPs in Guinea (Camara et al. 2018, Sayre et al. 2021) and Mozambique to investigate anomalous trends in malaria cases and these protocols and data collection instruments are available for adaptation in other settings. Please contact your PMI entomological backstop in order to access these materials and discuss how they might be adapted to your setting.

Anopheles stephensi may also persist during dry periods when other malaria vectors do not. Monitoring malaria case data for increases in urban areas during dry periods is also recommended in high risk countries. Different approaches in high and low burden settings may be necessary as the impact of *An. stephensi* may not be as apparent in high burden settings. Monitoring health center data in urban and peri-urban areas with low malaria burden may reveal increases in malaria due to *An. stephensi*; whereas a combination of epidemiological and larval surveillance may be necessary in high malaria burden settings to identify impacts of *An. stephensi*.

In high risk countries, identifying existing biological surveillance strategies along borders and in ports may provide opportunities to understand how *An. stephensi* could enter the country or entry could be prevented.

Vector control

Coordination with transportation and commerce sectors to identify major ports and points of entry along borders can be used to establish *An. stephensi* sentinel surveillance sites where larval surveillance should occur for detection of *An. stephensi.* Upon detection, immediate implementation of approved larvicides should occur and the types and volume of goods and materials entering positive ports should be documented to determine the goods *An. stephensi* could potentially move through.

PMI should support NMPs to include *An. stephensi* surveillance and morphological identification of *An. stephensi* in malaria vector training programs. Specifically, adult *An. stephensi* may be misidentified morphologically as *An. gambiae* s.l. if the correct taxonomic key is not used. The updated key (Coetzee et al. 2020), is available in French and English (Annex 2), includes *An. stephensi* morphology and should be used for morphological identification to ensure suspect *An. stephensi* are detected. Pinned specimens of *An. stephensi* have been prepared at CDC and can be requested by countries as reference material for training.

When possible and if resources allow, entomologists from high risk countries should try to participate in *An. stephensi* larval surveillance, identification, and control trainings in countries with established populations (endemic or invasive) to become familiar with the vector and methods used.

As part of urban vector surveillance landscaping activity noted above for all countries, if existing *Aedes* or other container breeding mosquito surveillance programs are identified, high risk countries may consider offering *An. stephensi* morphological identification training activities to these programs to expand the network of public health entomologists on alert for *An. stephensi* detection. In addition, regular communication between the NMP and these programs to establish a rhythm for real-time alerts of suspect findings is recommended. The following countries at high risk of *An. stephensi* invasion are also a part of the WAASuN: Benin, Cote d'Ivoire, Ghana, Senegal (see Table 1 and Annex 10 for contact information for these groups). If any *Anopheles* spp. larvae are found in *Ae. aegypti* habitats, larvae should be reared and stored for confirmation. Any confirmed *An. stephensi* detection should be responded to with larvicide implementation at the site of detection.

Social and behavior change

In areas at elevated risk of invasion, it is important to promote and increase core malaria-related behaviors, including prompt care seeking for fever to identify potential spikes in cases. Areas at risk of invasion should also be informed about *An. stephensi* identified in neighboring countries and the associated impacts of the invasion on malaria transmission to highlight the need to engage in malaria-related behaviors. A country's response to the threat of *An. stephensi* will vary based on local context as there is not one set of guidance that is representative of all contexts.

Building off of the SBC prioritization activity, certain behaviors may be likely to reduce *An. stephensi* populations. These behaviors may need to be clearly defined and sufficient detail provided to perform these behaviors effectively. As such, if resources allow, high risk countries should compile data on relevant behaviors (i.e., information on water storage behaviors and conditions, proximity to livestock) that may be needed to implement prioritized SBC behaviors if *An. stephensi* is detected.

There may be distinctions in human behaviors performed to avoid mosquitoes during the rainy and dry seasons. These human behaviors should be identified to be able to promote mosquito prevention behaviors during the dry season.

Multisectoral coordination – One Health

Anopheles stephensi is often found to be associated with livestock through feeding or resting in animal shelters and one of the initial detections of *An. stephensi* in Africa was found in a livestock quarantine station near a seaport. Countries at high risk of *An. stephensi* invasion should therefore consider animal-associated routes of introduction, transport corridors, and housing structures for the early detection of *An. stephensi*. High risk countries with livestock importation or exportation should consider coordinating with National Veterinary Services programs for *An. stephensi* surveillance in livestock quarantine stations (through backpack aspiration of resting adults and larval surveillance in surrounding areas). Similarly, coordinating with transport and port authorities to determine existing processes to minimize the introduction of livestock- associated invasive species could be leveraged for *An. stephensi*. If resources allow, animal shelters or structures found in urban areas and/or suitable habitats for *An. stephensi* establishment should be sampled for resting *An. stephensi*.

As in the scenario in countries with *An. stephensi* detections, the use of rapidly deployable geospatial mapping toolkits, such PopCAB (Annex 8) may also be beneficial in countries at high risk to identify major transport routes for humans, animals, and goods in country and along borders. If borders are shared with countries with known detections, these transport routes may be used to identify critical locations for entomological surveillance for early detection of *An. stephensi.* If *An. stephensi* is traveling with livestock or along livestock trade corridors, this multi-sectoral activity could be used to identify locations that would otherwise be missed along major cargo or human transport routes.

Regulatory needs

While all countries should identify insecticide registration status for WHO PQ-listed larvicides which may be used if *An. stephensi* is detected, high risk countries should identify what action may be needed to obtain the approvals necessary for control and environmental compliance documentation as soon as possible to expedite control if *An. stephensi* is detected. Rapid response following detection is necessary to limit further expansion of the species.

An assessment should be conducted of existing regulations for covering of water storage containers, mitigating larval habitats, and removing standing water from construction sites in urban areas. If no such regulations exist, coordination with local governments and WASH programs may be beneficial to determine strategies that may be beneficial for improving urban health.

Drug-based interventions

There are no evidence-based drug or vaccine interventions specific to the *An. stephensi* response at this time point.

Community health and engagement

In high risk countries, as in countries with known *An. stephensi* detections, community engagement to establish and strengthen public health entomology networks may provide beneficial opportunities to improve detection for *An. stephensi* if it is introduced while simultaneously establishing resilient local entomological surveillance capacity.

Several PMI countries have implemented community mosquito collector programs to collect community-level data for cost-effective, resilient, and high-resolution vector monitoring and control decision making. High risk countries with existing community entomology programs can leverage this existing infrastructure by adding *An. stephensi* training modules. High risk countries without existing community mosquito collector programs can initiate these programs using existing course and training materials (Annex 6).

Activities to prioritize

While all of the activities described above should be considered for *An. stephensi* early detection and rapid response in high risk countries, if resources are limited the following activities should be prioritized:

- Close monitoring of malaria cases in urban and peri-urban areas and larval vector surveillance for *An. stephensi* in response to any increase. Country specific habitat suitability maps in Annex 11 can be used to identify high risk locations and guide efforts.
- Rapid molecular follow up of suspect *An. stephensi* collected during routine surveillance
- Register insecticides needed for *An. stephensi* vector control for rapid implementation alongside detection.
- Establish or expand community based entomological surveillance and control capacity.

Knowledge needs

Despite heightened surveillance and active research in Ethiopia and neighboring countries on invasive *An. stephensi* , major gaps in knowledge remain regarding the vector and its impact on malaria in this context. To make the most informed decisions about which activities to pursue for the mitigation, containment, or elimination of *An. stephensi* and its impacts on malaria, it is essential to address the unknowns through surveillance, monitoring and evaluation, and research. To accelerate the timeline to address this global challenge to malaria, PMI recommends that this knowledge generation be conducted in parallel with the surveillance and mitigation activities PMI is actively implementing. A list of ongoing *An. stephensi* research activities globally can be found through MESA malaria and activities supported by PMI should be added to this list to ensure global coordination to address unknowns. The outcomes of this evidence will improve decisions about which interventions in the existing toolkits can be used and where heightened surveillance and innovative approaches should be applied. If resources are constrained, surveillance activities to define the extent of the invasion in Africa should be prioritized as described above.

Surveillance

PMI concluded that mitigation with enhanced surveillance, coordinated intervention implementation, and close monitoring at minimum would be the most feasible approach to *An. stephensi* in PMI partner countries because the extent of the distribution of *An. stephensi* in Africa remains unknown, and standard adult entomological collection methods do not work as well for this species as for other malaria vectors. This means that information on specific biting behaviors and biting times of adult *An. stephensi* are not known. For other malaria vectors, data on biting and resting behaviors is used to inform vector control decision making. PMI has identified the need for surveillance on: the distribution and population genetics of *An. stephensi*, identification of ideal adult *An. stephensi* collection tools, identification of when and where *An. stephensi* is biting in relation to human behaviors and animal proximity, understanding the impact of seasonality on *An. stephensi* abundance and transmission of *Plasmodium* spp., in countries with confirmed *An. stephensi*, what environmental differences exist between areas with and without *An. stephensi,* and understanding how *An. stephensi* presence across a rural/urban gradient impacts malaria cases. Since *An. stephensi* is also known to feed on animals, using blood meal and sporozoite analysis could address questions about *Plasmodium* transmission risk in livestock- versus human-dense settings and zooprophylaxis. Further investigation of the species and larval habitats being occupied in the Arabian Peninsula, especially Yemen, Oman, and Saudi Arabia, will help to identify the extent of the distribution of the species beyond Africa for additional coordination.

Control and elimination

Due to the unique ecology of *An. stephensi* when compared to other malaria vectors in Africa, the control tools are also different. There are several essential research questions that should be addressed before pursuing widespread intervention strategies. Some of these research questions include: What impact does larval source management (LSM), including larviciding, have on *An. stephensi* in urban areas? Can *An. stephensi* be eliminated from isolated rural locations once it is detected as a pilot elimination strategy? With data from Ethiopia showing that resting *An. stephensi* are often found in livestock shelters, the impact of IRS, which targets resting mosquitoes, in livestock shelters should be explored as a control tool for *An. stephensi*.

Global vector control response

Anopheles stephensi has been found to share larval habitats with *Aedes* mosquito vectors responsible for transmitting dengue, chikungunya, Zika, and yellow fever viruses. Evaluating how the control of these arbovirus vectors can simultaneously control *An. stephensi* and vice versa is needed. In alignment with the WHO global vector control response (GVCR) strategy (WHO, 2017) to strengthen vector control capacity and surveillance worldwide, integrated vector approaches can be a cost-effective approach to improve overall health. Without coordination with *Aedes* programs for *An. stephensi* surveillance and control there is great potential for duplication of effort and non-optimal use of limited resources. Modeling the costs of coordinated IVM for arboviruses and malaria would be beneficial for decision making.

Control innovations

Several tools exist or have been developed for mosquito control which are outside of those typically used by PMI, but would be worth exploring for *An. stephensi.* These include: sterile insect technique and the development and release of genetically modified *An. stephensi* for population suppression, identification of wild *Wolbachia* strains and potential for use in *An. stephensi* control, ivermectin treatment in livestock to control *An. stephensi* populations and what dosages are needed for livestock and residual efficacy. Many of these tools are still under development and a strong caveat should be added that the epidemiological value of these interventions remains unknown.

Prevention

Identifying the sources of spread of *An. stephensi,* particularly if it is being transported along trade routes, is essential to limiting further spread and continuous reintroductions. There are many knowledge needs in this space, including examining *An. stephensi* population genetics from both its native and invasive range in Asia, Africa, and the Arabian Peninsula to identify patterns of movement and population connectivity, identifying key ports (sea ports for marine cargo, dry land ports, and air ports) in coordination with port authorities determining how to prevent entry and re-entry of *An. stephensi* into port cities. Biosurveillance in ports and cargo shipments may be necessary to identify specific goods associated with *An. stephensi* spread, and this could also be leveraged by other global programs for the detection of future invasive mosquito vectors of public health concern.

Modeling needs

To date, modeling exercises have provided a wealth of information on cost to control *An. stephensi* and predicted patterns of invasion. Additional revised models are needed that use data from Ethiopia and other countries where invasive *An. stephensi* is now established. With new detections in rural locations in Ethiopia, revised ecological niche modeling could be conducted. Improved models will better allow us to understand the potential impacts elsewhere, and guide preparedness activities in countries at high risk of *An. stephensi* introduction.

USG and global coordination

For the PMI action plan to align with organizational and domestic and international initiatives across all settings, there is high level advocacy on the topic of *An. stephensi* as a biosecurity and global health threat. As an interagency USG organization, PMI is working towards a USG coordinated response to *An. stephensi* and the PMI *An. stephensi* Task Force is expanding to include technical experts from USG agencies beyond USAID and CDC. Coordination with other USG agencies and partners will contribute to surveillance and the research agenda by addressing knowledge and response needs that are outside of PMI's scope. For example, U.S. naval bases in high risk countries may be an efficient way to enhance *An. stephensi* surveillance for early detection and rapid response. PMI is also currently coordinating with global stakeholders including WHO, PAMCA, and the RBM Partnership to End Malaria. Additional engagement and coordination with African Leaders Malaria Association (ALMA), Global Fund (GF), International Atomic Energy Agency (IAEA) and other global counterparts will continue to highlight *An. stephensi* and its threat to global malaria control and elimination.

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ANNEX

Annex 1. Molecular confirmation of suspect *An. stephensi*

Suspected *An. stephensi* specimens should be confirmed molecularly and Sanger sequencing confirmation is needed for a detection to be reported to WHO. There are several PCR-based several approaches which may be used to identify *An. stephensi*; however, at this time only Sanger sequencing confirming the species can be used to report the presence of *An. stephensi* to WHO.

A summary of the molecular methods that can be used to confirm An. stephensi are described in this manuscript by Singh et al. 2023. They also describe for the first time a method which can be used to identify the presence of An. stephensi in a pool of ~100-500 suspect specimens.

Annex 2. *An. stephensi* morphological identification keys in French and English

Adult *An. stephensi* (collected as adults or reared from collected larvae) may be misidentified morphologically as *An. gambiae* s.l. if the correct taxonomic key is not used. The updated Coetzee 2020 key, which includes *An. stephensi*, should be used for morphological identification to ensure any suspected *An. stephensi* are appropriately identified. The French and English versions of the key can be downloaded from the included hyperlinks. Pinned specimens of *An. stephensi* have been prepared at CDC and can be requested by countries for use in morphological identification through Entomology Leads.

Annex 3. WHO form to report *An. stephensi* positive and negative detection sites

If *An. stephensi* is detected in a new location, the information should be immediately shared with the Ministry of Health, WHO, and PMI simultaneously and reported to the WHO Malaria Threats Map using the standard reporting form which should be emailed to vectorsurveillance@who.int. However, even if *An. stephensi* is **not detected**, but surveillance was conducted, surveillance sites should be submitted to WHO to be added to the map to allow for tracking of *An. stephensi* spread. Any questions regarding reporting can also be directed to this email address. More information on the urgency of immediate reporting and potential negative impacts of not reporting can be found in Kolaczinski et al. 2021. The reporting form can also be used to submit negative detections as well.

Annex 4. Larval and pupal surveillance protocol SOP and data entry form

Protocols were developed for *An. stephensi* larval and pupal surveillance and data entry forms were developed to include the quantification of *Aedes* species as well. In countries without established *Aedes* programs like Ethiopia, these complementary data can easily be collected to support urban health strategies. Entomological equipment needed for adult and larval *An. stephensi* surveillance is listed below:

- Dippers
- Beakers
- Larval pans
- Bug Dorm 1 Rearing Cage, White
- Aspirator, WHO (World Health Organization)
- CDC light traps

- Prokopack backpack aspirators
- Petri dishes
- Forceps: fine point, straight tip, stainless steel
- Stereomicroscopes

Annex 5. Information needed to determine feasibility of containment and/or elimination

What information is needed by the end of 2023 to determine whether containment is possible?

- Extent of spatial distribution
  - Larval surveillance in urban areas designated as high risk of *An. stephensi* establishment according to habitat suitability models (Sinka et al. 2020) to determine the extent of *An. stephensi* detection
  - Updated species distribution and habitat suitability modeling using revised detection data for more accurate targeted surveillance and control
- Extent of duration on the continent
  - Desk review of existing entomological collections of *An. gambiae* s.l. at present and prior to 2012 and molecular confirmation of specimens in collections
- Mode of introduction and reintroduction
  - Identification of routes of entry and characterization of cargo imports and exports in high risk countries to determine potential movement routes and whether biosecurity measures limiting introductions are possible
- Vector control tools for containment feasibility
  - Desk review/meta-analysis of vector control tools (including genetically modified mosquitoes) for *An. stephensi* to determine best practices. Specific review of attempts/successes in *An. stephensi* containment/elimination (specifically on island settings)
  - Pilot elimination study- In an isolated setting where invasive *An. stephensi* has established in Ethiopia, is it possible to eliminate *An. stephensi* using existing vector control tools?

What information is needed to determine whether elimination is possible?

All of the following conditions for containment must be met in addition to:
- Rate of reintroduction
  - Through population genetics estimate number of introductions in known range
- Introduction prevention
  - Desk review of biosecurity strategies to limit invasive species introductions

Annex 6. Community-based Entomological Surveillance Curriculum for Mosquito Collectors

Building in-country entomological monitoring capacity is possible through community mosquito collector programs which could be leveraged for later *An. stephensi* surveillance capacity. These programs may exist in entomological monitoring sites or be implemented in ad hoc sites. PMI has implemented community entomological training in several countries and full course materials have been developed by PMI Vector Link and can be accessed here.
Some of the materials used for training community-based mosquito collectors can also be viewed below.

1. Facilitator Guide
2. Proposed Agenda
3. Participant Workbook
4. Day 1 Slide Deck
5. Day 2 Slide Deck
6. Workshop Review Slide Deck
7. End-of-Course Feedback Form
8. Community Mosquito Collectors Pre-Test
9. Community Mosquito Collectors Post-Test
10. Community Mosquito Collectors Pre-/Post-Test Answer Key
11. Certificate of Completion

Annex 7. Community-based larval surveillance SOP and data collection form

In Ethiopia, the existing Community Mosquito Collector (CMC) program was leveraged for *An. stephensi* larval surveillance. The protocol and data collection form used to train community larval surveillance collectors can be found here.

Annex 8. Population Connectivity Across Borders (PopCAB) Toolkit for mapping mobility of humans, animals, goods for targeted surveillance and control

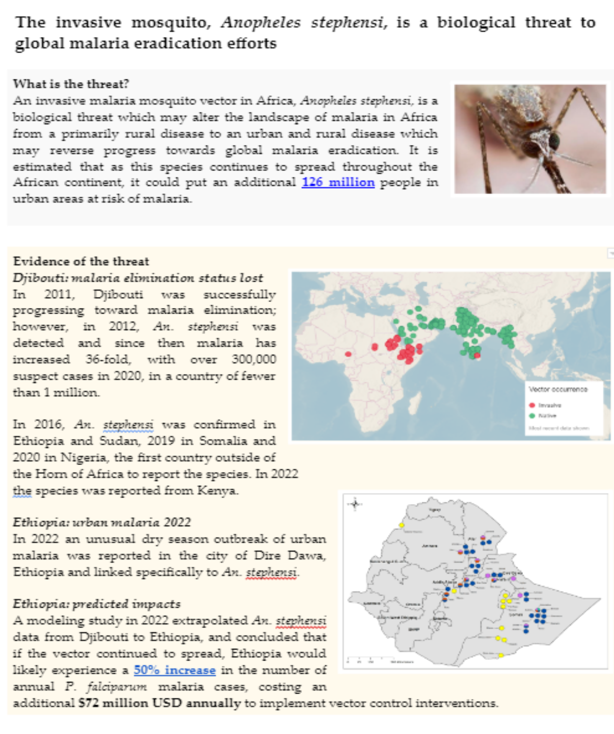
It is unclear how *An. stephensi* is being transported and introduced into new locations and what goods it may be transported with. Historically, other container breeding mosquitoes have been found to be transported through the used tire trade, shipments of ornamental bamboo, etc. Identifying major transport routes and the movement of goods and livestock can be used to identify critical transport pinch points (key points of entry) which could be used for targeted *An. stephensi* surveillance and control. A standardized mixed methods toolkit called PopCAB has been developed for deployment during outbreak responses to rapidly map and identify critical pinch points of human and animal mobility and these methods can also be adapted to map the movement of livestock and goods. This method is currently being implemented by FETP residents in Ethiopia and toolkit materials can be found here: Population Connectivity Across Borders (POPCAB) Toolkit; COVID-19 Preparedness and Response (Print-Only) (cdc.gov)

Annex 9. Developing a national action plan: lessons learned from existing documents

If NMPs are interested in developing a national action plan to respond to *An. stephensi* or prepare a response, many lessons learned can be gleaned from existing documents. To support the development of such documents, below is a table which highlights specific activities that may be considered. These are key activities where this PMI action plan, the National Ethiopian action plan, the WHO urban malaria framework align, and a number of these activities were implemented by the NMP in India, where the species is endemic and response to *An. stephensi* has been implemented for nearly a century.

Activity type	PMI action plan	WHO urban malaria framework	Ethiopia action plan
Integrated vector surveillance and in alignment with WHO Global Vector Control Response (GVCR) Integration of population mobility and trade/commerce hubs into surveillance and control activities Enhanced vector and disease surveillance for control planning Close epidemiological and entomological coordination to respond to increase in cases and/or detection of vector Multisectoral coordination/One Health approach Water storage strategies and regulations Community based surveillance and control systems and reinforcing SBC activities Cross-border coordination and collaboration	•Engaging in GVCR preparedness with integrated vector surveillance and control •Mapping movement of humans, animals, goods for targeted surveillance and control •Surveillance in ports and major transport hubs •Prioritization of surveillance to delineate extent of distribution •Larval surveillance where increases in malaria have been detected •Multisectoral coordination with different levels of government and communities/One Health •Implementing partner communication and coordination •Encouraging regulation strategies for water storage practices •Building, leveraging, and reinforcing community based systems •SBC activities to prevent further spread of *An. stephensi* •Community based vector programs encouraged for all countries •Engagement with FETP for increases in urban malaria •Training hubs for *An. stephensi*	•GVCR approach and integration of surveillance and control of different vector species •Human movement across the rural-periurban-urban gradient •Major pinch points and transport hubs (ports) as focal areas •Research emphasis on surveillance, modeling, and cost estimates •Increases in malaria cases followed up with vector surveillance •Coordinate with different levels of government •Multisectoral/One Health coordination •Develop regulatory strategies (such as by-laws for water storage practices) to limit vector habitats during urban development and expansion •Community involvement models •Vertical coordination (engagement between governments and non-state actors, including the private sector, civil society and the community)	•Clear integration of *An. stephensi* and *Aedes aegypti* surveillance and control at each step •Surveillance in major transport hubs/pinch points •Movement of humans, goods, and animals (ports, urban centers, peri urban environments) •Prioritization of surveillance to delineate extent of distribution •Larval surveillance where increases in malaria have been detected •Encourages regional coordination and collaboration and with different levels of government and One Health •Enacting by-laws for water storage practices •Leveraging community based HEP for larvicide implementation •Community engagement as a central theme for sustainable action •Coordination with neighboring countries in the Horn of Africa

Briefer documents summarizing the threat of *An. stephensi* have been developed by the PMI *An. stephensi* Task Force and can be shared and used to facilitate planning discussions. One such briefer can be found here. For additional materials you can reach out to the Task Force.


The invasive mosquito, *Anopheles stephensi*, is a biological threat to global malaria eradication efforts

What is the threat?
An invasive malaria mosquito vector in Africa, *Anopheles stephensi*, is a biological threat which may alter the landscape of malaria in Africa from a primarily rural disease to an urban and rural disease which may reverse progress towards global malaria eradication. It is estimated that as this species continues to spread throughout the African continent, it could put an additional 126 million people in urban areas at risk of malaria.

Evidence of the threat
Djibouti: malaria elimination status lost
In 2011, Djibouti was successfully progressing toward malaria elimination; however, in 2012, *An. stephensi* was detected and since then malaria has increased 36-fold, with over 300,000 suspect cases in 2020, in a country of fewer than 1 million.

In 2016, *An. stephensi* was confirmed in Ethiopia and Sudan, 2019 in Somalia and 2020 in Nigeria, the first country outside of the Horn of Africa to report the species. In 2022 the species was reported from Kenya.

Ethiopia: urban malaria 2022
In 2022 an unusual dry season outbreak of urban malaria was reported in the city of Dire Dawa, Ethiopia and linked specifically to *An. stephensi*.

Ethiopia: predicted impacts
A modeling study in 2022 extrapolated *An. stephensi* data from Djibouti to Ethiopia, and concluded that if the vector continued to spread, Ethiopia would likely experience a 50% increase in the number of annual *P. falciparum* malaria cases, costing an additional **$72 million USD annually** to implement vector control interventions.

Annex 10. West African *Aedes* Surveillance Network (WAASuN) contacts for PMI partner countries

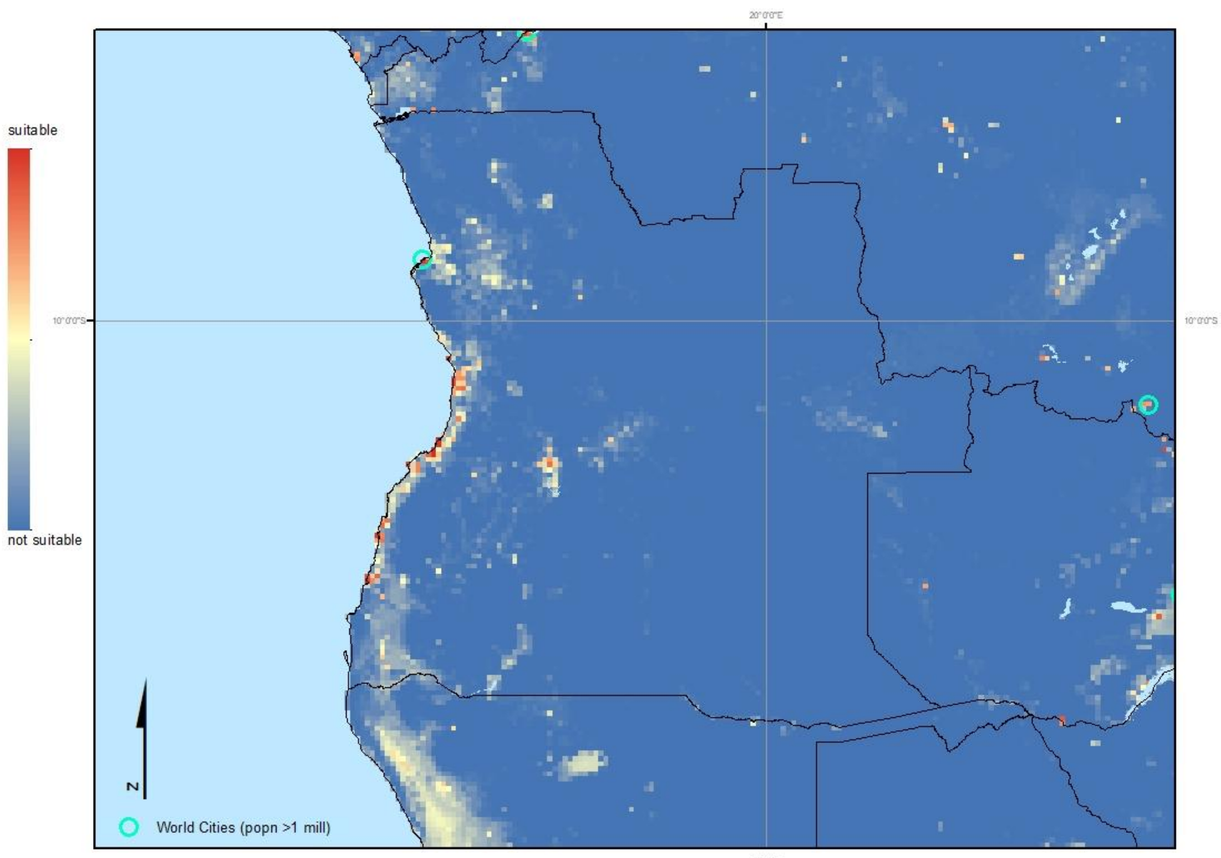
The following countries are participants in the WAASuN: Benin, Cote d'Ivoire

Ghana, Senegal, Burkina Faso, Guinea, Liberia, Mali, Niger, Nigeria, and Sierra Leone. Specific country focal points are in the process of being designated. Country specific contact information can be provided upon request at this point in time from Audrey Lenhart at ajl8@cdc.gov.

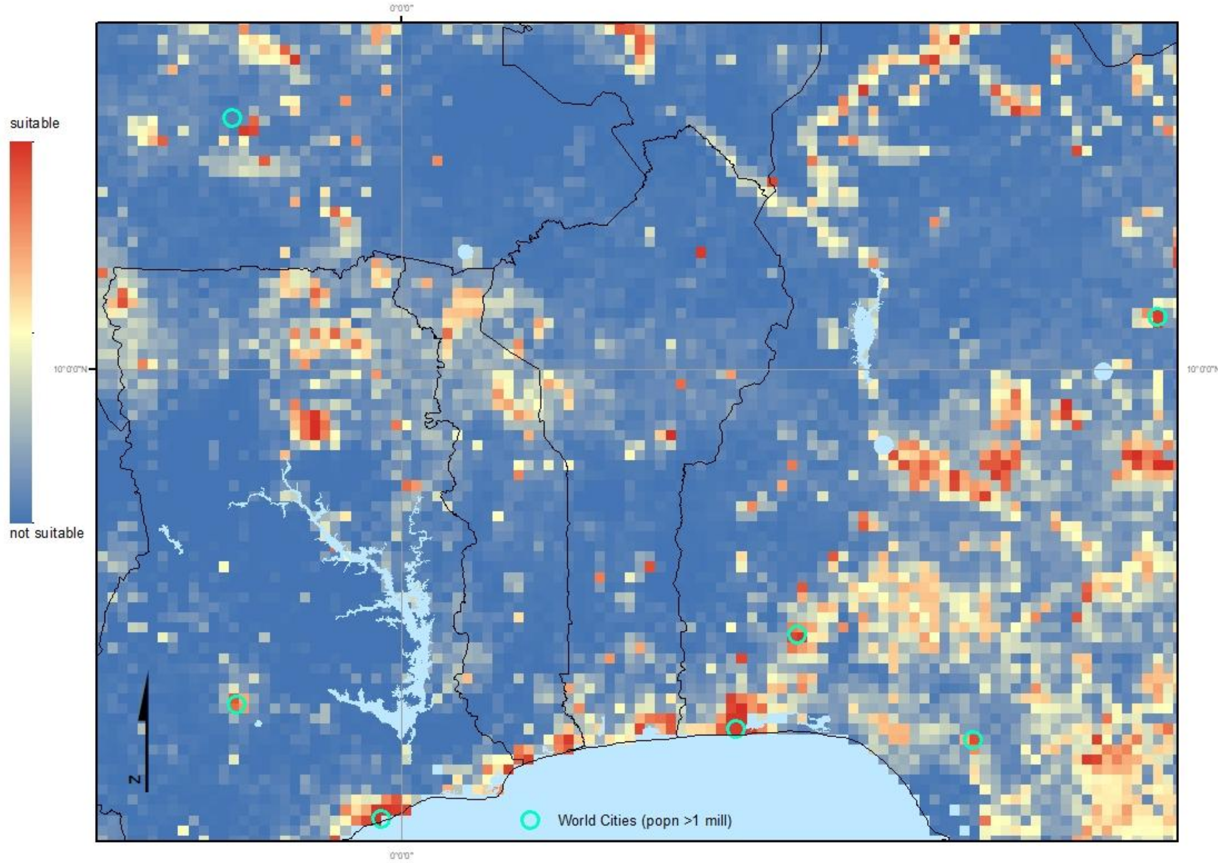
Annex 11. Maps of all PMI partner countries in Africa highlighting specific locations at risk of *An. stephensi* establishment based on habitat suitability

Maps of habitat suitability for *An. stephensi* establishment from Sinka et al. 2020. These maps can be used to identify locations which may be suitable for the establishment of introduced *An. stephensi* populations and could be used to guide surveillance efforts. The original high definition images can be provided to countries on request.

Angola



Benin



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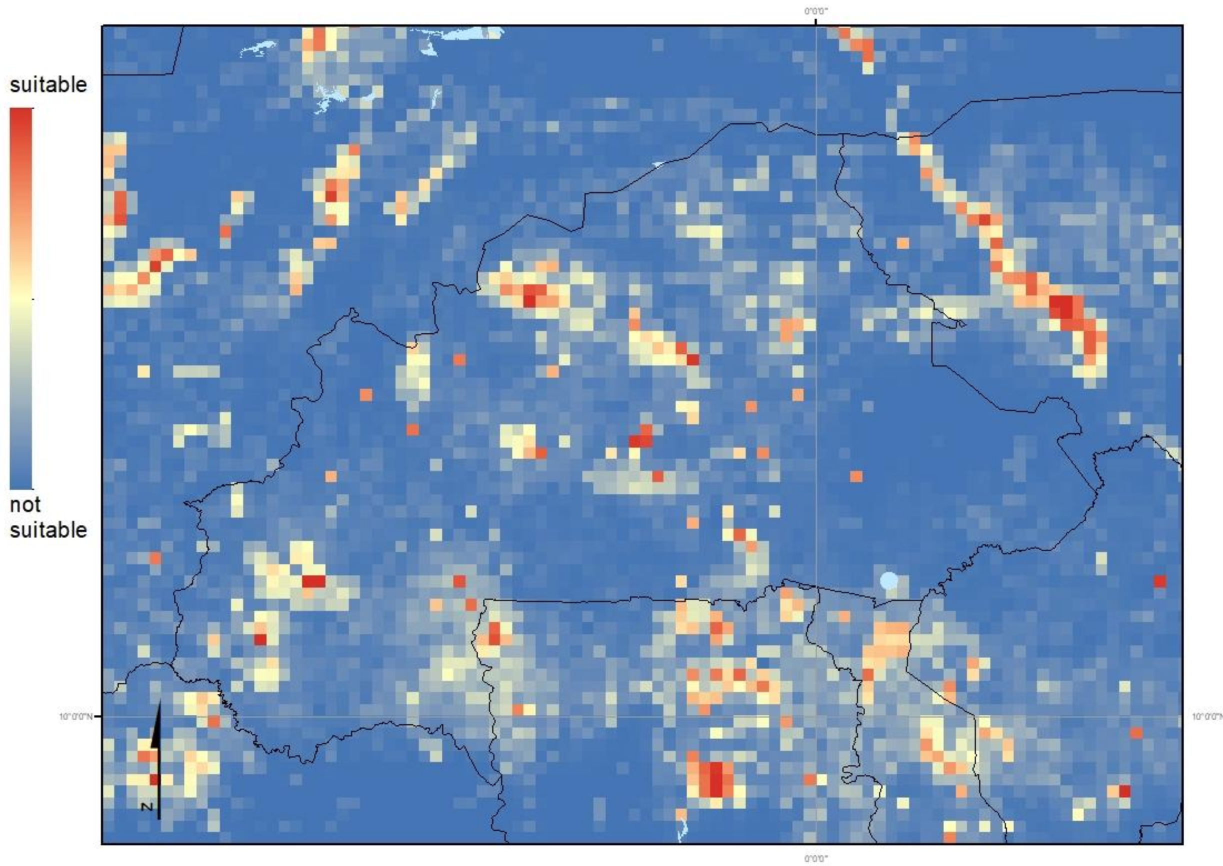
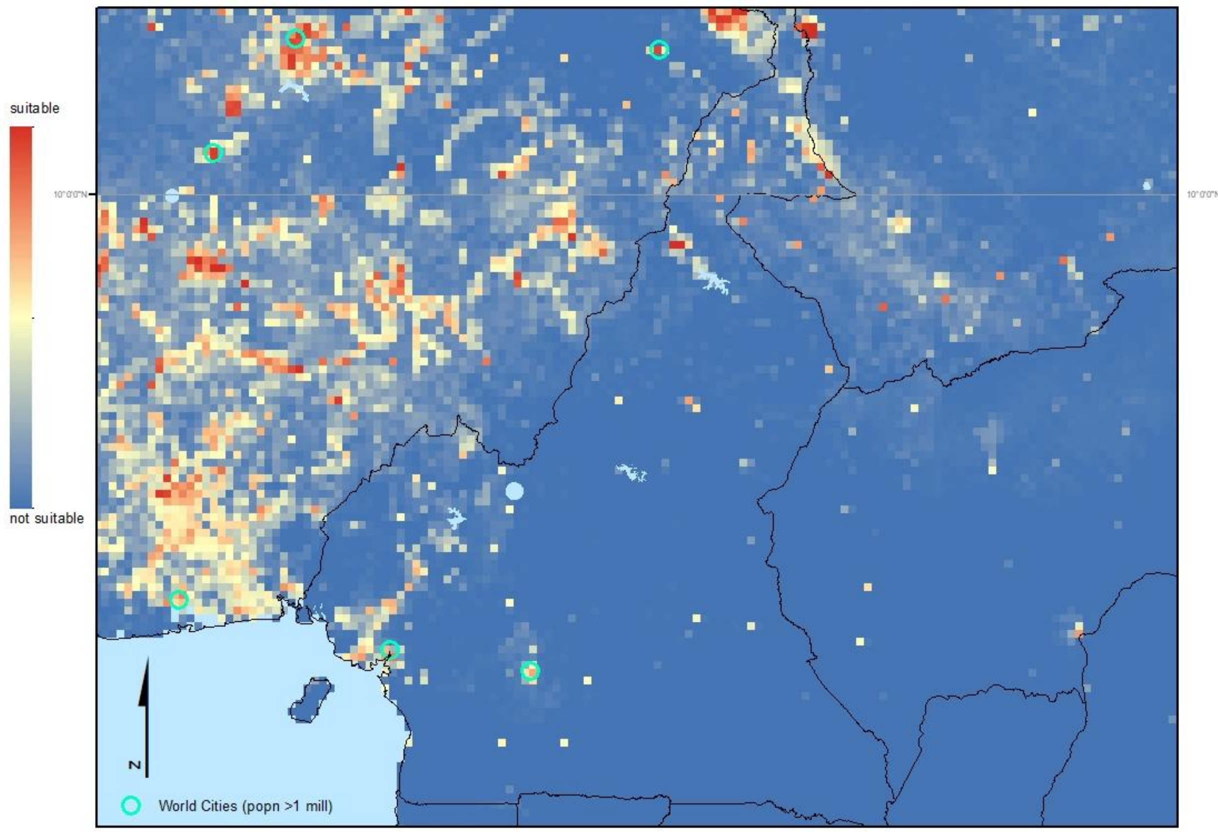
Burkina Faso



Cameroon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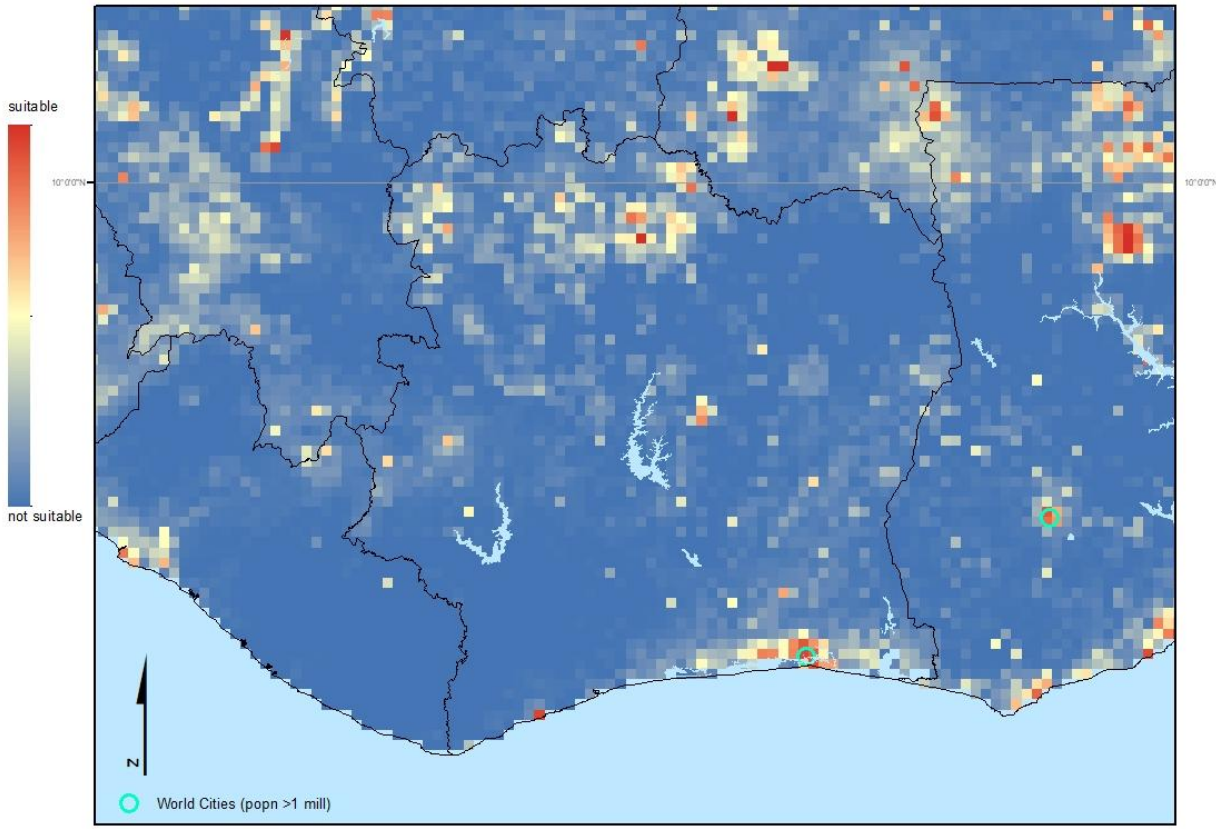
Cote d'Ivoire



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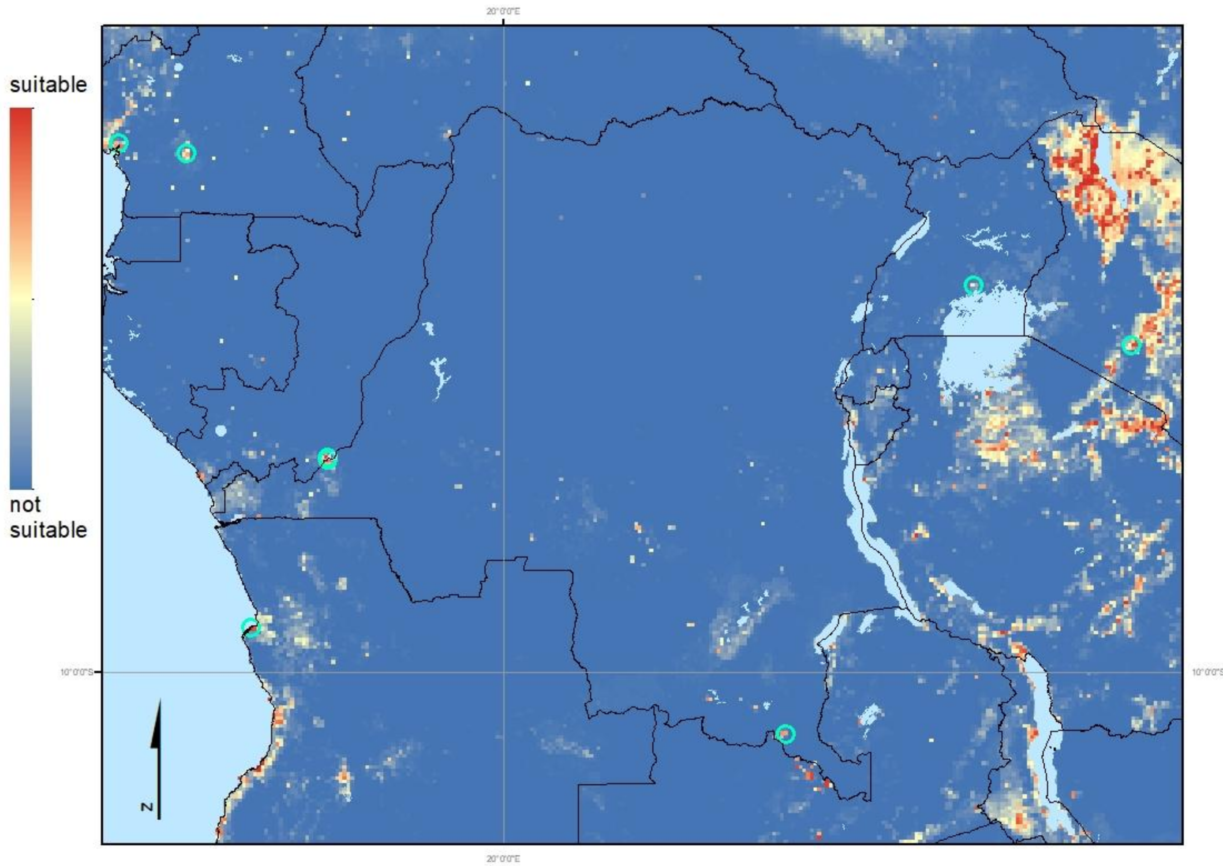
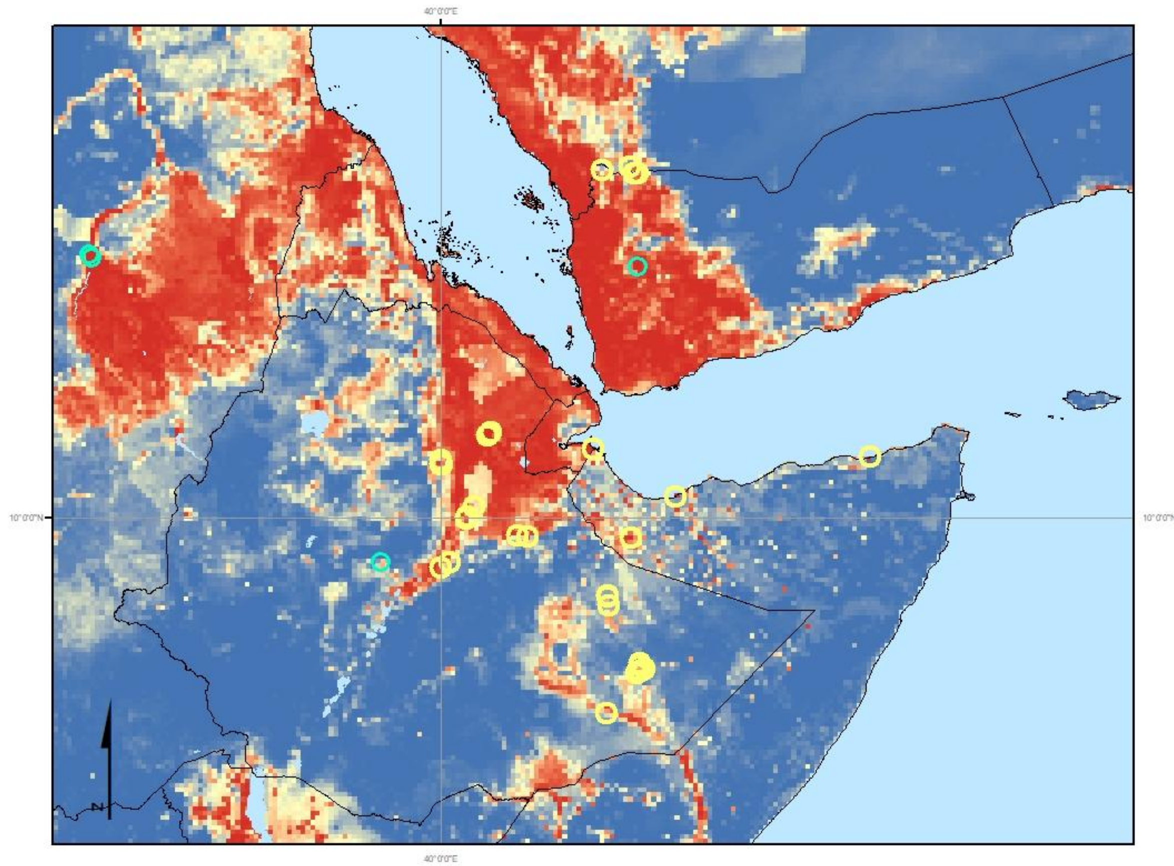
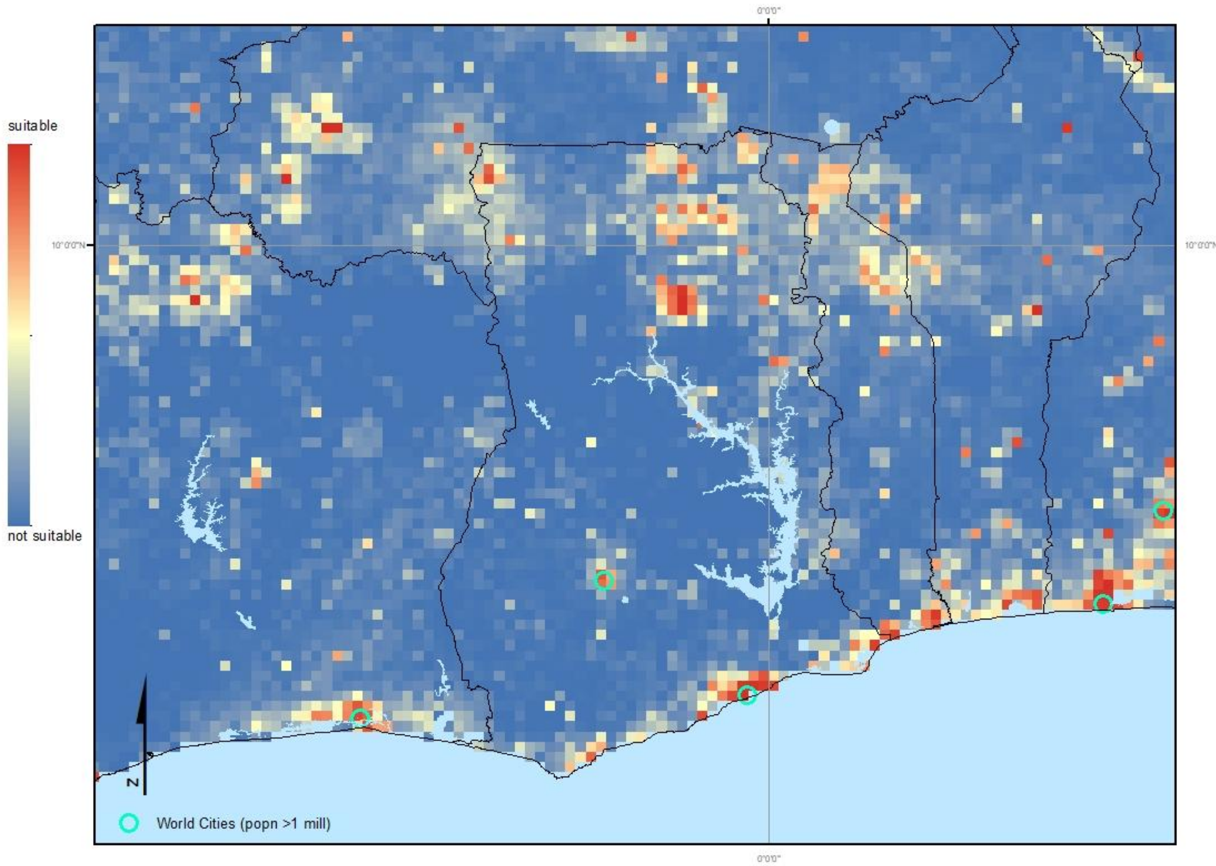


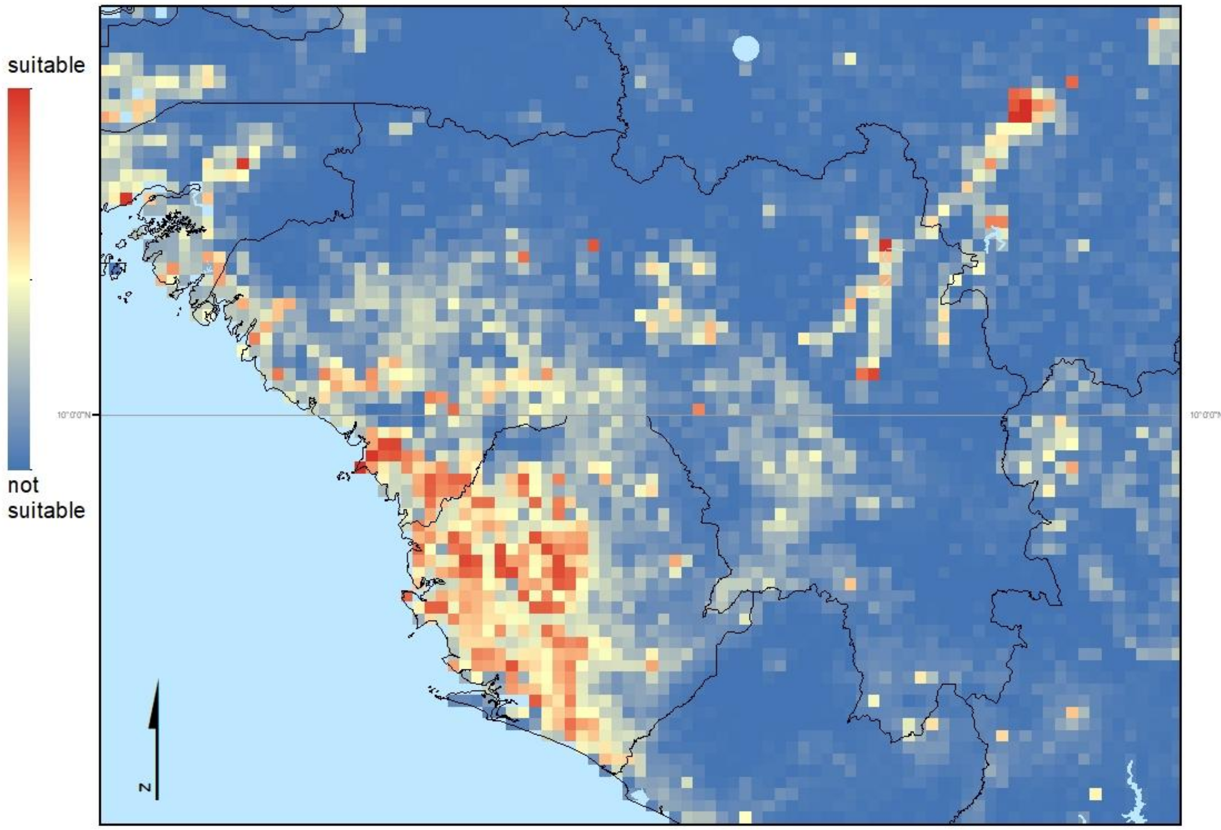
Ethiopia



Ghana



Guinea



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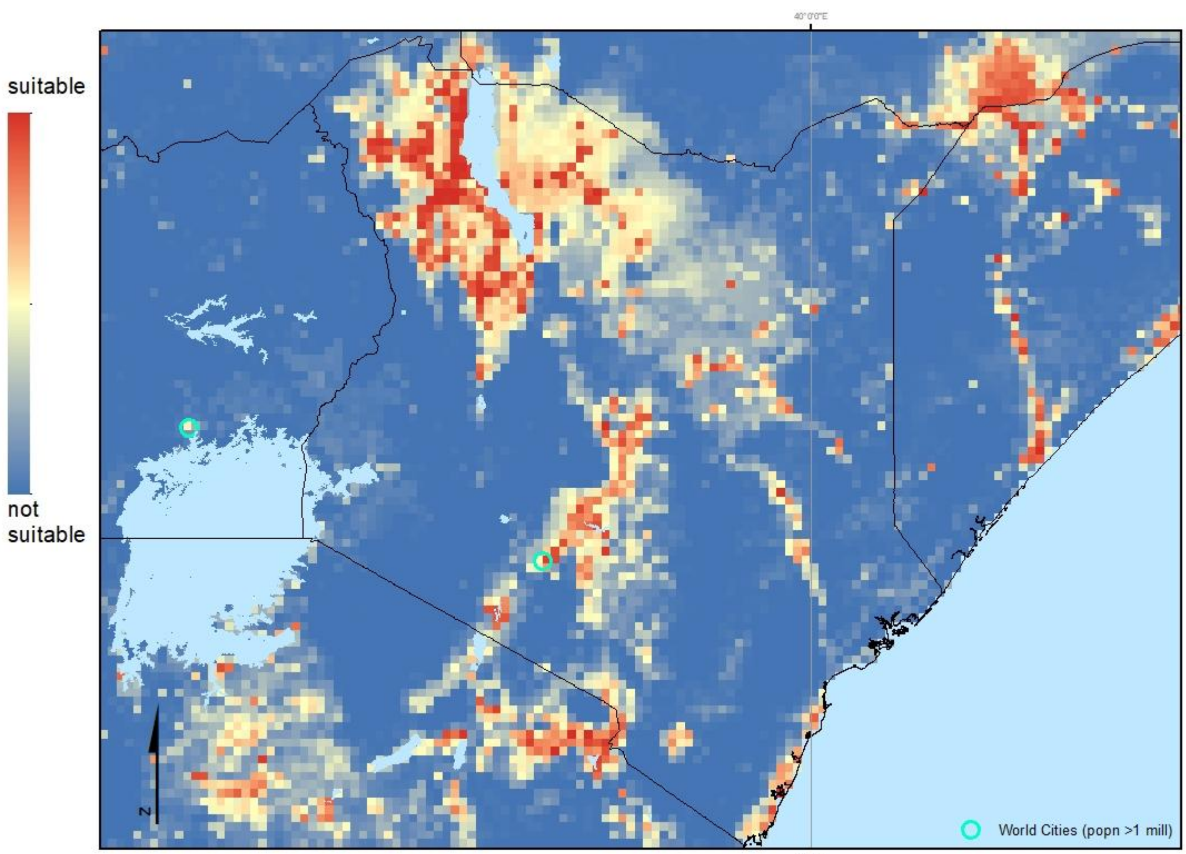

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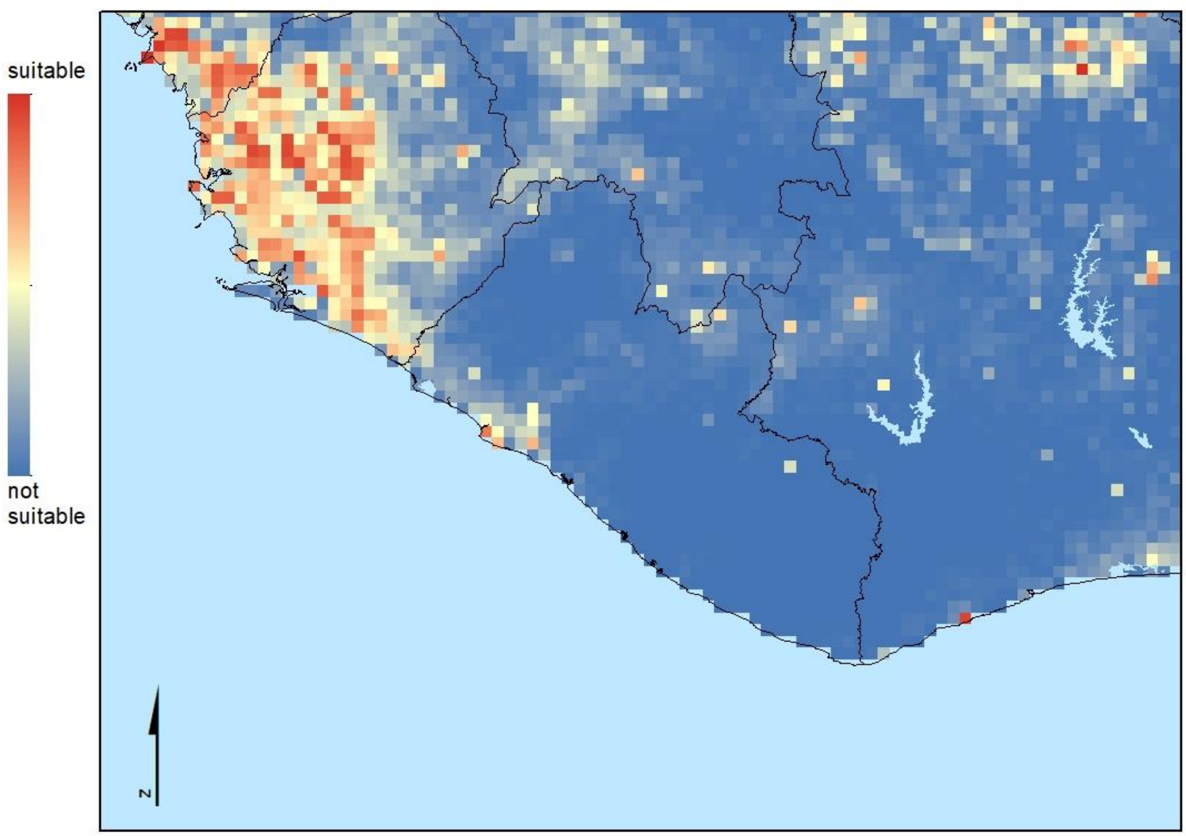

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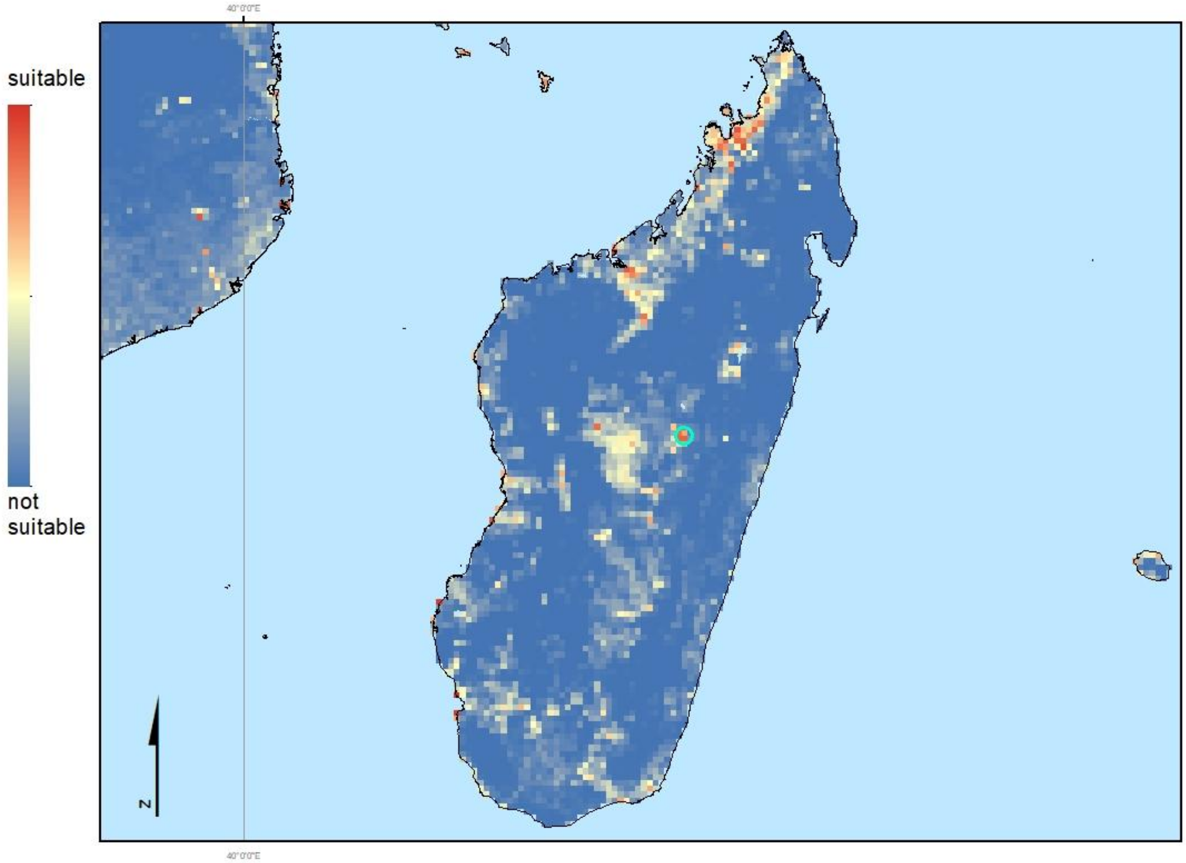

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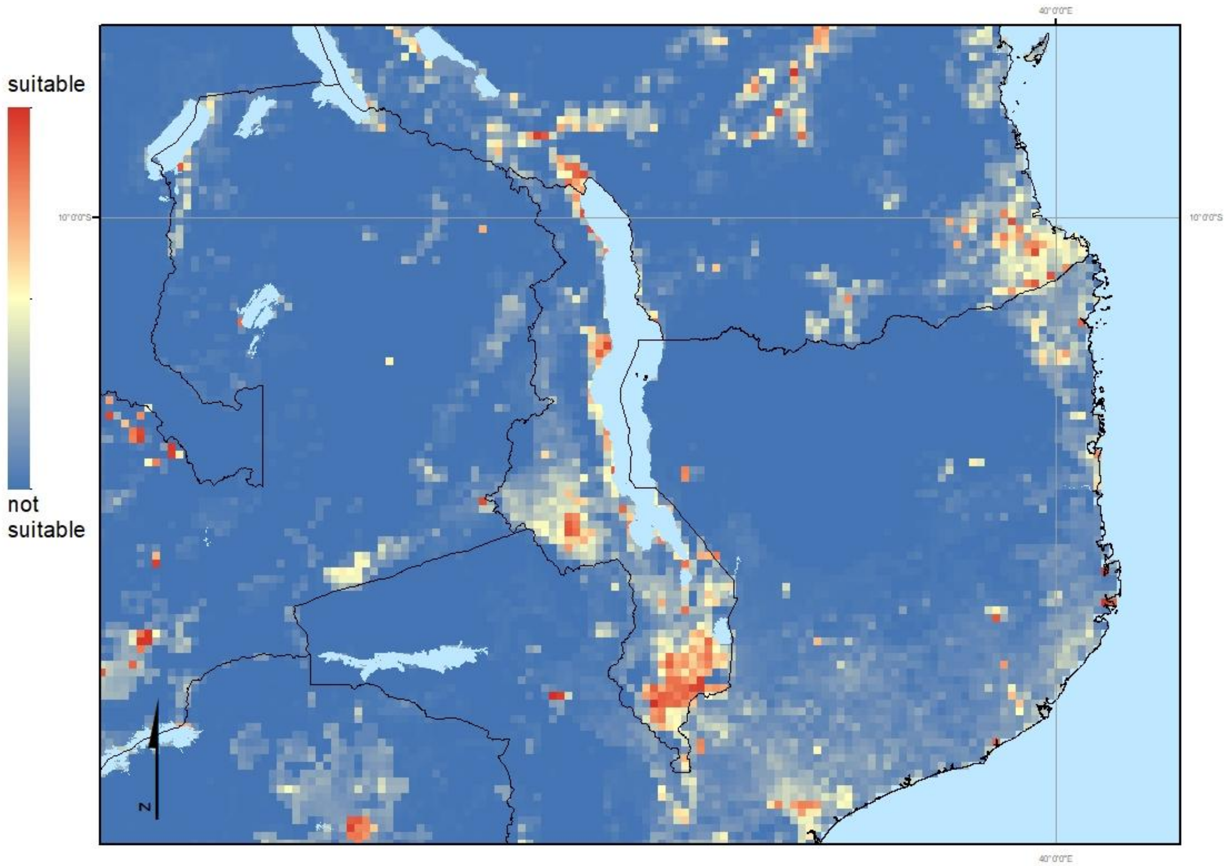

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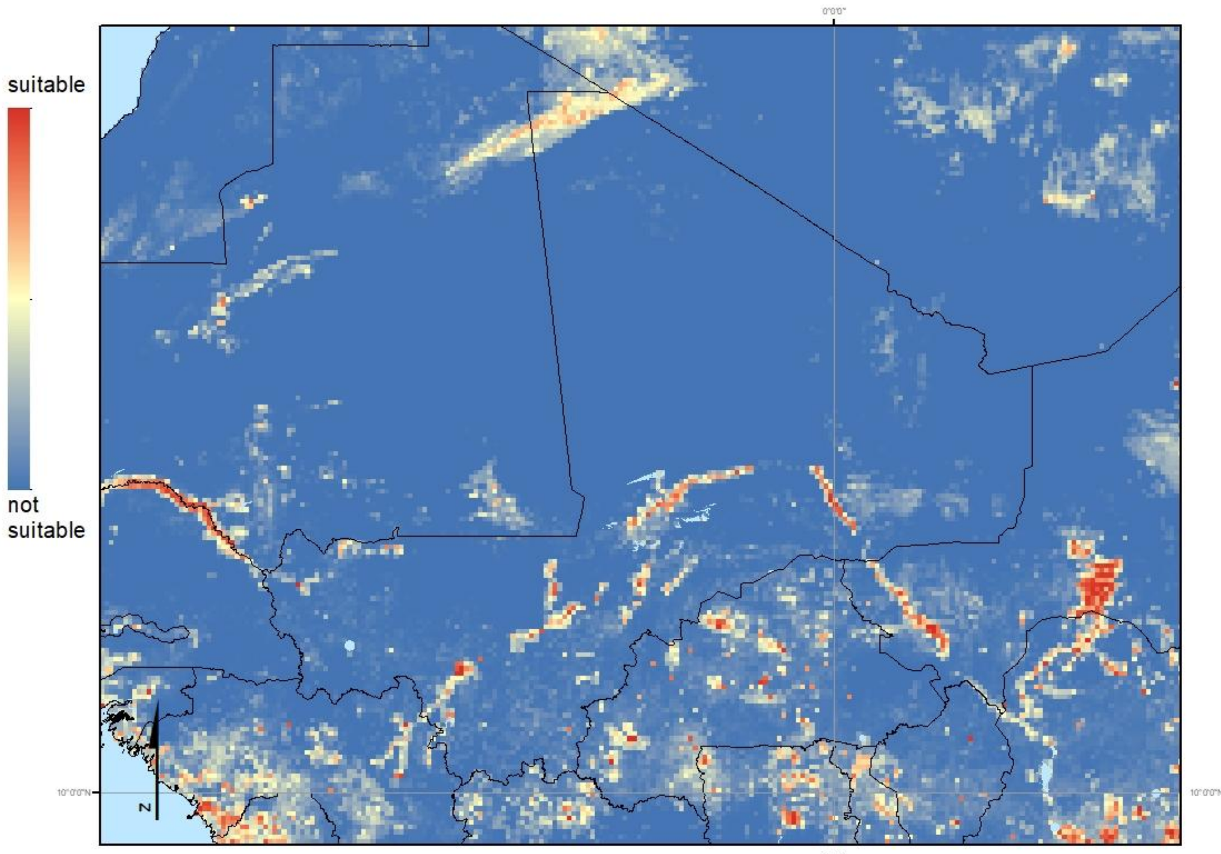

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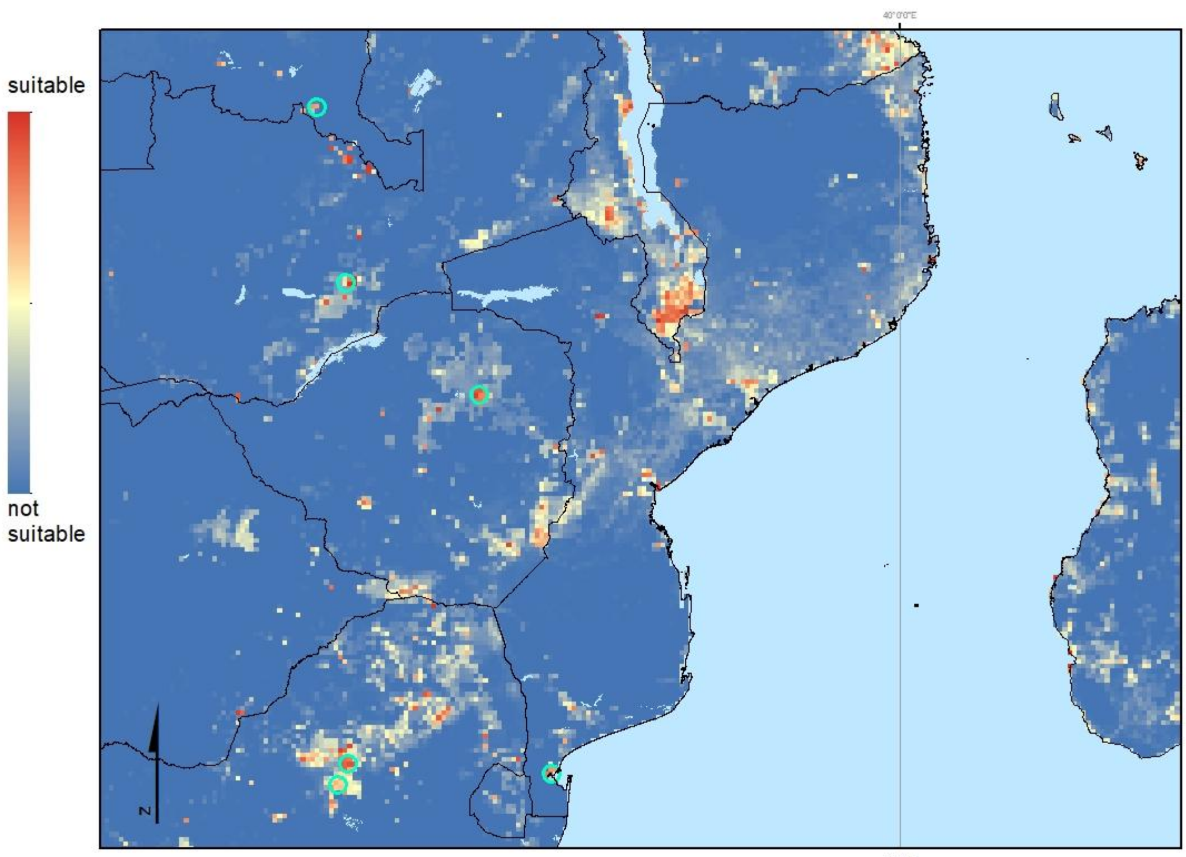

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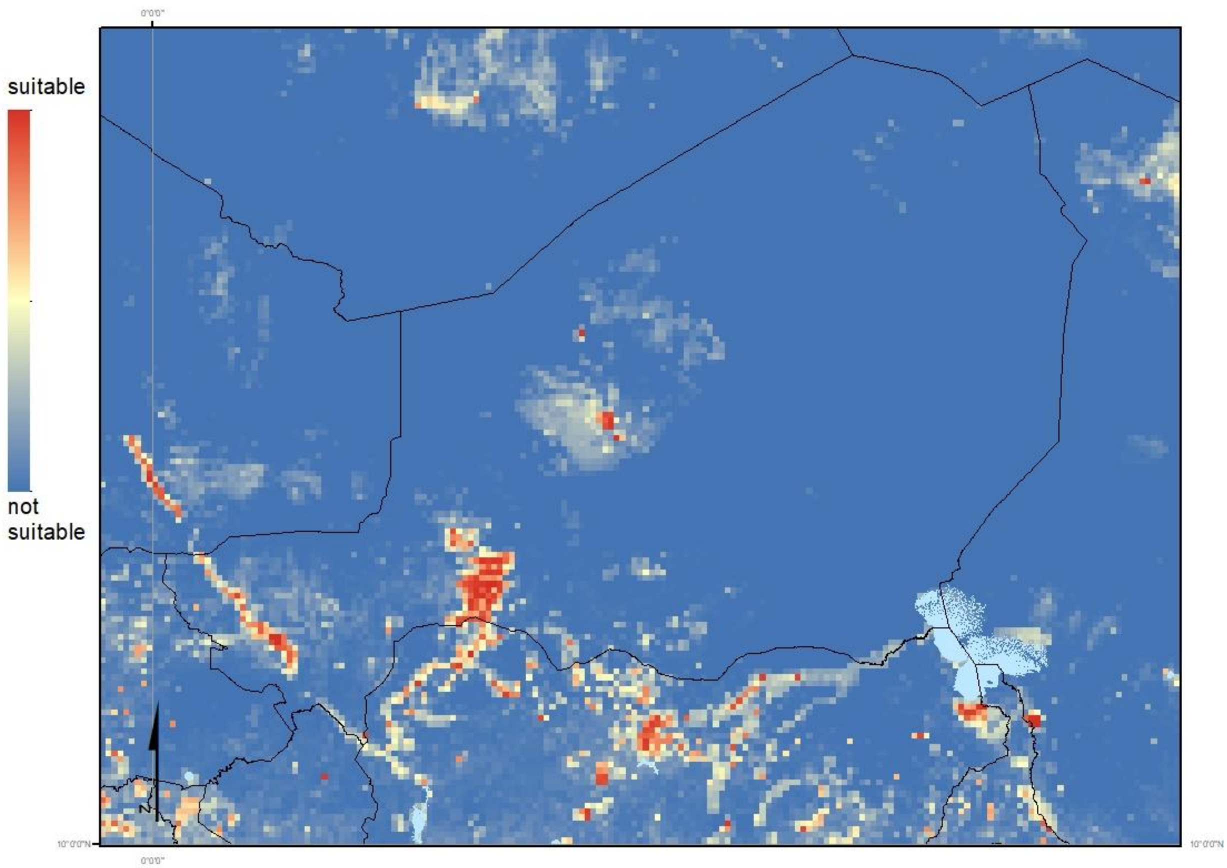

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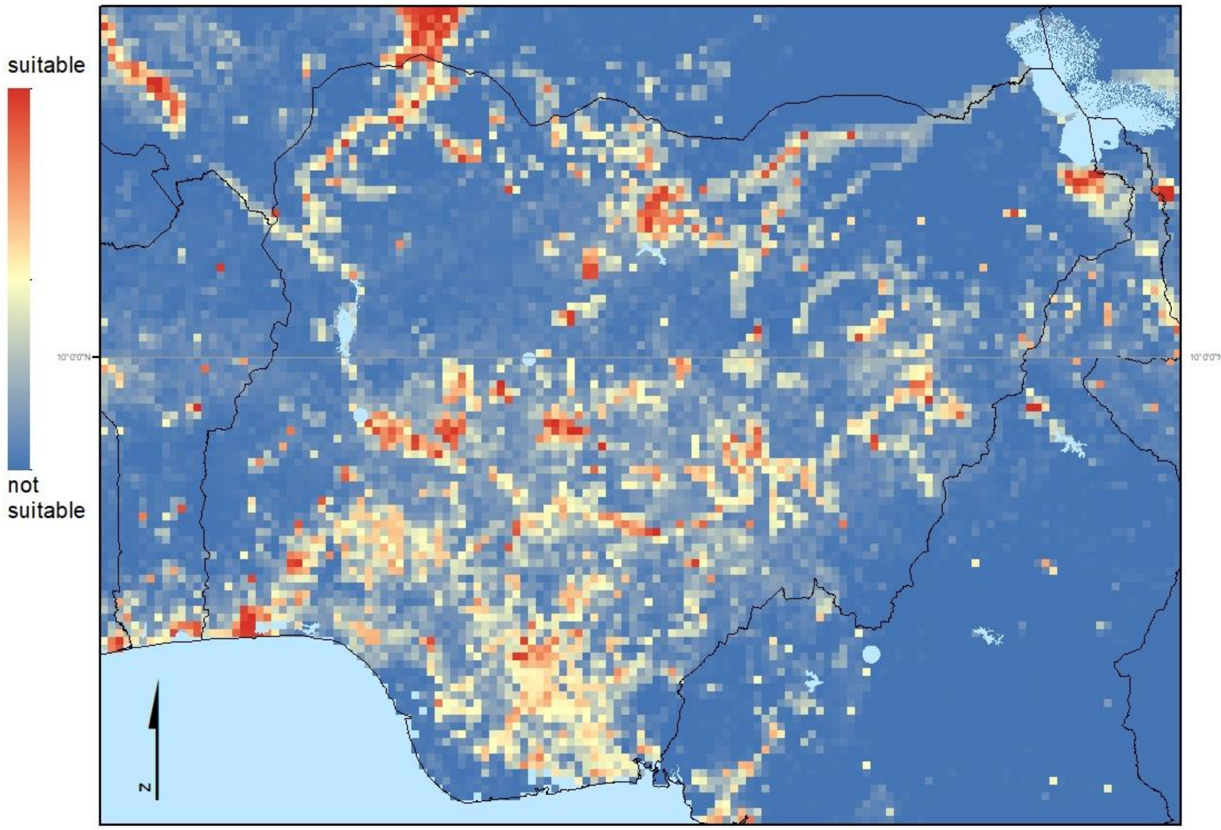

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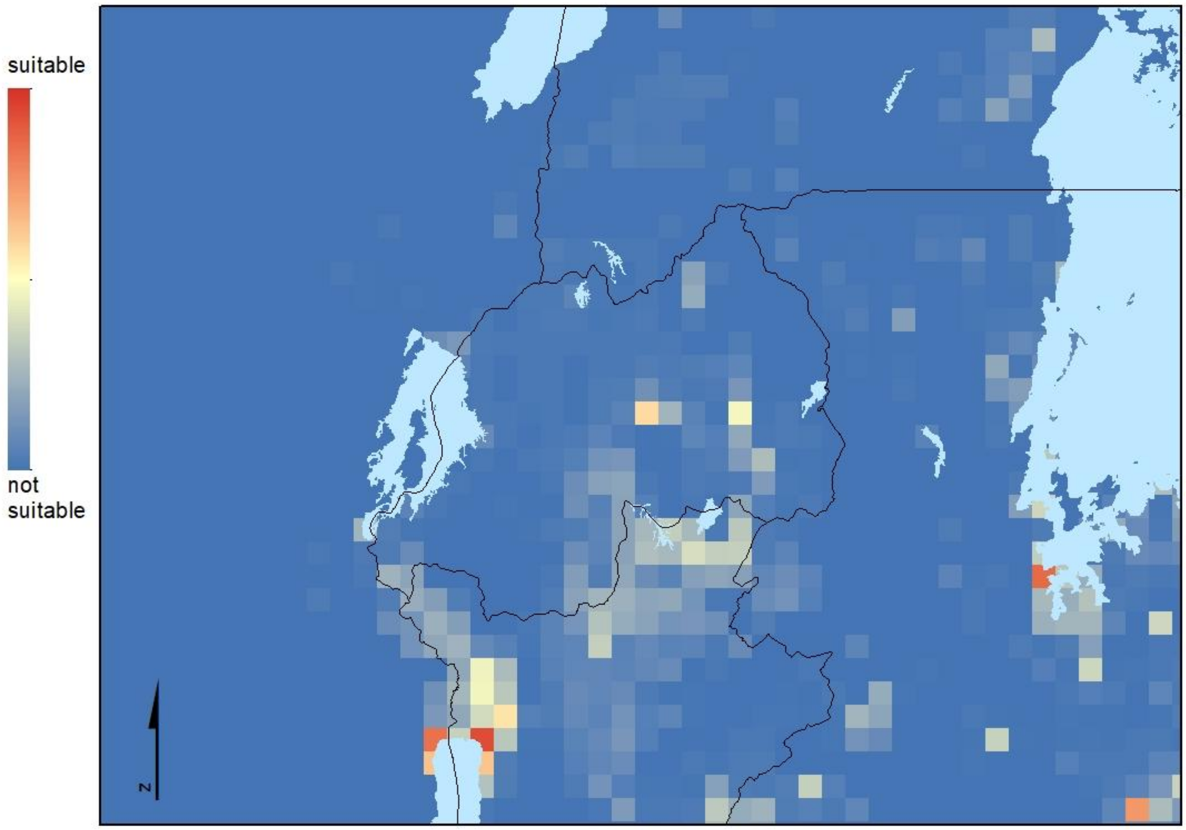

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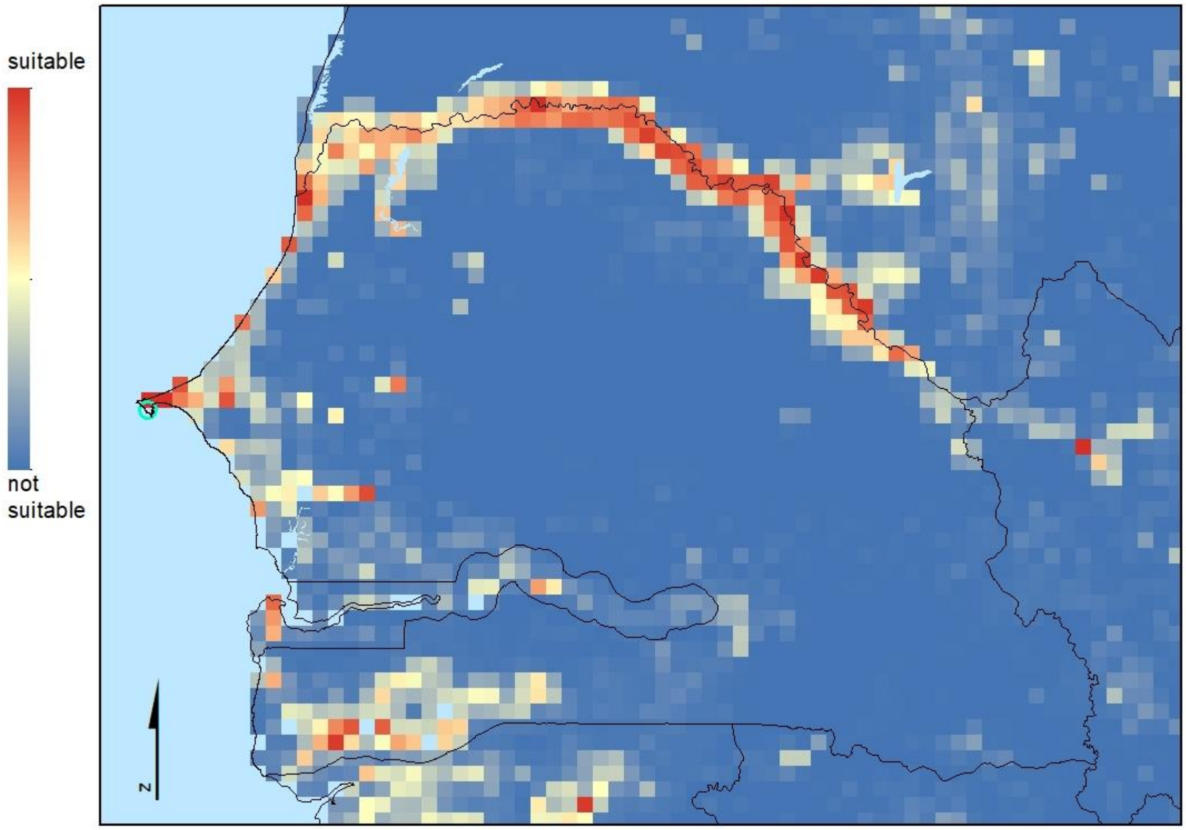

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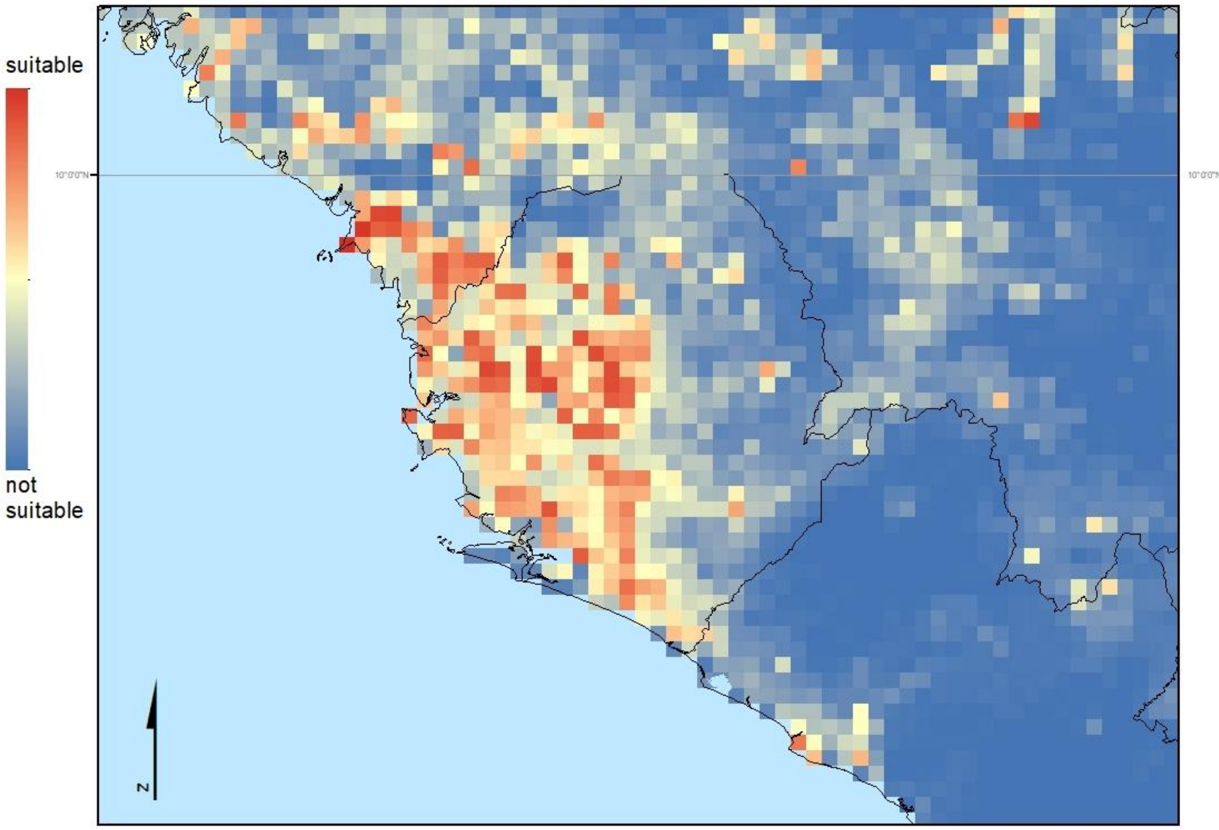

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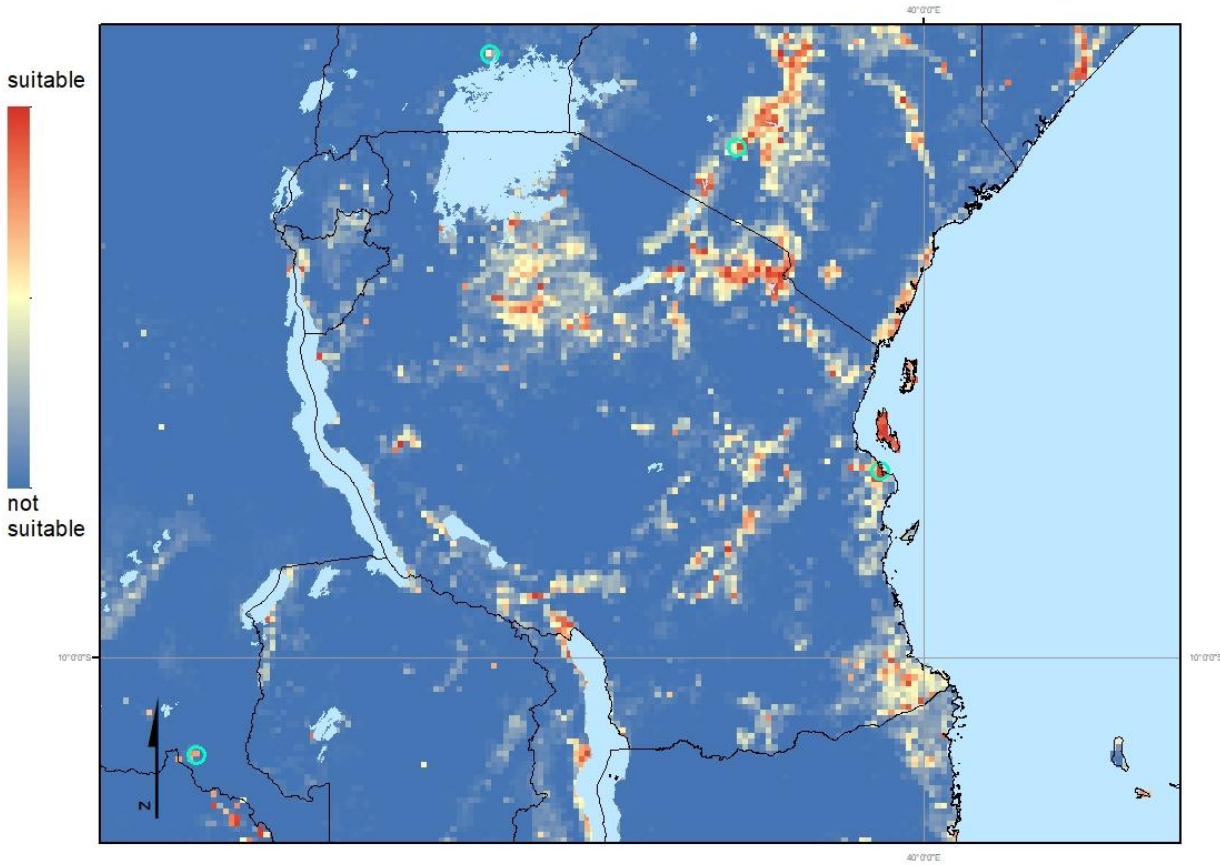

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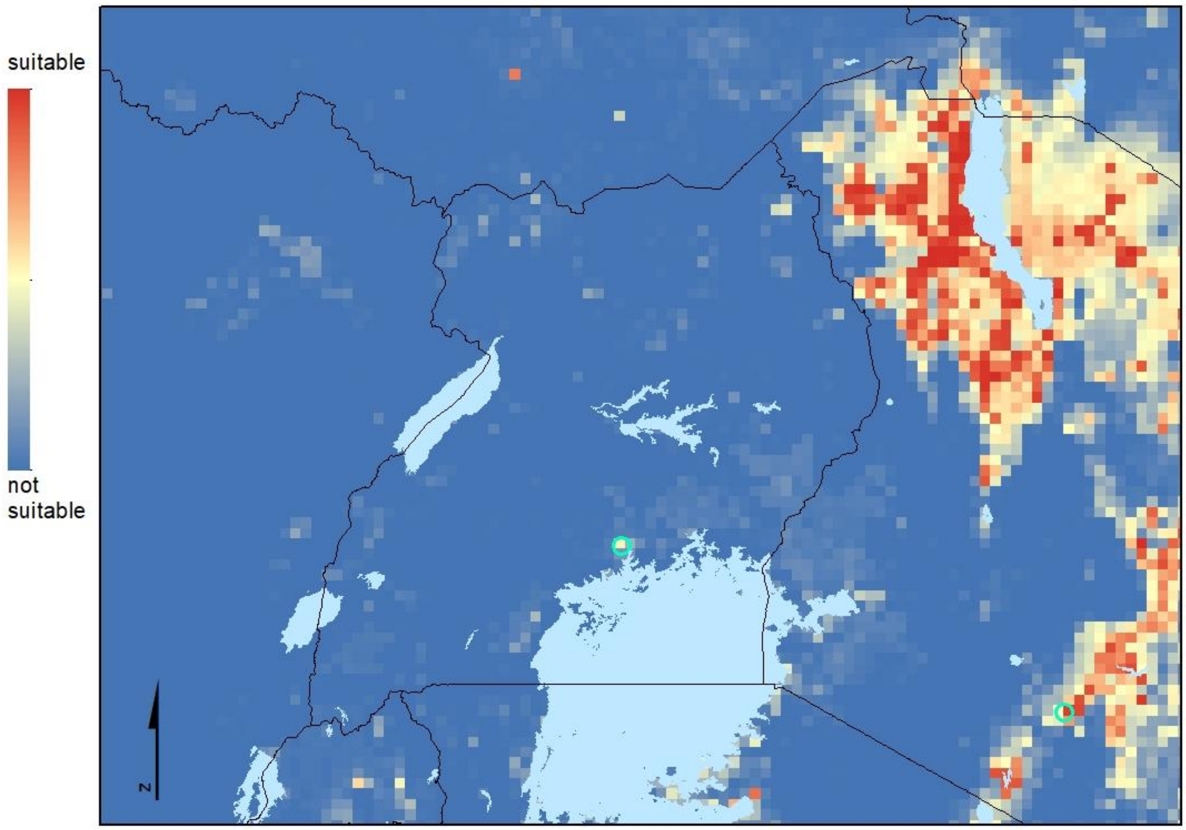

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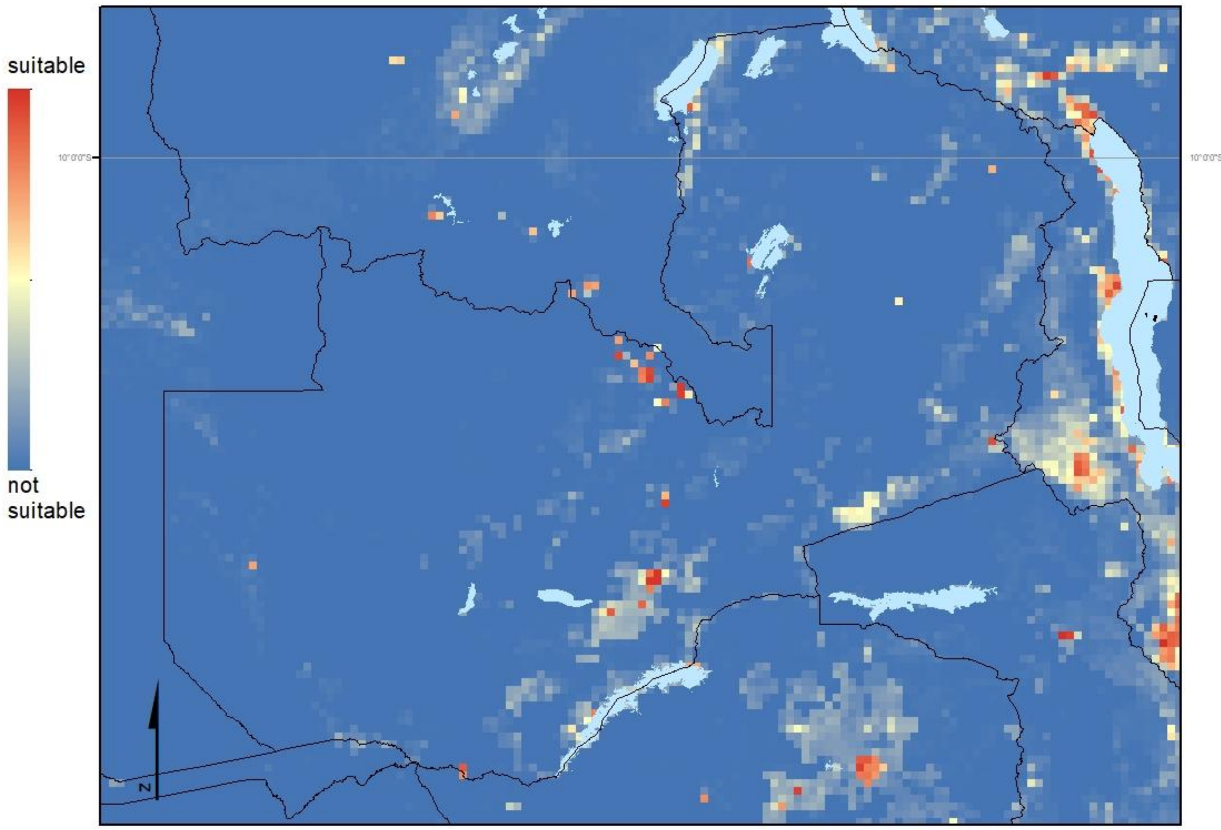

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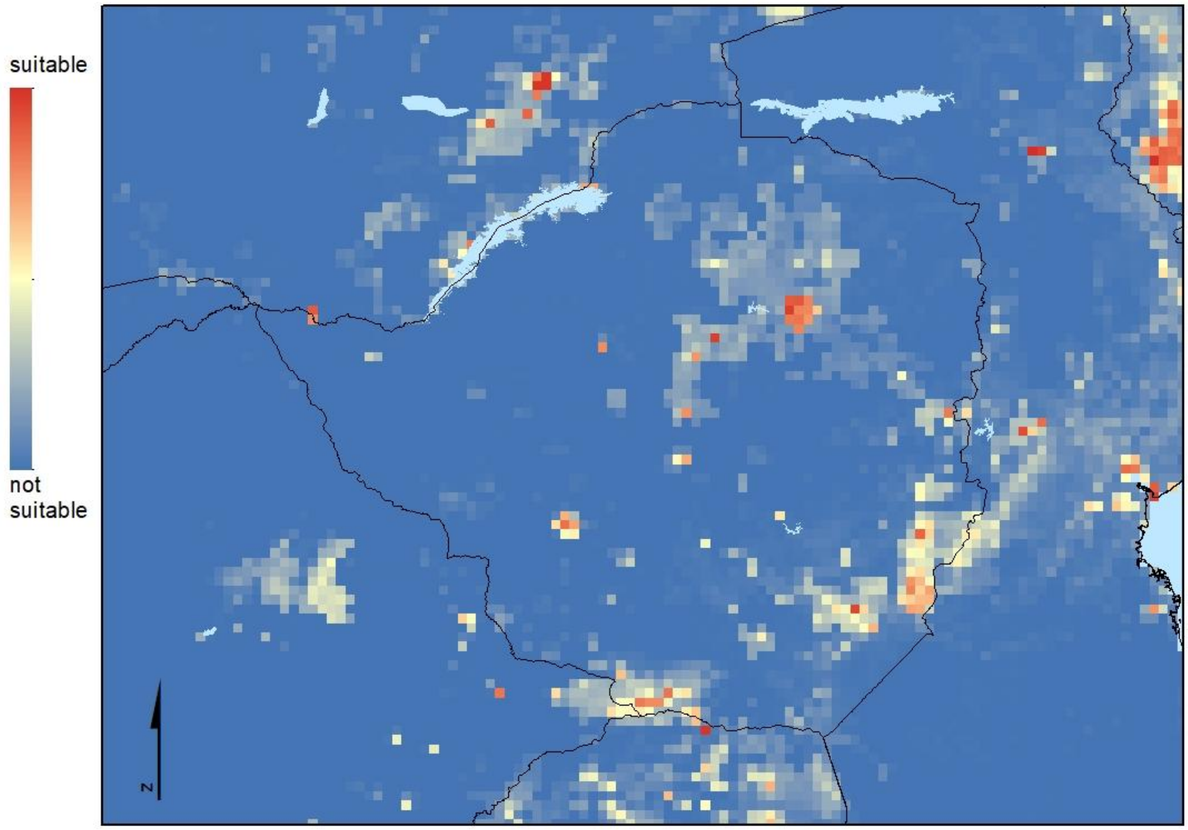

Annex 12. Estimated costs of larviciding in Ethiopia

Estimated costs of vector control interventions for *An. stephensi* in Ethiopia based on Hamlet et al. 2021. Different combinations of ITN/ITN-PBO/IRS/LSM and the associated annual malaria cases averted and costs, total and per person. Values in brackets for the cases averted refer to the difference in minimum and maximum 95% CI's and as such the cases averted median value does not always fall within this, or in numerical order.

ITN	ITN-PBO	IRS	LSM	Cases averted (thousands)	Total costs (million USD)	Cost per case averted (USD)
0%		0%	40%	68 (9-143)	9.9 (5.0-14.9)	18 (9-27)
80%		0%	0%	71 (29-31)	1.8 (1.7-1.9)	3 (3-3)
0%		80%	0%	75 (16-99)	58.6 (31.7 -84.2)	96 (52-138)
80%		80%	40%	172 (39-301)	70.3 (38.4-100.9)	50 (27-72)
	80%	0%	0%	139 (60-157)	3.5 (2.9-3.7)	25 (21-27)
	80%	80%		207 (70-33)	64.6 (37.2-90.8)	91 (52-127)
	80%		80%	210 (49-361)	16.0 (10.6-21.5)	22 (15-30)
	80%	80%	40%	302 (79-879)	72.0 (39.9-102.5)	239 (132-339)