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URBANIZATION DETERMINES THE ABUNDANCE OF DISEASE VECTOR MOSQUITOES IN MOOREA, FRENCH POLYNESIA

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ABSTRACT Mosquitoes are important disease vectors of infectious diseases. The effect of anthropogenic habitat modification is essential to the abundance and dissemination of mosquitoes. However, little is known about anthropogenic habitat modification's effect in the context of remote, oceanic, and tropical islands. This study assessed the impact that habitat modification, specifically urbanization, had on different mosquito species on the island of Moorea, French Polynesia. We measured the abundance of three mosquito species across a gradient characterized by the quantity of built environment and human population density. This urbanization gradient was represented by four low-elevation sites along the coasts of Opunohu Bay and Cook's Bay. A cluster of four different mosquito traps were rotated between these sites in 24-hour intervals. A total of 874 individual mosquitoes were collected and *Aedes aegypti* and *Culex quinquefasciatus* were found to be more abundant in urban areas. *Aedes polynesiensis* was found to be more abundant in forested areas. When comparing abundance between the rainy and dry season, *Aedes aegypti* numbers were five times greater during the rainy season. *Culex quinquefasciatus* and *Aedes polynesiensis* numbers were less than they were in the dry season. These findings suggest that the effects of urbanization were species-specific and dependent on a nexus of socioecological factors, with significant correlations between mosquito abundance, urbanization, and seasonal climate conditions. The study also showed that different sampling methods designed to capture flying adult mosquitoes differed in both efficacy and selectivity, suggesting that trapping systems must be considered in establishing a sound assessment of mosquito community composition.

Major, Year, and Department: Molecular Environmental Biology, 3rd Year, Department of Environmental Sciences, Policy, and Management

INTRODUCTION

The persistence and emergence of mosquito-borne diseases is a significant public health concern.[1] In the face of climate change, rising temperatures and precipitation levels provide increasingly favorable environmental conditions in which mosquitoes may thrive and reproduce.[2] These conditions facilitate longer transmission seasons, increase biting frequency, and shift spatial patterns, which ultimately amplify the emergence of mosquito-borne diseases in novel host populations.[3]

Similarly, new pathways for invasion created by an increasingly globalized network of travel and trade have made it possible for many pathogens and their respective disease vectors, the transmitters of the pathogen, to occupy novel human-modified habitats.[3,4] As the rate of introduction of new invasive alien species seems to be higher than ever before, it is critical to understand the factors that promote the colonization and coexistence of invasive disease-transmitting organisms. The resulting impacts of such invasions are of particular importance in the tropical regions of Sub-Saharan Africa, Central and South America, and South and Southeast Asia, where low-income communities are often disproportionately affected by the burden of mosquito-borne illnesses due to a number of socioecological factors.[5]

One such socioecological factor of considerable significance includes anthropogenic land-use change.[6] According to the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), land-use change caused by human activity today has had the largest relative negative impact on the environment since that of the 1970s.[7] Aside from agricultural expansion—the most common form of land-use change—the doubling of urbanization in urban areas since 1992 and the expansion of infrastructure are major contributors to the decline of biodiversity worldwide.[7] These changes continue to impact the global ecosystem as the human population continues to grow, so it is essential to understand how different community assemblages react following these disturbances.

While the effect of habitat modifications on local ecosystems and community assemblages of disease vectors has been studied, there remains a lack of understanding of these effects specifically on remote oceanic islands.[6] Previous studies on isolated island systems have analyzed larval mosquito distributions, interspecific competition, and the container types that are preferred oviposition sites for gravid females.[8,9,10] However, little is known about how urban modification on islands affects the community structure of mosquitoes. This is especially important considering the rate at which human populations are growing in certain island communities. For instance, in the Commune of Moorea-Maiao, French Polynesia, the population experienced a 13% growth rate from 2002 to 2007, according to the French Polynesia Statistical Institute (2007).

The island of Moorea, French Polynesia is home to nine species of mosquitoes, including *Toxorhynchites amboinensis*, a species introduced in 1975 as a biocontrol agent against *Aedes* mosquitoes.[8,11] Two of the nine species are more recent introductions and breed in artificial containers: *Aedes aegypti*, which is the primary disease vector of Dengue virus, and *Culex quinquefasciatus*, which is a significant vector of West Nile Virus in other parts of the world.[10,11] *Aedes polynesiensis*, which was most likely introduced by the early Polynesians, can carry *Wuchereria bancrofti*, a parasitic worm that causes lymphatic filariasis and elephantiasis, or the condition of extreme swelling and hardening of one's limbs.[11] The remaining six species are more elusive and are not recognized as salient disease vectors in Moorea.[11]

Disease vector mosquitoes are especially important to study considering the history of infectious disease outbreaks that have occurred in French Polynesia in the past.[12] In 1996 and 1997, a dengue virus serotype 2 (DENV-2) outbreak arose in this region of the South Pacific. From October 2013 to April 2014, a Zika outbreak occurred, resulting in 66% of the entire population of French Polynesia to have been infected, followed by a number of microcephaly cases.[12] The Society Islands are currently undergoing another dengue outbreak, with at least 2400 cases of dengue fever between Tahiti and Moorea alone as of early January 2020.[13] The outbreak history in French Polynesia prompts further research on not only mosquitoes and their disease vector capabilities, but also how the man-made environment contributes to the prolongation of mosquito-borne disease epidemics on remote oceanic islands.

The objective of this study is to understand the role of urbanization–in terms of the relative increase in both the human population and the built environment within a geographical location–on the community composition of mosquitoes, using the island of Moorea as a study system. This study aims to determine the relative abundance and distribution of existing mosquito species in Moorea by utilizing a system of mosquito traps to monitor host-seeking *Aedes* mosquitoes. Each trap within this system, incorporating a combination of two different trap types equipped with or without a human scent imitator, was then assessed upon its effectivity and selectivity in terms of capturing flying adult mosquitoes, regardless of sex. Due to inconsistent capture methodologies in previous Moorea-based studies, different trapping combinations were evaluated to the most effective method for future studies.[8,9,10]

We postulate that the species introduced more recently to the island will predominate urban areas, characterized by more dense human populations and a more extensively built environment that supports a greater amount of artificial breeding sites. This may be due to a multitude of factors, but considering the absence of large mammalian species outside of human-inhabited areas on the island, urban areas may be the only, not just probable, sites for mosquitoes to find a blood meal.

METHODS

Study Site

This study took place on the island of Moorea, French Polynesia. We sampled four different coastal, low-elevation sites along the northern stretch of the island (Fig. 1). The climate of this region is characterized by high temperatures and humidity throughout the year, with daily temperatures averaging a maximum of 30°C and a minimum of 20°C. The rainy season occurs from November to March while the dry season extends from April to October.

At each of the four sampling sites, we captured flying adult mosquitoes using different combinations of Biogents (BG) mosquito traps for professional use and BG-Lures, which are small

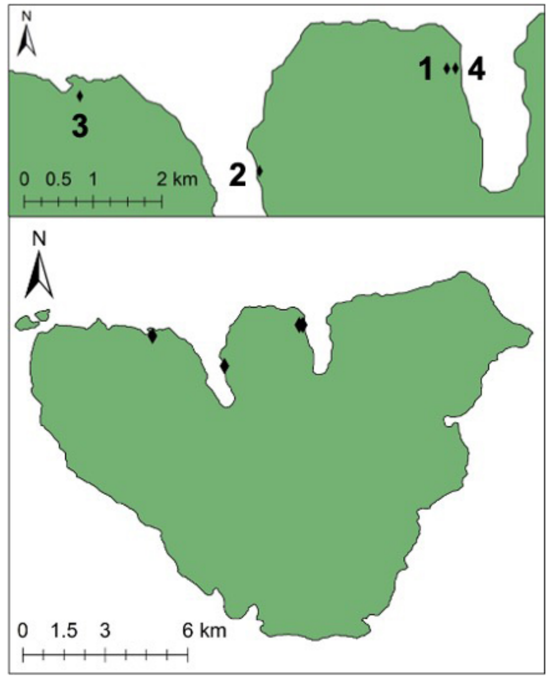

***Figure 1: (Bottom) Outline of Moorea, French Polynesia** marked with sampling sites. (Above) Close-up of sampling sites marked in order of increasing degrees of urbanization (1 least, 4 most urbanized).*

cartridges of chemicals that emit artificial human skin odors and are effective for up to five months. The four trap-lure combinations used in this study were as follows: a BG-Sentinel Trap (BG), a BG-Sentinel Trap with a BG-Lure cartridge (BGL), an EVS-style BG-Pro Trap (MTS), and an EVS-style BG-Pro Trap with a BG-Lure cartridge (MTSL).

We deployed each trapping system onto tree branches or supporting artificial structures marked with colored tape, indicating which trap-lure combination was placed there. All four trapping systems were placed together at the same sampling site and hung approximately 1 meter off of the ground. Traps were placed approximately 30 meters apart from each other at the modified forest site and the Gump Research Station, and approximately 10 meters apart at the rural house sites due to limited space.

Mosquito abundance across a rural-to-urban gradient

For the first part of the study, we treated the four trapping systems as one large, conglomerate trap that was rotated between the four sites. Traps, lures, and batteries were provided by the Institut Louis Malardé (ILM) based in Tahiti. The sites were selected to represent an urbanization gradient. Degree of urbanization was established based upon visual inspection of factors including vegetation type and coverage, land-use, quantity and categorization of built environment, number of human inhabitants, and local human population density (Table 1). Each site was sampled three times and traps were deployed for 24-hours starting and ending at the same time between 0900 and 1600 h from October 14, 2019 to November 9, 2019 (Table 1).

After every 24-hour treatment, labeled catch nets were transferred to a small cardboard box, and then frozen along with their

Site	Description	GPS Location	Sampling Dates
1. Lowland, modified forest	Abandoned coconut plantation, *Hibiscus spp.*, *Coco nucifera*, high canopy coverage, large quantity of dead organic matter (DOM), no human inhabitants, bisected by a road.	17°29'25.9"S 149°49'40.9"W	Oct. 14-15, 20-21, 27-28
2. Rural House: Opunohu	Rural residential house in cul-de-sac-like neighborhood, *Hibiscus spp.*, *M. indica*, assortment of house plants and pottery, intermediate canopy coverage, ~5-10 human inhabitants, dogs present, children present, built environment consists of living quarters and roads.	17°30'14.0"S 149°51'09.0"W	Oct. 16-17, 22-23 Nov. 8-9
3. Rural House: Papeto'ai	Rural residential house in a relatively dense, "city" setting, *Hibiscus spp.*, *M. indica*, assortment of house plants and pottery, intermediate canopy coverage, ~2-3 human inhabitants, built environment consists of living quarters, nearby road, outhouses, and cement platform in yard.	17°29'38.8"S 149°52'33.5"W	Oct. 29-30 Nov. 3-4, 6-7
4. Gump Research Station	Coastal research station with well-manicured lawn, *Hibiscus spp.*, *C. nucifera*, *G. taitensis*, *A. altillis*, little to no canopy coverage, ~25-30 human inhabitants, high population density, dogs present, lots of built environment including dormitories, dry and wet laboratories, boating dock, and road.	17°29'25.7"S 149°49'36.8"W	Oct. 17-18, 21-22, 28-29

Table 1: Table of sampling sites and their descriptions.

contents for at least 1 hour. Individual mosquitoes were then identified by species and sexed using identification keys provided by the ILM. The abundance of non-mosquito species caught was also recorded. Although *A. aegypti* larvae were discovered at the Gump Research Station, larvae and rafts were not included in the study due to difficulty in finding them elsewhere.

Seasonal comparison of mosquito abundance

In order to assess mosquito abundance in dry and wet conditions, trapping systems were set around the Gump Research Station during the rainy season on November 12, 13, and 14. Catch abundance during this period was compared to that of the urbanization study data collected at the Gump Research Station during the dry season in mid-October. The sampling approach was identical to that of examining a rural-to-urban gradient. Specifically, all four trap-lure combinations were considered one large, conglomerate trap in order to avoid trap bias and were deployed for 24-hour treatments, starting and ending at the same time between 0900 and 1100 h.

Trap-Lure system evaluation

This study evaluated the different trap-lure combinations in their effectiveness and selectivity in capturing mosquitoes of different species and sex by comparing abundance data for each trap-lure combination taken from both the rural-to-urban gradient and seasonal comparison traps.

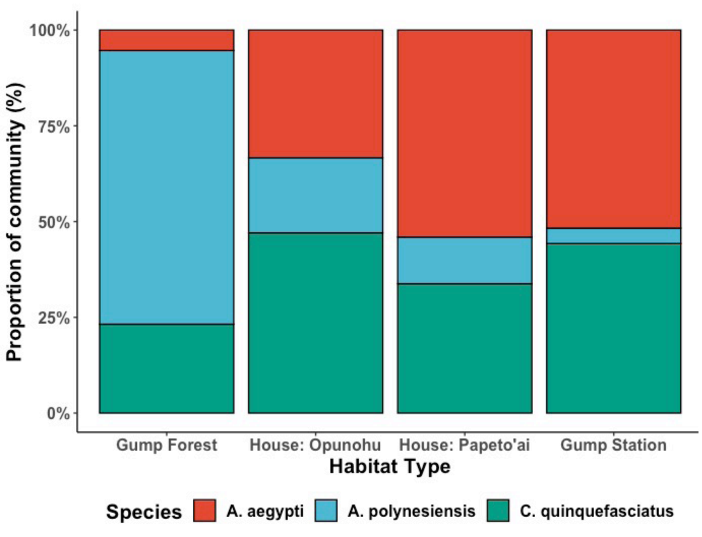

Figure 2: Overall mosquito community composition by species at each sampling site*, organized from least to most urbanized.*

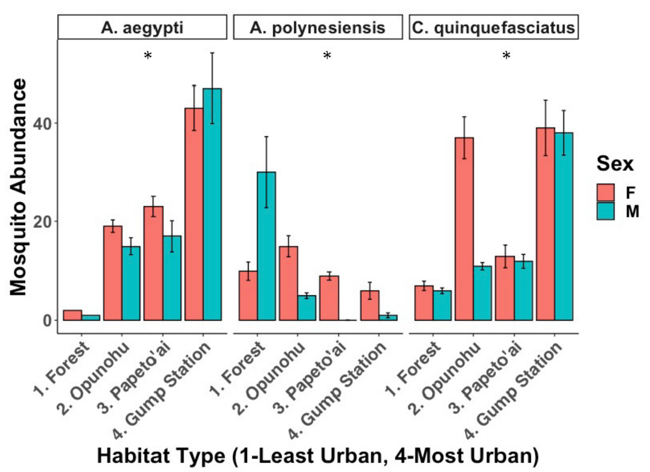

Figure 3: The effect of urbanization on mosquito abundance. *The abundance of* A. aegypti *and* C. quinquefasciatus *are higher in urban areas, whereas the abundance of* A. polynesiensis *is higher in forested areas. Error bars show standard deviation (SD).*

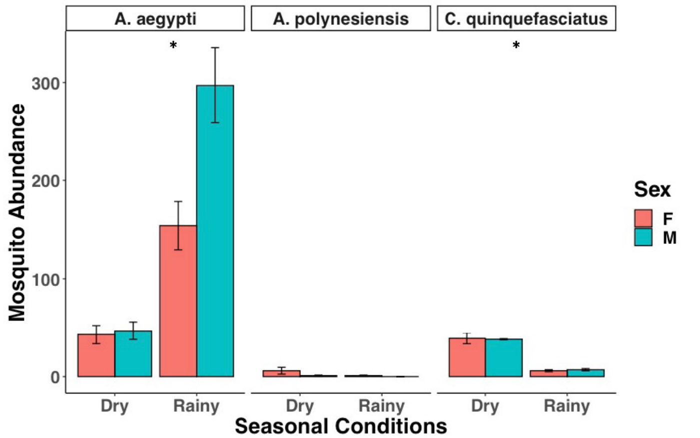

*Figure 4: **Mosquito abundance** during the transition from the dry season to the rainy season.* A. aegypti *abundance increases while* A. polynesiensis *and* C. quinquefasciatus *decrease. Error bars show standard deviation (SD).*

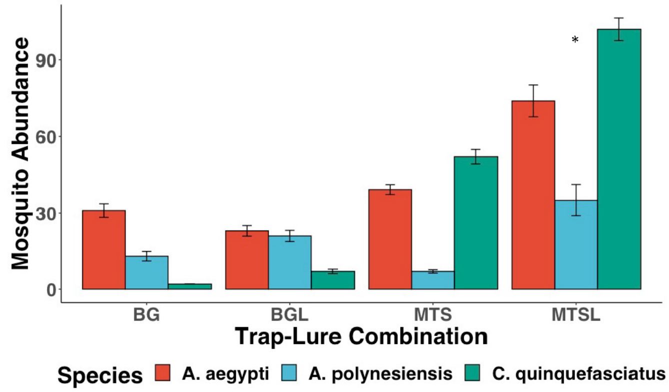

*Figure 5: **The evaluation of different mosquito trapping systems.** The trap-lure combination that caught the greatest number of mosquitoes was the MTS trap equipped with a BG-Lure. Error bars show standard deviation (SD).*

Statistical Analysis

To test for the differences in mosquito abundance, species richness, and sex across different urban habitat sites and captured via different trap-lure combinations, we used the X^2 (Chi-Square) Test of Independence and Generalized Linear Mixed-Effects Models (GLMM) with Poisson link function in the R package lme4 (Bates et al., 2015). ANOVA tests were used to compare GLMM's with and without the variable of interest. All statistical analyses were conducted using RStudio version 1.2.1335 (RStudio, Inc © 2009), with alpha = 0.05. Voucher specimens were deposited in the Essig Museum of Entomology (University of California, Berkeley, 94720).

RESULTS

Throughout the course of this study, we captured, identified, and sexed a total of 874 mosquitoes (Fig. 2). We observed *A. aegypti*, *A. polynesiensis*, and *C. quinquefasciatus* at all four sampling sites. Of all the caught mosquito species, *A. aegypti* (n=618) was the most abundant mosquito species, followed by *C. quinquefasciatus* (n=176), and *A. polynesiensis* (n=77). While *T. amboinensis* (n=2) and *Culex roseni* (n=1) were both present, these mosquito species were disregarded due to small sample sizes. Of all the mosquitoes captured at the lowland, modified forest region between the Gump Research Station and its hillside bungalows, *A. polynesiensis* (71.43%) was the most observed species, followed by *C. quinquefasciatus* (23.21%), and *A. aegypti* (5.36%). Of the mosquitoes trapped at the rural house located along the south eastern stretch of Opunohu Bay, *C. quinquefasciatus* was the most observed species (47.06%), followed by *A. aegypti* (33.33%), and *A. polynesiensis* (19.61%). At both the Gump Research Station and the rural house located along the outskirts of Papeto'ai, the most observed mosquito species were *A. aegypti* (house: 54.05%; station: 84.56%), followed by *C. quinquefasciatus* (house: 33.78%; station: 14.04%), and *A. polynesiensis* (house: 12.16%; station: 1.40%).

Mosquito abundance across a rural-to-urban gradient

The X^2 Test of Independence showed significant differences between the abundance of the three different mosquito species and habitat type (Fig. 3, X^2 =138.31, df=6, p<0.001). An ANOVA comparing two GLMMs, one with and one without habitat type, also showed significant differences (glmer, df=3, p<0.001). These tests determined that *A. aegypti* numbers increased significantly with increasing urbanization (glmer, df=3, p<0.001), as did the abundance of *C. quinquefasciatus* (glmer, df=3, p<0.001). On the other hand, *A. polynesiensis* numbers significantly decreased across this gradient (glmer, df=3, p<0.001).

Seasonal comparison of mosquito abundance

Significantly fewer mosquitos were caught during the dry season than the rainy season (Fig. 4, glmer, df=1, p<0.001). The number of *A. aegypti* caught during the rainy season (n =451) was five times greater than that of the dry season (n=90) (glmer, df=1, p<0.001). The number of *A. polynesiensis* (dry: n = 7; wet: n = 1) decreased during the transition from dry to wet conditions, but did not display a significant difference (glmer, df=1, p>0.05). The number of *C. quinquefasciatus* (dry: n = 32; wet: n = 13) significantly decreased during this transition (glmer, df=1, p<0.001).

Trap-Lure system evaluation

The X^2 Test of Independence showed significant differences between the abundance of the three different mosquito species and the trapping system used to capture the mosquitoes (Fig. 5, X^2=64.491, df=6, p<0.001). An ANOVA test comparing two GLMMs, one with and one without trap type, also showed significant differences (glmer, df=3, p<0.001). The MTSL trap-lure combination caught the largest total abundance of all three of the common mosquito species (n=211), followed by the MTS (n=98) and BGL traps (n=51). The BG trap caught the least mosquitoes overall (n=46). GLMMs indicated that the abundance of *A. aegypti* captured was significantly greater in the MTSL trapping system (glmer, df=3, p<0.001). These tests also indicated that the abundance of both *C. quinquefasciatus* captured (glmer, df=3, p<0.05), and *A. polynesiensis* captured (glmer, df=3, p<0.05) were greater in the MTSL trapping system.

DISCUSSION

In terms of the community composition of mosquitoes found on Moorea, *A. aegypti*, *C. quinquefasciatus*, and *A. polynesiensis* were the most abundant mosquito species on the island, respectively. Other species, such as *T. amboinensis* and *C. roseni*, were rarely seen. Some mosquito species known to have inhabited the Society Islands, including *C. annulirostris*, *C. atriceps*, *C. kesseli*, and *A. edgari*, were unobserved.[11]

The results of this study indicate that there is a correlation between the degree of urbanization and the abundance of mosquitoes. This correlation is specific to each species and in the context of remote, oceanic, tropical island systems. We found that *A. aegypti* and *C. quinquefasciatus*, two container-breeding species introduced most recently to Moorea, were more abundant in urban settings and increased in number along a rural-to-urban gradient. In contrast, we found that *A. polynesiensis*, introduced by early Polynesians, was more abundant in forest settings and decreased in number along the urbanization gradient. There was a greater abundance of mosquitoes overall in locations characterized by a higher human population density and a greater quantity of built environment. This supports the initial hypothesis made that *A. aegypti*, known to oviposit in artificial containers, and *C. quinquefasciatus* predominate urban areas due to a more extensive built environment that supports a greater amount of artificial breeding sites.

Mosquito abundance was also found to be correlated with environmental factors, such as seasonal climate conditions. During the transition from the dry season to the rainy season between October and November at the Gump Research Station, *A. aegypti* abundance rose five-fold while the abundance of *C. quinquefasciatus* and *A. polynesiensis* declined. It was also at this time towards the start of the rainy season that we found *A. aegypti* and *C. quinquefasciatus* mosquito larvae at two separate locations, both within the premises of the Gump Research Station. While larval sampling was disregarded in this study, future studies should incorporate larval sampling in addition to using trapping systems designed to catch flying adults. This will create a better and more comprehensive approach to studying the ecology of disease vector mosquitoes.

The results of the trap design evaluation showed that of the four different trap-lure combinations, the EVS-style BG-Pro trap equipped with a BG-Lure cartridge, or MTSL trap, was the most effective trap design for all of the observed mosquito species. The next best trapping system was the MTS trap without a lure, followed by the BG trap with a lure, and lastly the BG trap without a lure. These findings are consistent with the unpublished raw data released by Biogents in 2019 of a number of Latin square studies around the world indicating that the BG-Pro trap has been more effective than the BG-Sentinel when it comes to capturing *A. aegypti* mosquitoes.[14] This may in fact be due to the improvements in suction design and product quality on the BG-Pro, Biogents' newest development.

These trapping systems were far more efficient than those used in a previous trap-assessment study on Moorea, given that this past study was only able to capture 82 mosquitoes over 14 days, as opposed to the 874 mosquitoes caught in this study over 15 sampling days.[15] Despite its small sample size, the results of the previous study aligned with ours in that *A. aegypti* and *C. quinquefasciatus* were caught most often at urban sites.[15]

A majority of the other mosquito-related studies on Moorea determined that rain-filled, rat-chewed coconuts were the most common oviposit sites for gravid female mosquitoes to lay their eggs.[8,9] In terms of species eradication efforts and disease outbreak control programs, this would suggest that the removal of fallen coconut debris would be the most effective treatment.[8,9] However, this solution may not be as effective as initially expected. Due to the understanding that *A. aegypti* predominate urban sites and *A. polynesiensis* predominate forest sites, the proposed removal of fallen coconut debris could potentially reduce natural breeding grounds of *A. polynesiensis*. Furthermore, backed by Pocock's conclusions that *A. polynesiensis* larvae outcompete *A. aegypti* larvae in natural containers when food is present, this decrease of natural containers would then reduce the pressure of spatial competition on *A. aegypti*, allowing this disease vector species to spread, which would only advance the spread of dengue fever in the South Pacific.[10]

It must be taken into account that disease vector distribution within this island system differs in continental systems. The trend established by this study that mosquito abundance is higher in urban areas than natural areas in tropical islands largely contrasts with the findings of similar studies in continental settings.[7] In multiple regions of Europe including Spain, Belgium, and the Netherlands, mosquito communities are lower in abundance in urban and rural areas and higher in natural areas.[16,17,18] However, it is recognized in these studies that these results are species-specific responses. The mosquito species that favor artificial habitats regardless of being in continental or island systems include *Anopheles*, *Culex*, and *Aedes*–three major disease vector mosquito species.[6] This coincides with the findings of this study that disease vector mosquitoes, *C. quinquefasciatus* and *A. aegypti* in particular, had a positive relationship with urbanization.

Urbanization, however, is not the only form of anthropogenic land-use change influencing the distribution of disease vector mosquitoes and other invasive species. Increased trade, agricultural expansion, and other human population dynamics associated with urbanization have led to an increase in invasive species worldwide by 40% since 1980.[7] This leaves nearly one-fifth of the Earth's surface at risk for biological invasions, putting economies as well as human health at risk.[7] Therefore, it is more than necessary to be cognizant of this multitude of factors that come into play in this highly interconnected, globalized ecosystem.

Studying the factors that influence the abundance and distribution of mosquito vectors of human diseases also provides valuable information for public health management officials to identify priority areas for epidemiologic surveillance and vector control programs in preparation against large epidemics or outbreaks.

Having a better understanding of the associations between urbanization and the abundance and distribution of disease vector mosquitoes in Moorea can increase our awareness of how human-related changes such as urbanization are ultimately impacting the ecosystem and human health in the greater region of Oceania.

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