Effects of Seasonal Conditions on Abundance of Malaria Vector *Anopheles stephensi* Mosquitoes, Djibouti, 2018–2021

Alia Zayed, Manal Moustafa, Reham Tageldin, James F. Harwood

We describe the influence of seasonal meteorologic variations and rainfall events on *Anopheles stephensi* mosquito populations during a 40-month surveillance study at a US military base in Djibouti. Focusing surveillance and risk mitigation for *An. stephensi* mosquitoes when climatic conditions are optimal presents an opportunity for malaria prevention and control in eastern Africa.

Anopheles stephensi mosquitoes, an urban malaria vector, have established robust populations in the Horn of Africa. Since the mosquito's detection in 2012 (*1*), malaria cases in Djibouti increased 42.9-fold during 2013–2021, reaching ≈72,300 cases (*2*). Before introduction of *An. stephensi* mosquitoes, Djibouti was approaching the preelimination phase for malaria (*3*). Because *An. stephensi* mosquitoes are competent vectors for *Plasmodium falciparum* and *P. vivax* parasites (*3*), WHO considers this mosquito species a major threat to malaria elimination in Africa (*4*). *An. stephensi* mosquitoes have also been detected in Sudan, Ethiopia, and Somalia (*5–8*). Understanding *An. stephensi* mosquito adaptation to environmental conditions affecting population dynamics in urban settings is crucial in Africa. *An. stephensi* mosquitoes abundance (number of mosquitoes collected per trap night) changed from seasonal during fall–spring 2013–2016 to year-round in 2017 (*3*). Since *An. stephensi* mosquitoes were introduced, malaria cases have increased among military personnel, some immunologically naive, deployed as members of multinational militaries in Djibouti (*9*). Camp Lemonnier (CLDJ), a US naval base, has urban characteristics similar to the city of Djibouti, in which it is located. For this study, we monitored vector dynamics on the base, providing data to help inform health protection strategies among both military and civilian populations.

The Study

In coordination with the CLDJ Expeditionary Medical Facility, during January 2018–April 2021, we conducted weekly mosquito surveillance at 32 on-base sites covering 2 km^2 and stored information in dataset A. In October 2019, we began identifying monthly captures of *An. stephensi* mosquitoes specifically (i.e., identified at the species level) (dataset B). We set US Centers for Disease Control and Prevention (CDC) CO_2-baited Miniature Light traps (https://www.cdc.gov/mosquitoes/guidelines/west-nile/surveillance/environmental-surveillance.html) and Woodstream Mosquito Magnet (MM) propane-generated CO_2 traps (https://www.woodstream.com) overnight near dwellings, dining areas, sport facilities, and other areas frequented by humans. We identified *Anopheles* species on the basis of criteria published elsewhere (*10,11*). We analyzed abundance data in the context of specific weather events and seasonal climatic trends at the time of collection. We obtained meteorologic data from several sources (Appendix, https://wwwnc.cdc.gov/EID/article/29/4/22-0549-App1.pdf), using latitude 11.54733 N and longitude 43.15948 E (0.6–1.2 km from study sites) for location and a locally appropriate meteorologic calendar to determine seasons. We assessed the effects on *An. stephensi* mosquito abundance of monthly mean temperatures and rainfall amounts at time of precipitation and at 2-week, and 1- and 2-month lag times (i.e., time after rainfall). We did not consider longer lag times because of the likely effects of evaporation.

Author affiliations: US Naval Medical Research Unit No. 3, Cairo Detachment, Cairo, Egypt (A. Zayed, M. Moustafa, R. Tageldin); US Naval Medical Research Unit No. 3, Sigonella, Italy (J. Harwood); Cairo University, Cairo, Egypt (A. Zayed)

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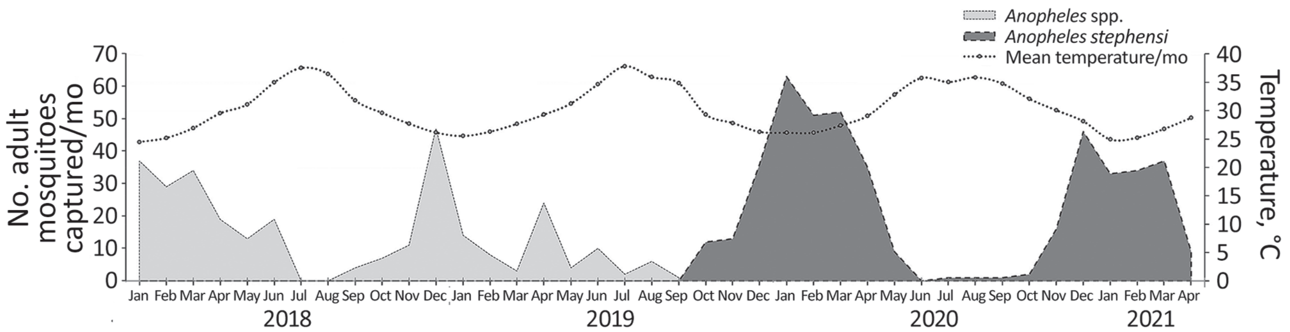

Figure 1. Associations between numbers of adult mosquitoes captured and mean temperature, by month, US military base, Djibouti, September 2019–August 2020. (We began identifying *Anopheles stephensi* mosquitoes specifically in October 2019.

We used the Shapiro-Wilk test to check normal distribution of *An. stephensi* mosquito data and Pearson correlation coefficient to evaluate relationships between mosquito abundance and climatic variables. We categorized temperatures as either above or equal to or below median annual temperature (30°C). We

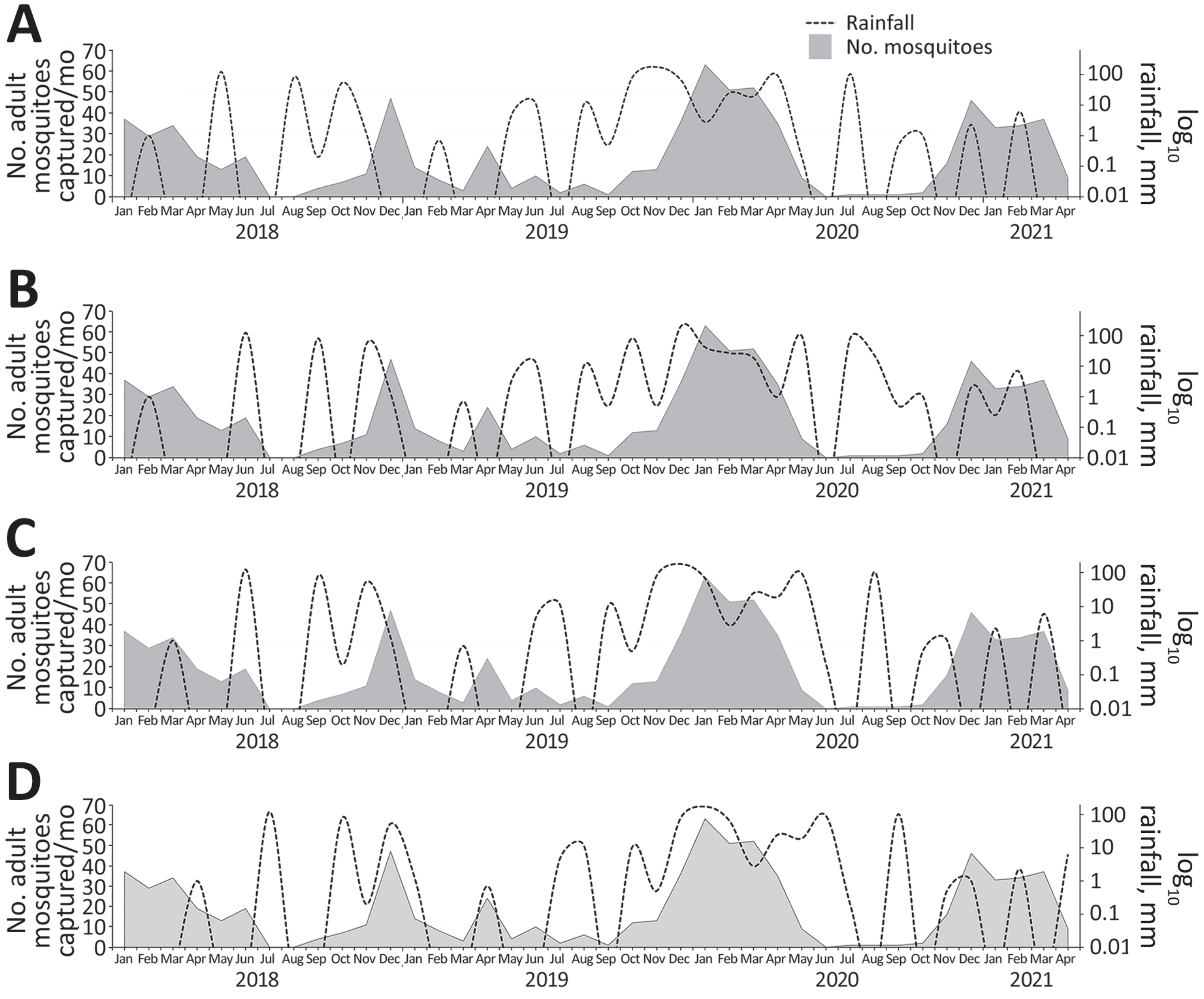

Figure 2. Associations between monthly collected numbers of *Anopheles stephensi* mosquitoes captured and precipitation rates, US military base, Djibouti, September 2019–August 2020. A) At time of rainfall; B) 2 weeks after rainfall; C) 1 month after rainfall; D) 2 months after rainfall.

Table 1. Univariate Poisson regression analysis of lagged effects of rainfall on abundance of *Anopheles stephensi* mosquitoes 2 weeks, 1 month, and 2 months after rainfall periods, US military base, Djibouti, September 2019–August 2020*

		Regression analysis		
Time after rainfall	Rainfall level, mm/wk	IRR (95% CI)	p value	Abundance
2 wk	40–155	0.56 (0.3–1.1)	0.09	2.3
	21.1–39.9	2.4 (1.7–3.4)	<0.0001	9.6
	5–21	1.5 (0.9–2.5)	0.11	6
	0.2–4.9	2.59 (2–3.4)	<0.0001	10.4
	0	Referent		4
1 mo	40–155	1.86 (0.9–2.2)	0.009	7
	21.1– 39.9	2.99 (2–3.8)	<0.0001	11.3
	5–21	1.13 (0.9–2.4)	0.6	4.3
	0.2–4.9	2.58 (1.5–2.7)	<0.0001	9.8
	0	Referent		3.8
2 mo	40–155	1.37 (1.2–3)	0.17	5.5
	21.1–39.9	2.75 (2.1–4.2)	<0.0001	11
	5–21	1.42 (0.7–1.9)	0.18	5.7
	0.2–4.9	2 (1.9–3.5)	<0.0001	8
	0	Referent		4

*Abundance is the average number of mosquitoes per trap night. IRR, incidence rate ratio.

grouped rainfall data according to frequency at each of 5 levels: 0, 0.2–4.9, 5–21, 21.1–39.9, and 40–155 mm/week. We used Poisson regression for univariate and multivariate analyses to determine associations between mosquito abundance and predictor variables, and used PROC GENMOD in SAS version 9.4 (SAS Institute, Inc., https://www.sas.com) to perform logistic regression. We expressed results in incidence rate ratios (IRR) and used p = 0.05 as the cutoff for statistical significance.

An. stephensi represented 95.6% of all *Anopheles* spp. mosquitoes we identified. Using dataset B to compare effectiveness of trap types, we found that MM traps captured 25.6% more *An. stephensi* mosquitoes than did CDC traps (IRR 2.3; p<0.0001) (Appendix Table, Figure). Univariate regression analysis of datasets A and B (Appendix Table) demonstrated that *An. stephensi* mosquito populations persisted year-round. Related to seasonal distribution, in dataset A, winter accounted for 56.4% of *Anopheles* spp. mosquito captures; spring, 28.1%; fall, 9.8%; and summer, 5.7%. In dataset B, winter accounted for 55.2% of *An. stephensi* mosquito captures; spring, 37.1%; fall, 6.9%; and summer, 0.8%. Associations between *An. stephensi* mosquito abundance and monthly mean temperatures (Figure 1) were positive for temperatures ≤30 (IRR 5.5 for dataset A, 7.4 for dataset B; p<0.0001). In dataset A, 85% of *Anopheles* spp. mosquitoes were collected at temperatures ≤30°C; for dataset B, the percentage was 94% of *An. stephensi* mosquitoes (Appendix Table).

Mosquito abundance increased 4–8 weeks after flooding in November 2019 (Figure 2). We also analyzed data on mosquito abundance 2 weeks and 1 and 2 months after rainfall throughout September 2019–August 2020, during which time 2 floods occurred (Table 1). Regression analysis showed significant associations between rainfall and *Anopheles* mosquito abundance recorded 2 weeks (IRR 2.4), 1 month (IRR 2.99), and 2 months (IRR 2.75) after periods of rainfall 21.1–39.9

Table 2. Multivariate Poisson regression analysis of seasonal and climatic factors associated with *Anopheles stephensi* mosquito abundance with and without lag effect after rainfall periods, US military base, Djibouti, September 2019–August 2020*

	No lag effect		1-mo lag effect	
Variable	IRR (95% CI)	p value	IRR (95% CI)	p value
Seasons				
Winter	4.2 (2.7–6.3)	<0.0001	4.12 (2.7–6.2)	<0.0001
Spring	2.8 (1.9–4.2)	<0.0001	2.86 (1.9–4.2)	<0.0001
Fall	1.3 (0.8–1.9)	0.3	1.19 (0.8–1.8)	0.42
Summer	Referent		Referent	
Temperature, °C				
≤30	2.4 (1.9–3.1)	<0.0001	2.2 (1.7–2.9)	<0.0001
>30	Referent		Referent	NA
Rain amounts, mm/wk				
40–155	0.33 (0.2–0.7)	0.004	1.2 (0.8–1.8)	0.4
21.1–39.9	1.1 (0.8–1.5)	0.6	1.5 (1.2–2.1)	0.0024
5–21	0.9 (0.6–1.3)	0.53	0.9 (0.6–1.5)	0.7
0.2–4.9	0.7 (0.6–0.9)	0.005	1.4 (1.2–1.7)	0.0002
0	Referent		Referent	

*NA, not applicable; IRR, incidence rate ratio.

mm/week ($p<0.0001$), corresponding to average mosquito counts of 9.6 (2 weeks), 11.3 (1 month), and 11.0 (2 months) after the rainfall. Unexpectedly, mosquito abundance also increased significantly 2 weeks (IRR 2.59), 1 month (IRR 2.58), and 2 months (IRR 2.00; $p<0.0001$) after periods of rainfall of just 0.2–4.9 mm/week. Multivariate analysis indicated that season and temperature were the variables most significantly associated with mosquito abundance when analyzed with no lag or 1-month rainfall lag effect. Winter (IRR 4.2 [no lag], 4.1 [1-month lag]; $p<0.0001$) and spring (IRR 2.8 [no lag], 2.9 [1-month lag]; $p<0.0001$) were the factors most associated with increases in *Anopheles* mosquitoes, followed by temperatures ≤30°C (IRR 2.4 [no lag], 2.2 [1-month lag]; $p<0.0001$) (Table 2).

Conclusions

We speculate that the slow continuous release of CO_2 of MM traps contributed to higher captures of *An. stephensi* mosquitoes than for CDC traps. In a study in Malaysia, MM traps performed 3-fold better than CDC traps for capturing *Anopheles* spp. mosquitoes (*12*), demonstrating the suitability of MM traps for *An. stephensi* mosquito surveillance in urban settings and areas with limited or no access to dry ice (*13*).

An. stephensi mosquitoes were present year-round but at substantially higher populations during winter (mean temperature 26°C, average rainfall 2.3 mm/week) and spring (mean temperature 29°C, average rainfall 7.3 mm/week). A previous study observed a similar link between temperature and *An. stephensi* mosquito populations, with 29°C assessed as optimal (*14*). We linked the bionomics of *An. stephensi* mosquito abundance in urban areas to human-modified conditions, such as air conditioning–produced condensation, water storage tanks, open jerry cans, and water-filled tires following rainfall, all of which increased favorable mosquito habitats (*1*) and in which we observed larval habitats around CLDJ. Flash flooding in Djibouti did not increase *An. stephensi* mosquito abundance. In fact, flooding might have destroyed laid eggs, hatched larvae, and temporary larval habitats, as was reported in China (*15*), possibly explaining higher population growth after periods of rainfall of 21.1–39.9 mm/week than 40–155 mm/week. Because breeding sites in urban areas depend as much on human-generated water sources as rainfall, adult mosquitoes were able to persist even during periods of low precipitation (*14*). We found that periods of rainfall at 21.1–39.9 mm/week and temperatures slightly <30°C were optimal for adult *An. stephensi* mosquito abundance. Therefore, surveillance and control efforts should be most intense during times of the year when these conditions are common. However, because *An. stephensi* mosquitoes are present year-round, prevention and control measures cannot be relaxed during any season (Appendix).

Although our study was set at CLDJ facilities, conditions were comparable to other urban settings in Djibouti, which should encourage local health authorities to benefit from our data. The persistence of mosquito populations at CLDJ, which regularly monitors and employs control efforts, should raise the alarm for increased malaria risk in densely populated city areas with fewer public health and disease control resources. Given limited resources, we recommend targeted reduction of *An. stephensi* larval habitat in this area.

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About the Author

Dr. Zayed is an entomologist at the US Naval Medical Research Unit-3, Cairo, with academic and research involvement in Middle Eastern countries. Her primary research interests are vector surveillance and control.

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Address of correspondence: Alia Zayed, US Naval Medical Research Unit No. 3, Cairo Detachment, Cairo, Egypt; email: alia.m.zayed.ln@health.mil

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Appendix

Additional Methods

Mosquito Magnet

Operational manual: https://www.mosquitomagnet.com

Meteorological Records

Daily records were acquired from US Air Force 14th Weather Squadron, (https://climate.af.mil/product_locator/station_page?networktype=AMIL&platformid=KQRH;) with Tuitempo archived weather supplementing missing events, (https://en.tutiempo.net/climate/10-2019/ws-631252.html) and flash flooding data acquired from the United Nations' Reliefweb site (https://reliefweb.int/sites/reliefweb.int/files/resources/Joint%20Flash%20Report%20Djibouti%20Flood%20-%20Nov%202019.pdf).

Results

Univariate analysis of abundance of *Anopheles stephensi* corresponding to predictor variables, and the efficiency of collection methods:

Univariate regression analysis of datasets A and B records (Table 1) proved the abundance of *An. stephensi* in all seasons with lower numbers in summer. Winter scored the highest records among seasons representing 56.4% of total mosquito numbers of dataset A and 55.2% of B. Compared to numbers in summer, density of vector in A was 9.7-fold higher in winter ($P<0.0001$), 4.9-fold higher in spring ($P<0.0001$) and 1.7-fold higher in fall ($P=0.01$), referring to IRR and parameter estimate values of the regression analysis. Number of mosquitoes in B was 52-fold higher in winter (P<0.0001), 36.6-fold higher in spring (P<0.0001) and 8.6-fold higher in fall (P=0.003) compared to summer records.

Trap Type Comparison

Comparison of the two trap types was conducted on surveillance data from October 2019 to April 2021 (Fig. 1).

Conclusion

The optimum temperature for *An. stephensi* adult population falls within the thermal breadth for transmission of *Plasmodium falciparum* and *P. vivax* (15.3-37.2 C and 15.7-32.5 C, respectively). This further exacerbates the year-round malaria transmission risk in Djibouti, knowing that *An.stephensi* is currently present in all seasons (*1*).

Reference

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Appendix Table. Univariate analysis of abundance of *Anopheles stephensi* corresponding to predictor variables, and the efficiency of collection methods

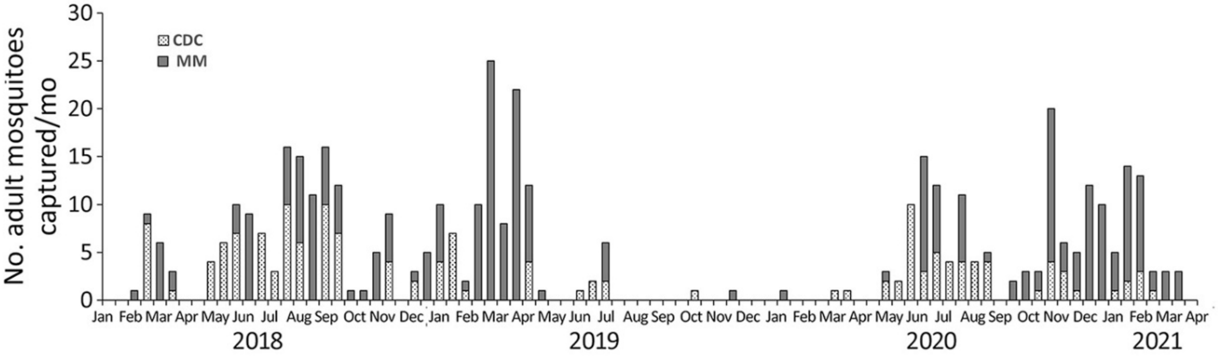
	Regression analysis (A)*			Regression analysis (B)*		
Variables/ Methods	IRR (95%CI)	P	n (%)†	IRR (95%CI)	P	n (%)†
Seasons†						
Winter	9.7 (6.9, 13.4)	<0.0001	387 (56.4)	52 (13, 210)	<0.0001	143 (55.2)
Spring	4.9 (3.4, 6.8)	<0.0001	193 (28.1)	36.6 (9, 148.3)	<0.0001	96 (37.1)
Fall	1.7 (1.1, 2.5)	0.01	67 (9.8)	8.9 (2.1, 38.3)	0.003	18 (6.9)
Summer	Reference (1)		39 (5.7)	Reference (1)		2 (0.8)
Temperature, °C						
≤30	5.5 (4.5,6.6)	<0.0001	722 (85.1)	7.4 (5.0, 11.0)	<0.0001	407 (94)
≥30	Reference (1)		126 (14.6)	Reference (1)		24 (6)
Trap type§						
MM				2.3 (1.8, 2.7)	<0.0001	258 (62.8)
CDC				Reference (1)		153 (37.2)

*Univariate Poisson regression analysis of data of the genus Anopheles from January 2018-April 2021 (Regression analysis (A)) and the precisely identified An. stephensi data from October 2019-April 2021 (Regression analysis (B)); CI: confidence intervals. IRR: Incidence Rate Ratio.

†Total number of mosquitoes with percentage of the collected mosquitoes represented for the variable in parentheses.

‡Seasonal analysis was conducted on data representing the four seasons of dataset A (March 2018-Febraury 2021) and the four seasons of dataset B (December 2019-November 2020).

§Trap types: MM: Mosquito Magnet. CDC: Disease Control and Prevention miniature light traps. Winter (December; January; February), spring (March; April; May), summer (June; July; August) and fall (September; October; November).



Appendix Figure. Weekly numbers of collected *Anopheles stephensi* from October 2019 to April 2021 using CDC or Mosquito Magnet (MM) traps.