Mosquito surveillance in maritime entry ports in Miami-Dade County, Florida to increase preparedness and allow the early detection of invasive mosquito species

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Abstract

Invasive mosquito vector species have been inadvertently transported to new areas by humans for decades. Strong evidence supports that monitoring maritime, terrestrial, and aerial points of entry is an essential part of the effort to curb the invasion and establishment of invasive vector mosquito species. Miami-Dade County, Florida is an important operational hub for the cruise ship industry and leisure boats that routinely visit nearby areas in the Caribbean, and freight cargo ships transporting goods from Miami-Dade to Caribbean countries and vice versa. To deal with the increasing public health concern, we hypothesized that mosquito surveillance in small- and medium-sized maritime ports of entry in Miami-Dade is crucial to allow the early detection of invasive mosquito species. Therefore, we have selected 12 small- and medium-sized maritime ports of entry in Miami-Dade County with an increased flow of people and commodities that were not covered by the current mosquito surveillance system. Collection sites were comprised of two distinct environments, four marinas with international traffic of leisure boats, and eight maintenance and commercial freight cargo ship ports. Mosquitoes were collected weekly at each of the 12 collection sites for 24 hours for 6 weeks in the Spring and then for 6 additional weeks in the Summer using BG-Sentinel traps. A total of 32,590 mosquitoes were collected, with Culex quinquefasciatus and Aedes aegypti being the most abundant species totaling 19,987 and 11,247 specimens collected, respectively. Our results show that important mosquito vector species were present in great numbers in all of the 12 maritime ports of entry surveyed during this study. The relative abundance of Cx. quinquefasciatus and Ae. aegypti was substantially higher in the commercial freight cargo ship ports than in the marinas. These results indicate that even though both areas are conducive for the proliferation of vector mosquitoes, the port area in the Miami River is especially suitable for the proliferation of vector mosquitoes. Therefore, this potentially allows the establishment of invasive mosquito species inadvertently brought in by cargo freights.
Introduction

Invasive vector mosquito species are a growing major public health concern [1]. Current estimates indicate that 129 countries and territories have conducive environments able to support the proliferation of invasive mosquito vector species [2–4]. The levels of mosquito-borne disease transmission continue to grow due to increased levels of proliferation and dispersion of invasive mosquito vector species across spatiotemporal scales greatly driving their presence and abundance in new areas [5–7].

Invasive mosquito vector species are being passively transported to new areas by humans for decades [8]. However, current levels of mobility with a worldwide increased flow of people and commodities have raised the risk of the introduction of invasive mosquito species considerably [9]. Several countries, such as New Zealand, The Netherlands, and Portugal, have been able to early detect and prevent the invasion of mosquito species that were being passively transported in tires, machinery, and by cars and commercial airplanes [10–12].

Unfortunately, that was not the case in many other countries where invasive mosquito vector species, such as *Aedes albopictus*, were able to successfully invade and become established [7]. *Aedes albopictus* is a primary vector of chikungunya, dengue, yellow fever, and Zika viruses [13], and subsequently to its invasion and establishment in most of Europe, arbovirus outbreaks were reported in Croatia, France, and Italy [14–16]. Strong evidence supports that monitoring maritime, terrestrial, and aerial points of entry is an essential part of the effort to curb the invasion and establishment of invasive vector mosquito species [17]. Different invasive mosquito species use different mechanisms to invade new areas and reliable and effective mosquito surveillance systems that can generate consistent and actionable data are paramount to avoid the spreading of invasive mosquito species into new areas [10, 18, 19].

Miami-Dade County, Florida is one of the most important gateways into the United States. Miami-Dade is located at a strategic geographic location being an important touristic and commercial entry point. Miami-Dade is one of the most important touristic destinations in the United States, receiving an average of over 120 million visitors every year [20]. Importantly, Miami-Dade has an increased flow of people and goods coming and going from the Caribbean region and Latin America. Miami-Dade is an important operational hub for the cruise ship industry, serving as the main port not only for cruise ships supplying the Caribbean and Gulf of Mexico region but also for leisure boats that routinely visit nearby areas in the Caribbean. Furthermore, freight cargo ships routinely transport goods from Miami-Dade to Caribbean countries and vice versa, including Haiti, Dominican Republic, and Cuba. Such high levels of human mobility and commodity trade increase the risk of the introduction of invasive species to Miami-Dade.

*Culex coronator* was able to successfully invade and colonize Miami-Dade County in less than 10 years from its first detection becoming one of the most abundant mosquito species with epidemiological relevance [21, 22]. Furthermore, the highly invasive species *Aedes vittatus* (Bigot, 1861), has been detected in Cuba and the Dominican Republic [23, 24]. In face of the high levels of human mobility and commodity trade between Miami-Dade and the Caribbean region, the introduction of *Ae. vittatus* to Miami-Dade is considered imminent.

Florida was the most affected state in the contiguous United States during the 2016 Zika virus outbreak totaling 256 locally transmitted cases [25, 26] with the Zika virus being introduced multiple times [27]. In 2020, 59 locally transmitted human cases of West Nile virus and 6 cases of dengue virus were reported in Miami-Dade by the Florida Department of Health and the CDC [28, 29]. To deal with the increasing public health concern, we hypothesized that mosquito surveillance in important small- and medium-sized maritime ports of entry in Miami-Dade is crucial to allow the early detection and elimination of invasive mosquito
species. Therefore, our objective was to survey 12 small- and medium-sized maritime ports of entry in Miami-Dade County, Florida with an increased flow of people and commodities that were not covered by the current mosquito surveillance system [30].

**Methods**

**Collection sites**

Mosquitoes were collected in 12 small- and medium-sized maritime entry ports in Miami-Dade County, Florida. Collection sites were comprised of two distinct environments: (i) four marinas with international traffic of leisure boats–Marinas 1–4; and (ii) eight maintenance and commercial freight cargo ship ports–Miami River 1–8: **Marina 1**: Medium-sized marina owned and operated by the City of Miami. Adjacent to the Miami City Hall; **Marina 2**: Small-sized private marina with regular international boat traffic; **Marina 3**: Medium size marina with increased flow of tourists and leisure boats to the Caribbean region. Adjacent to the U.S. Coast Guard Sector Miami; **Marina 4**: Medium-sized private marina located in a touristic area with increased flow of international leisure boats; **Miami River 1**: Residential area adjacent to the Miami River with increased traffic of small and medium commercial and leisure boats; **Miami River 2**: Commercial medium-sized port specialized in the trade of used goods to Haiti; **Miami River 3**: Commercial medium-sized port specialized in the trade of goods to the Caribbean region; **Miami River 4**: Small-sized boat maintenance port adjacent to a car yard; **Miami River 5**: Medium-sized boat maintenance port adjacent to a car yard; **Miami River 6**: Medium-sized boat maintenance port adjacent to a scrap yard; **Miami River 7**: Medium-sized cargo port and storage; **Miami River 8**: Medium-sized scrap metal port (Fig 1).

**Mosquito collection**

Mosquitoes were collected weekly at each of the 12 collection sites for 24 hours for 6 weeks in the Spring from April to May 2021 and then for 6 additional weeks in the Summer from June to July 2021 using BG-Sentinel traps (Biogents AG, Regensburg, Germany) baited with dry ice [31]. Mosquitoes were transported to the Miami-Dade County Mosquito Control Laboratory and subsequently morphologically identified to species using taxonomic keys [32]. Since this
study posed less than minimal risk to participants and did not involve endangered or protected species the Institutional Review Board at the University of Miami determined that the study was exempt from institutional review board assessment (IRB Protocol Number: 20161212).

To compare the mosquito species composition between seasons (i.e., Spring and Summer) as well as marinas and commercial ports (i.e., Marinas 1–4 and Miami River 1–8) we performed a PERMANOVA with 9,999 permutations based on Bray-Curtis (abundance-based) and the Jaccard (incidence-based) indices [33–36]. Data was subsetted into two groups to compare variations in the mosquito species composition between Spring and Summer, and then we subsequently reorganized into 2 groups comprising marinas and commercial ports. Then we used the SIMPER method based on the Bray-Curtis index to assess which species has contributed the most to the observed differences between groups of samples [37]. Analyses were done using PAST v3.2 [38].

Results

A total of 32,590 mosquitoes were collected, in the 12 maritime ports of entry surveyed during this project. *Culex quinquefasciatus* and *Aedes aegypti* were the most abundant species totaling 19,987 and 11,247 specimens collected, respectively. A total of 2,837 mosquitoes from 10 species were collected in the 4 marinas surveyed. *Culex quinquefasciatus* was the most abundant species with 1,925 specimens collected followed by *Ae. aegypti* with 633 specimens collected. Only one specimen of *Ae. albopictus* and one of *Cx. coronator* were collected in the Marinas. A total of 29,753 from 11 species were collected in the 8 ports in the Miami River. *Culex quinquefasciatus* was the most abundant species with 18,062 specimens collected followed by *Ae. aegypti* with 10,614. Differently from the Marinas, 122 *Ae. albopictus* and 16 *Cx. coronator* were collected in the Miami River. *Aedes bahamensis* and *Culex erraticus* were only found at the Marinas and *Anopheles atropos* was only collected at the Miami River (Table 1).

The relative proportion of mosquitoes collected in the Marinas and the Miami River varied greatly. On the one hand, *Aedes taeniorhynchus, Anopheles Atropos, Anopheles quadrimaculatus,* and *Deinocerites cancer* were more abundant at the Marinas. On the other hand, *Ae. aegypti, Ae. albopictus, Ae. bahamensis, Ae. tortillis, Cx. coronator, Cx. erraticus, Culex nigripalpus,* and *Cx. quinquefasciatus* were more abundant at the Miami River (Fig 2).

The PERMANOVA failed to yield significant results for the comparison between mosquito species composition collected in the Marinas and Miami River in different seasons, Spring and Summer based on both the Bray-Curtis (F = 1.225; *P* = 0.3008) and Jaccard (F = 0.9495; *P* = 0.432) indices. However, The PERMANOVA yielded significant results for the comparison between mosquito species composition collected in the Marinas and Miami River based on the Bray-Curtis (F = 6.767; *P* = 0.0037) and Jaccard (F = 3.373; *P* = 0.01) indices. The SIMPER analysis comparing the mosquito community composition of the Marinas and Miami River showed that *De. cancer, Ae. bahamensis, Ae. albopictus, Ae. tortillis,* and *Cx. coronator* contributed the most to the observed differences (Table 2).

Discussion

Mosquito surveillance in maritime ports of entry is crucial to allow the early detection of invasive mosquito species. Our results show that important mosquito vector species were present in great numbers in all of the 12 maritime ports of entry surveyed during this study, with *Cx. quinquefasciatus* and *Ae. aegypti* being the most abundant species. However, more importantly, the relative abundance of *Cx. quinquefasciatus* and *Ae. aegypti* was substantially higher in the Miami River than in the Marinas. These results are indicating that even though both areas are conducive for the proliferation of vector mosquitoes, the port area in the Miami
River is especially suitable for the proliferation of vector mosquitoes. Therefore, potentially allowing the establishment of invasive mosquito species inadvertently brought in by cargo freights.

Our results also indicate that there were no significant differences in the mosquito community composition between the Marinas and the Miami River between spring and summer as seen previously as an overall trend for Miami-Dade County, in which Cx. quinquefasciatus usually peaks in abundance in March and April and Ae. aegypti in June and July [30]. On the other hand, despite the fact that Ae. aegypti and Cx. quinquefasciatus were found to be the dominant species in both the Marinas and the Miami River, the mosquito community composition was significantly different between these areas according to both the Bray-Curtis (abundance-based) and the Jaccard (incidence-based) indices.

The intense traffic of boats from Miami-Dade to the Caribbean region in the maritime ports of entry surveyed in this study alongside the elevated presence and abundance of vector species revealed a worrisome scenario. People coming and going from endemic areas may inadvertently become infected and introduce arboviruses to Miami-Dade to areas where workers spent a disproportionate amount of time outdoors and are exposed to large numbers of vector mosquito species. A similar scenario was documented during the Zika virus outbreak,
in which construction workers were exposed to Zika-infected *Ae. aegypti* mosquitoes breeding in large numbers in a construction site [39, 40]. Furthermore, added to the risk of arbovirus introductions to Miami-Dade and the United States, there is also the risk of introduction of

![Fig 2. Relative proportion of mosquitoes collected in the two categories of maritime entry ports in Miami-Dade County, Florida, Marinas and commercial ports at the Miami River.](https://doi.org/10.1371/journal.pone.0267224.g002)

| Species                      | Average dissimilarity | Contribution % | Cumulative % |
|------------------------------|-----------------------|----------------|--------------|
| *Deinocerites cancer*        | 5.794                 | 15.13          | 15.13        |
| *Aedes bahamensis*           | 5.033                 | 13.14          | 28.27        |
| *Aedes albopictus*           | 4.872                 | 12.72          | 40.99        |
| *Aedes tortilis*             | 4.59                  | 11.98          | 52.97        |
| *Culex coronator*            | 4.108                 | 10.73          | 63.7         |
| *Aedes taeniorhynchus*       | 3.704                 | 9.669          | 73.37        |
| *Culex nigripalpus*          | 3.104                 | 8.105          | 81.47        |
| *Anopheles quadrimaculatus*  | 3.1                   | 8.093          | 89.56        |
| *Anopheles atropos*          | 2.803                 | 7.318          | 96.88        |
| *Culex erraticus*            | 1.194                 | 3.118          | 100          |
| *Culex quinquefasciatus*     | 6.98E-06              | 1.82E-05       | 100          |
| *Aedes aegypti*              | 4.18E-06              | 1.09E-05       | 100          |

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invasive mosquito species that are adapted to urban environments and are primary vectors of
arboviruses (e.g., Ae. vittatus) that would further increase the risk of outbreaks in the region.

Our results indicate that all the surveyed ports of entry have (albeit at different levels) the
potential to support high levels of mosquito vector populations and have the suitable condi-
tions to allow a successful invasion and subsequent proliferation of invasive mosquito species
that are adapted to urban environments. Invasive species could be brought to Miami-Dade
either passively (i.e., transport of eggs or immature mosquitoes withing goods transported
from the Caribbean region to Miami-Dade) or actively (i.e., adult mosquitoes seeking host
actively board leisure boats and commercial freights and are inadvertently brought back to
Miami-Dade). Therefore, mosquito surveillance should be intensified in small and medium
maritime ports of entry to allow not only the early detection of invasive species but also to
allow the development of effective control efforts to reduce the presence and abundance of
dangerous mosquito vector species in these areas. Mosquitoes were not collected through all
seasons, which may have led to the underestimation of species richness by not collecting rare
species and failing to detect natural fluctuations in the mosquito community composition over
time. However, our experimental design was appropriate to detect important mosquito vector
species that should be considered by mosquito control strategies.

Conclusion
Our results indicate that mosquito surveillance in small- and medium-sized maritime ports of
entry are key not only to inform mosquito control operations to more effective control strate-
gies to reduce populations of vector mosquito species in those areas and decrease the risk of
arbovirus introductions but also to allow the early detection and elimination of invasive mos-
quito species inadvertently transported by leisure boats and cargo freight ships.

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