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A quantitative and systematic analysis of *Anopheles stephensi* bionomics and control approaches

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Abstract

Anopheles stephensi is a mosquito endemic to South Asia and the Arabian Peninsula that has recently been detected in eight African countries, posing a significant threat to global malaria control efforts. A challenge with *An. stephensi* is that it requires unique surveillance and control tools when compared to other malaria vectors. Through a systematic literature review, we investigated the efficacy of trapping methods and controls for *An. stephensi* mosquitoes, with a focus on studies of its behavior and biology. Data from 83 articles (native range: Afghanistan, India, Iran, Iraq, Pakistan, and Qatar; invasive range: Djibouti, Ethiopia, Sri Lanka, Saudi Arabia, and Sudan) met our study inclusion criteria. Data from these studies revealed that using host-seeking animal baited traps increased the number of mosquitoes collected per trap per day in the native range when compared to host-seeking human baited traps. However, these differences were not present in data collection rate assessments from the invasive range. We also found that *An. stephensi* equally used a large variety of breeding habitats in the native range, but that it tended to prefer water reservoirs and wastewater in the invasive range. Finally, we found that temephos, fenthion, *Bacillus thuringiensis israelensis*, and *Beauveria bassiana* were more effective at reducing larvae in their native range compared to the approaches found in our systematic

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Declaration of competing interest

The authors have no conflict of interest.

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Tabeth Mwema: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Sarah Zohdy:** Writing – review & editing, Methodology, Conceptualization. **Mekala Sundaram:** Writing – review & editing, Methodology, Formal analysis. **Christopher A. Lepczyk:** Writing – review & editing, Methodology. **Lana Narine:** Writing – review & editing, Methodology. **Janna R. Willoughby:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Disclaimer

The findings and conclusions expressed herein are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention (CDC), US Agency for International Development (USAID), or US President's Malaria Initiative (PMI).

Supplementary materials

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literature search, but the relative effectiveness of these approaches in the invasive range was less clear. Understanding proven historical surveillance and control approaches is essential to the advancement of invasive *An. stephensi* mitigation efforts, but continued investigations in the invasive range are critical to reducing the impacts of malaria morbidity and mortality.

Keywords

Malaria vector; Mosquito control; Invasive species

1. Introduction

An estimated 608,000 malaria deaths and 249 million cases occurred globally in 2022, with Africa accounting for 95 % of deaths and 94 % of global cases (World Health Organization, 2023). Although considerable progress has been made in reducing the malaria burden in Africa through various intervention programs, primarily by controlling mosquitoes that can transmit human malaria-causing *Plasmodium* parasites, numerous threats remain, including vector insecticide resistance (Sougoufara et al., 2014), malaria drug resistance (Dondorp et al., 2009), and diagnostic challenges (Golassa et al., 2020; Niyukuri et al., 2022; Okanda et al., 2023). Compounding these threats is the recent invasion of a malaria vector species in Africa (*Anopheles stephensi*). This mosquito species has the potential to halt or even reverse the progress towards malaria elimination due to its ability to persist year-round instead of seasonally (Basseri et al., 2010; Ravishankaran et al., 2021), thrive in urban environments instead of just rural ones (Sinka et al., 2011), and modified biting behaviors (Sinka et al., 2020), resulting in catastrophic health outcomes for many African communities. If *An. stephensi* continues to spread across the continent, an additional 126 million people per year will be at risk of malaria (Sinka et al., 2020).

An. stephensi is a highly adaptable species, capable of surviving and reproducing in diverse and novel environments, increasing this species ability to survive and transmit malaria causing pathogens. *An. stephensi* is a competent vector for both *Plasmodium falciparum*, the deadliest malaria-causing pathogen, and *P. vivax*, the most prevalent malaria-causing pathogen on the African continent (Ashine et al., 2020; Tadesse et al., 2021; World Health Organization, 2023). The transmission of these pathogens by *An. stephensi* is compounded by this species' use of both rural as well as urban areas, indicating a shift from the typical rural preferences of other common African malaria vector species and increasing interactions with humans due to the increased human density in urban relative to rural settings (Sinka et al., 2011). Reports from the endemic range suggest *An. stephensi* will bite humans both indoors and outdoors, which can complicate control efforts (Ghosh et al., 2010; Mojahedi et al., 2020; Waka et al., 2006). Furthermore, *An. stephensi* is highly flexible in breeding site selection, with reported preferences ranging from wells and overhead tanks to freshwater natural habitats (Manouchehri et al., 1976; Surendran et al., 2019; Thomas et al., 2016). It has also been reported to display high salinity tolerance, highlighting its ability to thrive in a diverse range of physiochemical conditions (Surendran et al., 2019).

Due to its adaptability, the presence of *An. stephensi* poses a challenge to primary malaria vector control tools, including insecticide-treated bed nets and indoor residual spraying. These methods target indoorbiting and indoor-resting mosquitoes, but changes in feeding and resting behaviors may reduce their effectiveness. In addition, this mosquito shows high resistance to pyrethroids, the main chemicals used in insecticide-based bed nets and indoor residual spraying (Eshghy and Janbakhsh, 1976; Safi et al., 2019; Vatandoost and Hanafi-Bojd, 2012). Adding to the unique bionomics of the species, reports suggest that invasive *An. stephensi* populations in Africa show resistance to several classes of insecticides commonly used for adult malaria vector control (e.g., DDT, malathion, pirimiphos-methyl, bendiocarb, propoxur, deltamethrin, and permethrin; (Balkew et al., 2021; Enayati et al., 2020; Yared et al., 2020). Given *An. stephensi*'s reported bionomical flexibility and its range expansion into Africa, it is critical to identify the species' biting and resting behaviors, preferred larval habitat characteristics, and optimal surveillance and control tools that can facilitate rapid, effective, and efficient response efforts in Africa.

In response to the expansion of *Anopheles stephensi*'s range in Africa, the World Health Organization (World Health Organization 2024) has encouraged countries to conduct targeted surveillance, increase scientific collaboration, improve information exchange, develop public health guidance resources, and prioritize research to understand control strategies. One approach to accelerating the response to this species and preventing its further contribution to malaria epidemics is to identify critical surveillance and control tools in its endemic range (Emiru et al., 2023). This would provide baseline information about *An. stephensi* and synthesize nearly a century of data and lessons learned about the species from its endemic range to inform the invasive context. To contribute to this effort, we sought to address four questions related to its bionomics and control. First, what adult collection techniques are effective in capturing *An. stephensi*? Second, based on the literature, does *An. stephensi* exhibit a significant preference for biting and resting indoors or outdoors? Third, which larval habitat characteristics predict *An. stephensi* presence and indicate likely breeding sites? Fourth, which *An. stephensi* control interventions have been shown to be effective for adults and larvae? Answering these questions is critical to advancing mitigation efforts across Africa and ultimately reducing the impacts on malaria in the region.

2. Methods

We used a systematic literature review and subsequent regression analyses of *An. stephensi* surveillance and control interventions used in its endemic and invasive range. This was conducted to understand how these surveillance and control approaches can be applied to Africa for a swift response to this expanding pest species (Table 1).

2.1. Data collection and reclassification

To identify relevant literature on *An. stephensi* behavior and biology, we conducted searches in three databases, PubMed, Web of Science, and Google Scholar, following the PRISMA guidelines. Specifically, we used the keyword string: ("*Anopheles stephensi*" OR "*An. stephensi*") (All Fields) AND "vector" (All Fields) AND ("control" OR "intervention*") (All Fields) AND ("bionomic*" OR "ecolog*" OR "biolog*") (All Fields) between 1959

and 2022. All articles were then imported into Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia). We limited studies to those written in English, but there was no restriction on the country in which the study was conducted. From the included articles (Supplementary Fig. 1), we extracted the following data on surveillance and control measures: descriptions of the location in which adult biting and resting behavior occurred (i.e., indoor or outdoor), larval breeding sites (as defined by the authors of the papers), larval and adult sampling methods (e.g., larval dipping surveys, human landing catches, and CDC light traps), and adult and larval control interventions (e.g., indoor residual spray and larvicide). We also streamlined our adult collection methods into broader categories based on trap function and baits (Supplementary Table 1). For adulticides, synthetic organic compounds, bacterial larvicides, and biocontrols (larvivorous fish), we recorded *An. stephensi* mortality or adult inhibition rates. We excluded studies that used plant extracts not listed by the WHO PQ for control interventions. Additionally, studies that did not specify abundance and/or density for *An. stephensi*, but rather reported these metrics collectively for all species, were excluded. Reviews and meta-analyses were also excluded to avoid data duplication in our analyses. Studies based in Afghanistan, India, Iran, Iraq, Pakistan, and Qatar were considered representative of the native range whereas studies from field sites in Djibouti, Ethiopia, Sri Lanka, Saudi Arabia, and Sudan were considered part of the invaded range.

Although all breeding sites noted in the literature were initially included, these descriptions varied across almost every study and were therefore reclassified into broader categories for further analysis. For instance, drainage, flooding sewage ponds, pits, unused wells, and waste ponds as breeding sites were recategorized as ‘wastewater’ whereas barrels, birkas, and concrete water containers were classified as water reservoirs. (A complete list of all breeding grounds found in our surveyed data and how they were reclassified is available in Supplementary Table 2.) We also collected larval density in each of these breeding sites. Since some studies did not report larval density or the volume of water in the containers, we assumed that collectors adhered to WHO recommendations of standardized dip counts at 10 or multiples of 10 (e.g., 20, 30) dips per breeding site based on larval density (World Health Organization, 1975). Therefore, unless otherwise specified, we assumed a minimum of 10 dips per site. We then used this assumption to calculate the larval density per 10 dips by dividing the total number of larvae collected by the number of dips.

To enable downstream analysis of the control interventions, we also grouped adulticides and larvicides into chemical classes based on their active properties. For larval source management control tools, the WHO operational manual was used to group interventions into biological larval control or larvicide categories (World Health Organization, 2016). Since different chemicals are used for adults and larvae, we analyzed the different chemicals/biopesticides separately for the two life stages. We grouped the 25 chemical insecticides and larvicides identified in our dataset into several categories. For adults, we considered organochlorines, organophosphates, pyrethroids, carbamates, and pyrroles. For larvae, we considered biological controls (larvivorous fish), bacterial, fungal and chemical larvicides - organochlorine, organophosphate, and pyrethroids. As only a single datapoint of carbamate adulticide use was noted, it was ultimately excluded from statistical analyses (Supplementary Table 3).

From each included study, we also recorded the number of collected samples and the duration of the collection period. Prior to analyzing our data, we standardized our measurement of the number of mosquitoes collected across studies to account for differences in collection effort and duration across studies, dividing the total reported number of mosquitoes collected by the number of collection days. For studies reporting collection periods in units larger than a day (e.g., two months), we calculated the number of days by subtracting two days per week to factor in rest time (Tadesse et al., 2021). For simplicity, we refer to these standardized values as collection rates.

2.2. Data analysis

We used sample collection rate per sampling location as the effect size for our first set of analyses. We separated our data according to the methods used for collecting biting and resting adult mosquitoes and analyzed them separately based on their native or invasive range. We conducted independent linear regressions for each of these categories. We performed a comparative analysis of adult collection methods by examining the collection rate between each pair-wise collection method in our dataset. This analysis was performed using a logged linear regression model, with the collection method serving as the predictor variable. To ensure that our results were not a result of chance, we permuted the response variable and repeated regressions 1000 times to create a null distribution of t-values. We then interpreted the directionality of these differences by comparing the mean collection rates to the random permutations. Since larvae were only collected using dipping, we did not run any statistical analyses on the corresponding dataset.

We evaluated the preferred resting and biting sites of *An. stephensi* based on collection methods using log linear regression. We posited that higher collection rates from particular locations would indicate a higher prevalence of individuals engaging in said behavior. For instance, if the collection rates for two resting methods were 2.5 and 0.2, the method with the higher rate would be deemed more effective for collecting resting mosquitoes. We next extracted the residuals from a linear model in which the collection rate served as the response variable and the collection method served as the predictor variable. Subsequently, these residuals were treated as response variables in a second set of linear regressions, where the only predictor variable was the location of collection (specifically, whether it was conducted indoors or outdoors). For this analysis, we separately examined the regression models for the collection of biting individuals and for the collection of resting individuals.

In a similar manner to our assessment of collection methods, we used a series of pairwise regressions to ascertain which breeding sites contained more larvae, by extracting or calculating the density of larvae per dip. For this regression, we used density as our response variable and compared density values between each pair of breeding locations using a generalized linear model with a Gaussian family. To account for type I errors, we conducted a permutation test, where density values were shuffled while keeping the predictor variable (breeding site) unchanged to create a null distribution of t-values.

Finally, we compared the effectiveness of various control measures for *An. stephensi* adults, independently of larvae. We collected mortality rates for each insecticide, larvicide, and biocontrols (larvivorous fish). These were used to determine the median mortality rates for

each insecticide class with mortality rate as the response variable. All these data came from controlled laboratory bioassays. We scaled mortality rate values using the ‘scale’ function in R to aid data interpretation. The resulting vector had a mean of zero and a standard deviation of one. Since the assumption of normality was violated for all intervention controls, we applied a Kruskal-Wallis test (a nonparametric test) to compare the median mortality rates across different larvicides and larvivorous fish in the larvae stage. We then performed Dunn’s test as a post-hoc analysis with a Bonferroni correction to account for multiple comparisons (pairwise differences between specific larvicides/fish). All data analyses were conducted in R, and all data and analysis codes are available on GitHub (<https://github.com/tabethmwema/Anopheles-stephensi/>).

3. Results

A total of 3209 studies were initially targeted for consideration in this study. Of these, 798 duplicates were identified and excluded, and 1662 studies were determined to be irrelevant to our research questions. After applying exclusion criteria, a final subset of 749 studies remained for consideration, among which 83 ultimately met our inclusion criteria with 72 originating from native range countries and 11 from invasive range countries (Supplementary Fig. 1). Data on behavior and breeding habitats were extracted from 38 to 25 articles, respectively. Furthermore, 47 articles contained intervention data, while 11 articles included both bionomics and intervention data. Our database ultimately included data from studies conducted in *An. stephensi* native (Afghanistan, India, Iran, Iraq, Pakistan, and Qatar) and in invasive (Djibouti, Ethiopia, Sri Lanka, and Saudi Arabia, and Sudan) ranges.

The majority of collection methods were found to be equally effective in capturing mosquitoes, as indicated by the number of mosquitoes collected per day per method. In the native range, only one out of the possible pair-wise comparisons between biting methods exhibited significant differences in collection rate (Tables 1 and 2). These comparisons revealed that host-seeking animal-baited traps were a more efficient method of capturing biting mosquitoes than host-seeking human-baited traps such as human landing catches. Using host-seeking animal-baited traps, an average of 0.35 log transformed mosquitoes per unit effort were collected, and this was decreased by 1.86 per unit effort in human-baited traps ($p = 0.04$). Aspiration proved to be the most efficient method for resting mosquito collection in the native range, collecting 1.98 more log transformed mosquitoes per unit time than passive traps ($p = 0.04$). Passive traps, in turn, collected 3.71 more log transformed mosquitoes than pyrethrum spray catch ($p = 0.00$). There was no significant difference between aspirations and pyrethrum spray catches. In its invasive range, we did not find any significant differences between methods targeting biting mosquitoes. However, resting *An. stephensi* were collected more efficiently with passive traps, collecting 1.28 and 2.41 more log transformed mosquitoes than aspirations ($p = 0.03$) and pyrethrum spray catches ($p < 0.001$; Table 2), respectively. Finally, we also considered the influence of indoor compared to outdoor collection efforts in the native range of *An. stephensi*. (We did not have sufficient data to make this comparison in the invasive range.) We found that a similar number of adults were collected indoors and outdoors, using both biting and resting targeting methods (Table 3).

We compared larval habitat characteristics and larval density in *An. stephensi*'s native range in 21 total pairwise comparisons to understand breeding habitat preferences. In the native range, we found that mosquito larvae collected per day per breeding site was equal in all habitat categories (Table 4). We interpret this as a signal that these habitat types are all equally preferred breeding sites by *An. stephensi* in its native range. In the invasive range, we found the highest mean densities of 8.75 ($p = 0.01$) and 13.61 ($p = 0.01$) per 10 dips in water reservoirs and wastewater, respectively, in comparison to discarded human goods (Table 4). Since there were fewer studies of *An. stephensi* in the invasive range, our database included fewer breeding site categories compared to the native range breeding site analysis. Despite this, these early trends may indicate increased reliance on human-related water sources in the invasive range compared to *An. stephensi* populations in the native range.

We were also interested in understanding the relative effectiveness of synthetic organic compounds, bacterial larvicides, microbial fungi and biological controls used to reduce *An. stephensi* populations in their native and invasive ranges. To address this question, we separated control methods for the adults and larvae into the chemical class of their active ingredients or mode of action and analyzed them based on mosquito life stage. Specifically for the comparison of effectiveness of adulticides in the *An. stephensi* native range, organochlorine class chemicals had a lower median mortality than organophosphates ($p < 0.001$), pyrethroids ($p < 0.001$), pyrroles ($p < 0.001$) and carbamates ($p < 0.001$; Table 5). No significant differences were identified between insecticide comparisons in the introduced range, although we do note that reduced sample sizes from the invasive range dataset may have limited our ability to systematically detect these differences.

Although these results indicated the efficacy of some adulticides over others in the native range, instances of insecticide resistance have been reported across numerous insecticides both in the native and invasive ranges (Eshghy and Janbakhsh, 1976; Safi et al., 2019; Vatandoost and Hanafi-Bojd, 2012). Hence, we compared the effectiveness of controls for larvae. We compared the mortality effects of bacterial larvicides (*Bacillus sphaericus* and *Bacillus thuringiensis israelensis*), microbial fungus (*Beauveria bassiana*), biological control (larvivorous fish - *Gambusia affinis*, *Aphanius dispar*, and *Poecilia reticulata*), as well as synthetic organic compounds (organophosphate) fenthion and temephos. (We identified only two data points related to the use of *Aplocheilus blockii* so these data were excluded from the analysis).

In our comparison of the median mortality rate of synthetic organic compounds, bacterial larvicides, and biological control (larvivorous fish), we found that the Kruskal-Wallis rank sum test suggested a statistically significant overall difference in the variable mortality rate among the larval controls ($\chi^2 = 113$, $df = 7$, $p < 0.01$), indicating variability in their effectiveness. Follow-up pairwise comparisons using Dunn's test with Bonferroni adjustment revealed several significant differences (Table 6). *Bacillus thuringiensis israelensis* had a higher efficacy when compared to *Aphanius dispar* ($p = 0.02$) and *Bacillus sphaericus* ($p = 0.02$), but a lower efficacy when compared to *Beauveria bassiana* ($p < 0.01$) as well as synthetic compounds fenthion ($p < 0.01$) and temephos ($p = 0.01$). The synthetic compounds of fenthion and temephos were more effective than the biological controls *Aphanius dispar* ($p < 0.001$ for both compounds) and *Gambusia affinis* ($p < 0.001$

for both compounds) as well as *Bacillus sphaericus* ($p < 0.001$ for both compounds; Table 6). For the control of larvae in their invasive range, sufficient data were only available for comparison between *Bacillus thuringiensis israelensis* and temephos, but no significant difference between these two controls was detected.

4. Discussion

This systematic review highlights variations in the ecology and bionomics of *An. stephensi* between the native and invasive ranges. This may be due to the fact that many of the studies in the invasive range include only a small subset of the phenotypic and behavioral variation that is present in the native range. For example, it is likely that studies in our database were focused on the same urban ecotype (Faulde et al., 2014; Balkew et al., 2020; Ahmed et al., 2021; Balkew et al., 2021), which is found in the native range alongside a wide range of alternative ecotypes (Al-Ghoury et al., 2006; Amani et al., 2014; Ali et al., 2015; Alkhayat et al., 2020). We observed hints of this when evaluating the efficiency of *An. stephensi* mosquito collection methods that targeted biting mosquitoes; there were significant differences in method efficiency in the native range but no significant differences were observed between these trapping methods in the invasive range (Table 2), potentially due to the limited number of studies available as well as the limited number of effective options for trapping this species with a reduced diversity of phenotypes.

The variability in *An. stephensi* behavior and collection efficiency may also be influenced by local conditions such as host availability, including human and animal presence, and environmental factors. Although our results show that host seeking animal baited traps are more efficient at collecting adults than host seeking human baited traps, seasonal variations in feeding behavior have been observed; for instance, a study from Iran reported fluctuating host preferences of *An. stephensi* across different seasons (Basseri et al., 2010). Furthermore, the anthropophilic index shows considerable variability, ranging from as high as 100 % in urban areas (Hati, 1997) to as low as 0.5 % in rural settings (Basseri et al., 2005; Manouchehri et al., 1976). Contrasting findings from Iran (Edalat et al., 2020; Tyagi and Yadav, 2001) and rural Ethiopia (Ashine et al., 2024; Balkew et al., 2021) further exemplify this variability, underscoring the potential of *An. stephensi* to adapt its feeding behavior based on available hosts.

Some studies suggest that invasive *An. stephensi* in Africa may prefer biting outdoors over indoors (Sinka et al., 2020), thus evading current malaria control tools. Based on the literature in its endemic range and our analysis here (Table 3), *An. stephensi* tends to bite both indoors and outdoors in its native range. For example, Ghosh et al. (2010) reported a greater number of *An. stephensi* mosquitoes biting and resting indoors than outdoors in India. These behaviors may vary based on environmental conditions, as Ghosh et al. (2010) also showed that biting and resting behavior were strongly correlated with temperature and humidity. *Anopheles stephensi* may also breed in stagnant water found in roof gutters and on rooftops in its native range (Ravishankaran et al., 2021). Our data suggest that *An. stephensi* in the invasive range may be more reliant on water sources related to human needs (e.g. wastewater, water reservoirs) compared to populations in the native range (Table 4), potentially increasing contact with human populations. However, this early signal may be

refined as additional efforts in the invasive range collect data from wet and dry vegetated sites to form a basis for a more robust comparison.

In the control of *An. stephensi*, our systematic review underscores the differential efficacy observed with various intervention strategies across native and invasive populations. Analyzed across studies, we found that pyrethroids and carbamates reduce adult mosquito populations more effectively than other compounds in the native range (Table 5). However, the evolving resistance among mosquito populations necessitates the integration of resistance management strategies within control programs (Cohnstaedt et al., 2012). Although we did not have the statistical power to detect significant differences between adult control methods in the invasive range, we expect that similar patterns are present based on the evolution of resistance documented in some populations (Balkew et al., 2021).

Finally, control of *An. stephensi* is often accomplished by targeting larvae since insecticide resistance has been reported to most of the adult-targeting chemicals used in both the native (Enayati et al., 2020; Eshghy and Janbakhsh, 1976; Gorouhi et al., 2018; Safi et al., 2019; Vatandoost and Hanafi-Bojd, 2012) and invasive range (Ahmed et al., 2022; Ali et al., 2022; Balkew et al., 2021; Govoetchan et al., 2023; Yared et al., 2020). In our dataset, which focused exclusively on data from the native range, we found that synthetic organic compounds reduced larval populations better than the majority of bacterial larvicides, microbial fungus, and biological controls included in our dataset (Table 6). However, the continual application of these compounds can lead to increasing resistance levels among mosquito populations to these larvicides, necessitating periodic reassessment of chemical strategies (World Health Organization, 2016). Our analyses also suggest that biological controls, including larvivorous fish, are a viable alternative that avoids chemical resistance issues. Yet, these methods are not without their ecological risks, primarily when non-native species are introduced into new habitats, as such introductions can lead to unintended consequences on local biodiversity (Juliano and Lounibos, 2005; Walton, 2007). Given these complexities, the choice of larval control measures is likely to become increasingly situation-dependent and increasingly influenced by specific environmental contexts and community goals. As these variables change over time, so too may the preferred control strategies, underscoring the need for adaptive management practices that can evolve in response to changing ecological and social landscapes (Barrett and Arcese, 1995; Beier et al., 2008; Chaffin et al., 2016).

One further factor to consider is the design of surveillance and control programs in Africa. The challenges in understanding the behavior and implications of this relatively new invasive species, are intensified by the limited scope and geography of studies conducted on the African continent. Despite the ever-increasing spread of this mosquito to new regions, there is a lack of comprehensive research that captures the full extent of its ecological and behavioral characteristics. Although studies from the native range provide valuable insights into the biology and epidemiology of *An. stephensi*, it can be problematic to extrapolate these findings to invasive ranges due to differences in environmental conditions, host availability, and vector control measures. Therefore, the limited availability of data from Africa poses a significant barrier to accurately predicting the species' impact on malaria transmission dynamics and informing targeted surveillance and control strategies in regions

where it has recently invaded. Addressing this limitation requires concerted efforts to expand research initiatives in African countries affected by the invasion of *An. stephensi*. This can be achieved through fostering collaborations between researchers, public health agencies, and local communities to generate context-specific knowledge that is essential for effective malaria control interventions.

Based on the findings of this systematic and quantitative review, an increase in research efforts is needed to fill knowledge gaps about *An. stephensi* bionomics and control in its invasive range. Robust surveillance programs are crucial for monitoring the spread and population dynamics of *An. stephensi*. Our results suggest that aspiration and passive traps targeting adult mosquitoes, along with surveying larvae in water reservoirs and wastewater may be effective. Efficient monitoring should include molecular identification and geographic information systems to map the spread of *An. stephensi*, with special attention to potential ecological adaptations. In addition, identifying and managing potential larval habitats for *An. stephensi* control is important, focusing on habitats associated with human activities, such as water reservoirs and stagnant water in urban environments. In areas with prevalent water bodies, biological control (e.g., larvivorous fish), based on the literature here, may be promising. However, biological control efforts should be conducted cautiously, considering the ecological impacts of non-native species. An integrated approach combining chemical, biological, and environmental management strategies may enhance control efforts, while promoting community involvement and education can significantly contribute to the effectiveness of these measures (World Health Organization, 2024).

Finally, empowering and working alongside communities to eliminate potential mosquito breeding sites around houses and participate in surveillance activities may create sustainable and long-term solutions to malaria programs. Approaches focused on social and behavior change to encourage evidence-based actions at the individual, household, and community levels are essential in addressing the growing threat of *An. stephensi* populations in Africa (Hunter et al., 2023). By leveraging published literature to implement proven surveillance and control strategies, countries can improve their preparedness and response to *An. stephensi* invasions, ultimately reducing the risks of malaria transmission and protecting public health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

Data and code publicly available at the GitHub repository location included in the manuscript.

References

- Ahmed A, Abubakr M, Ali Y, Siddig EE, Mohamed NS, 2022. Vector control strategy for *Anopheles stephensi* in Africa. *Lancet Microb.* 3, e403.
- Ahmed A, Khogali R, Elnour MAB, Nakao R, Salim B, 2021. Emergence of the invasive malaria vector *Anopheles stephensi* in Khartoum State, Central Sudan. *Parasit Vect.* 14, 1–5.
- Al-Ghoury AA, El-Hashimi WK, Abul-Hab J, 2006. Epidemiology of malaria and predictions of retransmission in Babylon government, Iraq. *East Mediterr. Health J.* 12, 270–279. [PubMed: 17037694]
- Ali N, Noreen S, Khan K, Wahid S, 2015. Population dynamics of mosquitoes and malaria vector incrimination in district Charsadda, Khyber Pakhtunkhwa (KP) Pakistan. *Acta Trop.* 141, 25–31. [PubMed: 25255965]
- Ali S, Samake JN, Spear J, Carter TE, 2022. Morphological identification and genetic characterization of *Anopheles stephensi* in Somaliland. *Parasit. Vect.* 15, 247.
- Alkhayat FA, Ahmad HA, Rahim J, Dieng H, Ismail BA, Imran M, Sheikh UAA, Shahzad MS, Abid AD, Munawar K, 2020. Characterization of mosquito larval habitats in Qatar. *Saudi J. Biol. Sci.* 27, 2358–2365. [PubMed: 32884417]
- Amani H, Yghoobi-Ershadi MR, Kassiri H, 2014. The ecology and larval habitats characteristics of anopheline mosquitoes (Diptera: Culicidae) in Aligudarz County (Luristan province, western Iran). *Asian Pac. J. Trop. Biomed* 4, S233–S241. [PubMed: 25183088]
- Ashine T, Eyasu A, Asmamaw Y, Simma E, Zemene E, Epstein A, Brown R, Negash N, Kochora A, Reynolds AM, Bulto MG, Tafesse T, Dagne A, Lukus B, Esayas E, Behaksra SW, Woldekidan K, Kassa FA, Deressa JD, Assefa M, Dillu D, Assefa G, Solomon H, Zeynudin A, Massebo F, Sedda L, Donnelly MJ, Wilson AL, Weetman D, Gadisa E, Yewhalaw D, 2024. Spatiotemporal distribution and bionomics of *Anopheles stephensi* in different ecoepidemiological settings in Ethiopia. *Parasit. Vect.* 17, 166.
- Ashine T, Tekla H, Esayas E, Messenger LA, Chali W, Meerstein-Kessel L, Walker T, Behaksra SW, Lanke K, Heutink R, Jeffries CL, Mekonnen DA, Hailemeskel E, Tebeje SK, Tafesse T, Gashaw A, Tsegaye T, Emiru T, Simon K, Bogale EA, Yohannes G, Kedir S, Shumie G, Sabir SA, Mumba P, Dengela D, Kolaczinski JH, Wilson A, Churcher TS, Chibsa S, Murphy M, Balkew M, Irish S, Drakeley C, Gadisa E, Bousema T, Tadesse FG, 2020. *Anopheles stephensi* as an emerging malaria vector in the Horn of Africa with high susceptibility to Ethiopian *Plasmodium vivax* and *Plasmodium falciparum* isolates. *bioRxiv*. 10.1101/2020.02.22.961284.
- Balkew M, Mumba P, Yohannes G, Abiy E, Getachew D, Yared S, Worku A, Gebresilassie A, Tadesse FG, Gadisa E, Esayas E, Ashine T, Ejeta D, Dugassa S, Yohannes M, Lemma W, Yewhalaw D, Chibsa S, Tekla H, Murphy M, Yoshimizu M, Dengela D, Zohdy S, Irish S, 2021. An update on the distribution, bionomics, and insecticide susceptibility of *Anopheles stephensi* in Ethiopia, 2018–2020. *Malar. J.* 20, 263. [PubMed: 34107943]
- Balkew M, Mumba P, Dengela D, Yohannes G, Getachew D, Yared S, Chibsa S, Murphy M, George K, Lopez K, Janies D, 2020. Geographical distribution of *Anopheles stephensi* in eastern Ethiopia. *Parasit Vect.* 13, 1–8.
- Barrett CB, Arcese P, 1995. Are Integrated Conservation-Development Projects (ICDPs) sustainable? On the conservation of large mammals in sub-Saharan Africa. *World Dev* 23, 1073–1084.
- Basseri H, Raeisi A, Ranjbar Khakha M, Pakarai A, Abdolghafar H, 2010. Seasonal abundance and host-feeding patterns of anopheline vectors in malaria endemic area of Iran. *J. Parasitol. Res.* 2010, 671291. [PubMed: 21559055]
- Basseri HR, Moosakazemi SH, Yosafi S, Mohebbi M, Hajaran H, Jedari M, 2005. Anthropophily of malaria vectors in Kahnouj District, South of Kerman, Iran. *Iran. J. Public Health* 34, 27–35.
- Beier JC, Keating J, Githure JI, Macdonald MB, Impoinvil DE, Novak RJ, 2008. Integrated vector management for malaria control. *Malar. J.* 7 (1), S4. Suppl.
- Chaffin BC, Garmestani AS, Gunderson LH, Benson MH, Angeler DG, Arnold CAT, Cosens B, Craig RK, Ruhl JB, Allen CR, 2016. Transformative environmental governance. *Annu. Rev. Environ. Resour.* 41, 399–423. [PubMed: 32607083]

- Cohnstaedt LW, Rochon K, Duehl AJ, Anderson JF, Barrera R, Su N-Y, Gerry AC, Obenauer PJ, Campbell JF, Lysyk TJ, Allan SA, 2012. Arthropod surveillance programs: basic components, strategies, and analysis. *Ann. Entomol. Soc. Am.* 105, 135–149. [PubMed: 26543242]
- Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Arie F, Hanpithakpong W, Lee SJ, Ringwald P, Silamut K, Imwong M, Chotivanich K, Lim P, Herdman T, An SS, Yeung S, Singhasivanon P, Day NPJ, Lindegardh N, Socheat D, White NJ, 2009. Artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* 361, 455–467. [PubMed: 19641202]
- Edalat H, Mahmoudi M, Sedaghat MM, Moosa-Kazemi SH, Kheirandish S, 2020. Ecology of malaria vectors in an endemic area, southeast of Iran. *J. Arthropod Borne Dis* 14, 325–343. [PubMed: 33954207]
- Emiru T, Getachew D, Murphy M, Sedda L, Ejigu LA, Bulto MG, Byrne I, Demisse M, Abdo M, Chali W, Elliott A, Vickers EN, Aranda-Díaz A, Alemayehu L, Behaksera SW, Jebessa G, Dinka H, Tsegaye T, Tekla H, Chibsa S, Mumba P, Girma S, Hwang J, Yoshimizu M, Sutcliffe A, Taffese HS, Bayissa GA, Zohdy S, Tongren JE, Drakeley C, Greenhouse B, Bousema T, Tadesse FG, 2023. Evidence for a role of *Anopheles stephensi* in the spread of drug- and diagnosis-resistant malaria in Africa. *Nat. Med.* 29, 3203–3211. [PubMed: 37884028]
- Enayati A, Hanafi-Bojd AA, Sedaghat MM, Zaim M, Hemingway J, 2020. Evolution of insecticide resistance and its mechanisms in *Anopheles stephensi* in the WHO Eastern Mediterranean Region. *Malar. J.* 19, 258. [PubMed: 32680514]
- Eshghy N, Janbakhsh B, 1976. Insecticide resistance of *Anopheles stephensi* mysorensis in the province of Fars, southern Iran. 1975. *Mosq. New.* 36, 336–339.
- Faulde MK, Rueda LM, Khairah BA, 2014. First record of the Asian malaria vector *Anopheles stephensi* and its possible role in the resurgence of malaria in Djibouti, Horn of Africa. *Acta Trop.* 139, 39–43. [PubMed: 25004439]
- Ghosh A, Mandal S, Chandra G, 2010. Seasonal distribution, parity, resting, host-seeking behavior and association of malarial parasites of *Anopheles stephensi* Liston in Kolkata, West Bengal. *Entomol. Res.* 40, 46–54.
- Golassa L, Messele A, Amambua-Ngwa A, Swedberg G, 2020. High prevalence and extended deletions in *Plasmodium falciparum* hrp2/3 genomic loci in Ethiopia. *PLOS ONE* 15, e0241807. [PubMed: 33152025]
- Gorouhi MA, Oshaghi MA, Vatandoost H, Enayati AA, Raeisi A, Abai MR, Salim-Abadie Y, Hanafi-Bojd AA, Paksa A, Nikpoor F, 2018. Biochemical basis of cyfluthrin and DDT resistance in *Anopheles stephensi* (Diptera: culicidae) in malarious area of Iran. *J. Arthropod. Borne. Dis.* 12, 310–320.
- Govoetchan R, Ibrahim MM, Sovi A, Omar HM, Boulhan AO, Darar HY, 2023. *Anopheles stephensi*: the emerging vector of malaria in the Republic of Djibouti, Horn of Africa. *Int. J. Biosci.* 10.12692/ijb/22.1.8-17.
- Hati AK, 1997. Urban malaria vector biology. *India. J. Med. Res.* 106, 149–163.
- Juliano SA, Lounibos LP, 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecol. Lett.* 8, 558–574. [PubMed: 17637849]
- Hunter G, Hughes J, Zhody S, Cash S, Avrokotos A, Yoshimizu M, Ebihara LE, Higginbotham B, Tompsett A, Armistead J, & Monroe A (2023, February). Social and Behavior Change Guidance for *Anopheles stephensi* in Africa. Johns Hopkins University. <https://breakthroughactionandresearch.org/sbc-guidance-for-anopheles-stephensi-in-africa>.
- Manouchehri AV, Javadian E, Eshghy N, Motabar M, 1976. Ecology of *Anopheles stephensi* Liston in southern Iran. *Trop. Geogr. Med.* 28, 228–232. [PubMed: 1006792]
- Mojahedi AR, Safari R, Yarian M, Pakari A, Raeisi A, Edalat H, Beniardelan M, Poudat A, Zaim M, Basseri HR, 2020. Biting and resting behaviour of malaria vectors in Bandar-Abbas County, Islamic Republic of Iran. *East. Mediterr. Health J* 26, 1218–1226. [PubMed: 33103749]
- Niyukuri D, Sinzinkayo D, Troth EV, Oduma CO, Barengayabo M, Ndereyimana M, Holzschuh A, Vera-Arias CA, Gebre Y, Badu K, Nyandwi J, Baza D, Juma E, Koepfli C, 2022. Performance of highly sensitive and conventional rapid diagnostic tests for clinical and subclinical *Plasmodium falciparum* infections, and hrp2/3 deletion status in Burundi. *PLOS Global Public Health* 2, e0000828. [PubMed: 36962426]

- Okanda D, Ndwiga L, Osoti V, Achieng N, Wambua J, Ngetsa C, Lubell-Doughtie P, Shankar A, Bejon P, Ochola-Oyier LI, 2023. Low frequency of *Plasmodium falciparum* hrp2/3 deletions from symptomatic infections at a primary healthcare facility in Kilifi, Kenya. *Front. Epidemiol* 3.
- Ravishankaran S, Asokan A, Justin NAJA, Thomas S, Mathai MT, Eapen A, 2021. Are dawn collections of *Anopheles stephensi* a better method to estimate the resting vector density? A study from Chennai, India. *Am. J. Trop. Med. Hyg.* 105, 507–510. [PubMed: 34181571]
- Safi NHZ, Ahmadi AA, Nahzat S, Warusavithana S, Safi N, Valadan R, Shemshadian A, Sharifi M, Enayati A, Hemingway J, 2019. Status of insecticide resistance and its biochemical and molecular mechanisms in *Anopheles stephensi* (Diptera: culicidae) from Afghanistan. *Malar. J.* 18, 249. [PubMed: 31349836]
- Sinka ME, Bangs MJ, Manguin S, Chareonviriyaphap T, Patil AP, Temperley WH, Gething PW, Elyazar IRF, Kabaria CW, Harbach RE, Hay SI, 2011. The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic précis. *Parasit. Vect.* 4, 89.
- Sinka ME, Pironon S, Massey NC, Longbottom J, Hemingway J, Moyes CL, Willis KJ, 2020. A new malaria vector in Africa: predicting the expansion range of *Anopheles stephensi* and identifying the urban populations at risk. *Proc. Natl. Acad. Sci. U. S. A.* 117, 24900–24908. [PubMed: 32929020]
- Sougoufara S, Diédhiou SM, Doucouré S, Diagne N, Sembène PM, Harry M, Trape J-F, Sokhna C, Ndiath MO, 2014. Biting by *Anopheles funestus* in broad daylight after use of long-lasting insecticidal nets: a new challenge to malaria elimination. *Malar. J.* 13, 125. [PubMed: 24678587]
- Surendran SN, Sivabalakrishnan K, Sivasingham A, Jayadas TTP, Karvannan K, Santhirasegaram S, Gajapathy K, Senthilnathanan M, Karunaratne SP, Ramasamy R, 2019. Anthropogenic factors driving recent range expansion of the malaria vector *Anopheles stephensi*. *Front. Public Health* 7, 53. [PubMed: 30923705]
- Tadesse FG, Ashine T, Teka H, Esayas E, Messenger LA, Chali W, Meerstein-Kessel L, Walker T, Wolde Behaksra S, Lanke K, Heutink R, Jeffries CL, Mekonnen DA, Hailemeskel E, Tebeje SK, Tafesse T, Gashaw A, Tsegaye T, Emiru T, Simon K, Bogale EA, Yohannes G, Kedir S, Shumie G, Sabir SA, Mumba P, Dengela D, Kolaczinski JH, Wilson A, Churcher TS, Chibsa S, Murphy M, Balkew M, Irish S, Drakeley C, Gadisa E, Bousema T, 2021. *Anopheles stephensi* Mosquitoes as Vectors of *Plasmodium vivax* and *falciparum*, Horn of Africa, 2019. *Emerg. Infect. Dis.* 27, 603–607. [PubMed: 33496217]
- Thomas S, Ravishankaran S, Justin JA, Asokan A, Mathai MT, Valecha N, Thomas MB, Eapen A, 2016. Overhead tank is the potential breeding habitat of *Anopheles stephensi* in an urban transmission setting of Chennai, India. *Malar. J.* 15, 274. [PubMed: 27169513]
- Tyagi BK, Yadav SP, 2001. Bionomics of malaria vectors in two physiographically different areas of the epidemic-prone Thar Desert, north-western Rajasthan (India). *J. Arid Environ.* 47, 161–172.
- Vatandoost H, Hanafi-Bojd AA, 2012. Indication of pyrethroid resistance in the main malaria vector, *Anopheles stephensi* from Iran. *Asian Pac. J. Trop. Med.* 5, 722–726. [PubMed: 22805725]
- Waka M, Hopkins RJ, Glinwood R, Curtis C, 2006. The effect of repellents *Ocimum forskolei* and deet on the response of *Anopheles stephensi* to host odours. *Med. Vet. Entomol.* 20, 373–376. [PubMed: 17199748]
- Walton WE, 2007. Larvivorous fish including *Gambusia*. *J. Am. Mosq. Control Assoc.* 23, 184–220. [PubMed: 17853606]
- World Health Organization, 2023. World Malaria Report 2023. World Health Organization.
- World Health Organization, 2016. Test Procedures For Insecticide Resistance Monitoring in Malaria Vector Mosquitoes. World Health Organization.
- World Health Organization, 2024. Surveillance and Control of *Anopheles stephensi*: Country Experiences 2024 World Health Organization. CC BY-NC-SA 3.0 IGO.
- World Health Organization, 1975. Manual on Practical Entomology in Malaria.
- Yared S, Gebressielasie A, Damodaran L, Bonnell V, Lopez K, Janies D, Carter TE, 2020. Insecticide resistance in *Anopheles stephensi* in Somali Region, eastern Ethiopia. *Malar. J.* 19, 180. [PubMed: 32398055]

Table 1

Summary of statistical models used in the study. Here, each study question is noted with the data collected across our systematic review, the regression model we used to address the question, and the justification and hypotheses for each question.

Question	Data used	Model used	Justification
1. What <i>An. stephensi</i> adult collection techniques are successful in capturing <i>An. stephensi</i> ?	Collection rate per method	Logged linear regression, followed by permutation test	We log-transformed the data to normalize its distribution. We used a permutation test to test the hypothesis that mosquito collection rates differed between biting and resting collection methods.
2. Are there significant differences in <i>An. stephensi</i> preference for biting and resting indoors compared to outdoors?	Collection rate per method	We used a logged linear regression where the collection method was the predictor variable and collection rate was the response variable. Then residuals were extracted from the first model and used in the second model as the predictor variable, with collection methods as the response variable.	We log-transformed the data to normalize the distribution of skewed data. We used a permutation test to test the hypothesis that mosquito biting and resting rates differed between indoors and outdoors.
3. Which larval habitat characteristics predict the presence of <i>An. stephensi</i> and indicate likely breeding sites?	Breeding site characteristics and larval density	We log-transformed the effect size for our linear regression with breeding site as the predictor variable and larval density as the response variable. A permutation test was then used.	We log-transformed the data to normalize the distribution of skewed data. We used a permutation test to test the hypothesis that mosquito larval density differed between habitat types.
4. Which <i>An. stephensi</i> control interventions are effective for adults and larvae?	Insecticides, bacterial, fungal, and synthetic organic chemical larvicides, larvivoracious fish, and mortality rate.	Shapiro-Wilk test for normality. Kruskal-Wallis test and Dunn's test with Bonferroni adjustment.	The data were not normally distributed, so we used Kruskal-Wallis' test and Dunn's test with a Bonferroni adjustment to determine the median mortality rate for different insecticide, bacterial, fungal, and synthetic organic chemical larvicides, and larvivoracious fish.

Table 2

Pairwise regression comparing biting and resting collection methods of *An. stephensi* in its native and invasive ranges. Response variables in each regression are the log of the number of *Anopheles stephensi* captured per day per method.

Collection method comparison	Intercept estimate (t-value, p-value)	Slope estimate (t-value, p-value)	df	R ²
<i>Native range – biting targeting methods</i>				
Host-seeking animal baited trap vs. Host-seeking human baited	0.35 (0.58, 0.04)	-1.86 (-2.12, 0.04)	23	0.16
Host-seeking animal trap vs. Host-seeking mechanical baited trap	0.35 (0.60, 0.11)	-1.49 (-1.68, 0.07)	21	0.12
Host-seeking human baited trap vs. Host-seeking mechanical baited	-1.51 (-2.93, 0.00)	0.37 (0.49, 0.38)	20	0.01
<i>Native range – resting targeting methods</i>				
Aspiration targeting resting mosquitoes vs. Pyrethrum spray catch	0.50 (-0.96, 0.27)	1.73 (1.92, 0.053)	34	0.10
Passive trap vs. Pyrethrum spray catch	-2.48 (-2.23, 0.04)	3.71 (2.98, 0.00)	13	0.41
Aspiration targeting resting mosquitoes vs. Passive trap	-0.50 (-0.92, 0.00)	-1.98 (-1.23, 0.04)	25	0.06
<i>Invasive range – biting targeting methods</i>				
Host-seeking animal baited vs. Host-seeking mechanical traps	-1.89 (-4.98, 0.00)	-1.12 (-2.21, 0.16)	9	0.35
Host-seeking animal-baited vs. Host-seeking human baited	-1.89 (-5.90, 0.00)	-1.05 (-2.20, 0.15)	9	0.35
Host-seeking human baited vs. Host-seeking mechanical traps	-2.95 (-7.91, 0.00)	-0.072 (-0.14, 0.44)	8	0.00
<i>Invasive range – resting targeting methods</i>				
Aspiration targeting resting mosquitoes vs. Passive trap	-1.61 (-2.11, 0.00)	1.28 (0.84, 0.03)	6	0.11
Passive trap vs. Pyrethrum spray catch	-0.33 (-0.37, 0.00)	-2.41 (-1.13, 0.00)	6	0.48
Aspiration targeting resting mosquitoes vs. Pyrethrum spray catch	-1.61 (-2.27, 0.00)	-1.14 (-1.13, 0.12)	10	0.11

Significant comparisons are in bold.

Table 3

Propensity of biting and resting behavior of *An. stephensi* in its native range based on the biting and resting behaviors related to collection rates and methods. Response variables in each regression are the log of the number of mosquitoes captured per day per method.

Comparison	Estimate (CI)	Standard error	t-value	R ²	df	p-value
<i>Native range</i>						
Biting targeting methods: Indoor vs. outdoor	0.05 (−0.86, 0.97)	0.44	0.12	0.00	21	0.90
Resting targeting methods: Indoor vs. outdoor	0.04 (−1.09, 1.19)	0.55	0.08	0.00	27	0.94

Table 4

Estimates of median larval density in different breeding sites in the native ranges of *An. stephensi*, and their permuted p-values.

Contrasts	Estimate (CI)	Standard error	t-value	Permutated p-value
<i>Native range</i>				
Discarded human goods vs.				
Water reservoirs	0.01 (0.00, 0.95)	11.84	-1.98	0.10
Dry vegetated areas	0.20 (0.02, 1.96)	3.23	-1.39	0.18
Non-vegetated areas	0.21 (0.02, 2.89)	3.82	-1.17	0.29
Waste water	0.03 (0.00, 13.49)	21.59	-1.11	0.28
Wet vegetated areas	0.19 (0.00, 16.05)	9.58	- 0.73	0.49
Water reservoirs vs.				
Dry vegetated areas	26.17 (1.16, 590)	4.91	2.05	0.08
Non-vegetated areas	27.82 (0.07, 10,895)	21.04	1.09	0.37
Waste water	4.36 (0.02, 922)	15.37	0.54	0.59
Wet vegetated areas	25.50 (0.33, 1997)	9.25	1.46	0.18
Dry vegetated areas vs.				
Non-vegetated areas	1.06 (0.07, 16.4)	4.04	0.04	0.99
Waste water	0.17 (0.00, 7.16)	6.82	- 0.93	0.41
Wet vegetated areas	0.97 (0.05, 17.7)	4.39	0.02	0.98
Non-vegetated areas vs.				
Waste water	0.16 (0.00, 279)	45.57	- 0.49	0.79
Wet vegetated areas	0.92 (0.00, 212)	16.09	- 0.03	0.97
Waste water vs.				
Wet vegetated areas	5.85 (0.03, 1005)	13.81	0.67	0.50
<i>Invasive range</i>				
Discarded human goods vs.				
Water reservoirs	8.75 (1.56, 49.13)	2.41	2.46	0.01
Waste water	13.61 (2.86, 64.74)	2.22	3.28	0.01
Water reservoirs				
Waste water	1.56 (0.20, 12.07)	2.84	0.42	0.68

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Table 5

Comparison of the median mortality rate across different adulticides in their native range, where column categories were compared to each row category (e.g., a significant and positive mean indicates a larger column mortality compared to the row category).

	Carbamate	Organochlorine	Organophosphate	Pyrethroid
<i>Native range</i>				
Organochlorine	5.4 (<0.001)			
Organophosphate	2.4 (0.08)	-3.2 (<0.001)		
Pyrethroid	1.2 (1.00)	-7.7 (<0.001)	-2.0 (0.25)	
Pyrroles	-1.2(1.00)	-3.3 (<0.001)	-2.2 (0.13)	-1.7 (0.45)
<i>Invasive range</i>				
Organophosphate	-1.0(0.50)			
Pyrethroid	-1.3 (0.27)	- 0.01 (1.0)		

The numerical values presented in parentheses for each comparison indicate Bonferoni-adjusted p-values, with the bolded values representing statistical significance.

Table 6

Comparison of the column-row median mortality rate of synthetic organic compounds, biological larvicides, and larvivorous fish in the native range of *An. stephensi*, where column categories were compared to each row category (e.g. a significant and positive mean indicates a larger column mortality compared to the row category). A Kruskal-Wallis rank sum test was used to assess disparities in efficacy across five classes of insecticides and subsequent pairwise comparisons were carried out using Dunn’s test with Bonferroni adjustment.

	<i>Bacillus sphaericus</i>	<i>Bacillus thuringiensis israelensis</i>	<i>Beauveria bassiana</i>	<i>Aphanius dispar</i>	<i>Gambusia affinis</i>	<i>Poecilia reticulata</i>	Fenthion
Bacterial larvicide							
<i>Bacillus sphaericus</i>							
<i>Bacillus thuringiensis israelensis</i>	-3.1 (-0.02)						
Microbial fungus							
<i>Beauveria bassiana</i>	-7.7 (<0.01)	-4.6 (<0.01)					
Biological control (larvivorous fish)							
<i>Aphanius dispar</i>	2.2 (0.37)	3.2 (0.02)	4.8 (<0.01)				
<i>Gambusia affinis</i>	-0.06 (1.0)	2.4 (-0.24)	6.0 (<0.01)	-2.2 (0.42)			
<i>Poecilia reticulata</i>	-0.58 (1.00)	0.41 (1.0)	2.0 (0.68)	-2.0 (0.59)	-0.55 (1.00)		
Synthetic organic compound							
Fenthion	-5.1 (<0.01)	-3.8 (<0.01)	-1.8 (1.0)	-5.0 (<0.01)	-4.8 (<0.01)	-2.7 (0.09)	
Temephos	-6.0 (<0.01)	-4.0 (<0.01)	-1.1 (1.0)	-5.0 (<0.01)	-5.1 (<0.01)	-2.4 (0.24)	0.8 (1.0)

The numerical values presented in parentheses for each comparison indicate adjusted p-values, with the bolded values representing statistical significance.