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CHARACTERIZING DOMESTIC MOSQUITO POPULATIONS IN MONTSERRAT, WEST INDIES

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YALE SCHOOL OF PUBLIC HEALTH

EPIDEMIOLOGY OF MICROBIAL DISEASES

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ABSTRACT

Vector control in the Caribbean Island of Montserrat relies on routine residential inspections to count, identify, and treat standing water sources where mosquito larvae develop. *Aedes aegypti*, *Culex quinquefasciatus*, and *C. nigripalpus* are local domestic pests with competence to transmit several pathogens including dengue, Chikungunya, and Zika viruses. With the recent emergences of Chikungunya and Zika viruses in the Caribbean, evaluating infestation densities is an important step in identifying target areas for increased vector control. The current study aimed to depict the burden of domestic mosquito infestations in Montserrat based on infestation indices and the abundance of containers that serve as potential larval habitats. Data from residential inspections performed by the Vector Control Team of the Montserrat Environmental Health Department 2013-2015 were used to calculate infestation index measures. The House Index, Container Index, and Breteau Index were calculated for six Montserrat localities for each year. Container types noted during inspections were ranked by frequency to determine their relative abundance. The House Index ranged across localities from 2.5% to 11.4% in 2013, 5.1% to 11.5% in 2014, and 3.9% to 18.5% in 2015. The Container Index ranged from 4.2% to 11.7% in 2013, 2.4% to 9.2% in 2014, and 3.7% to 20.6%in 2015. The Breteau Index ranged from 7.6 to 25.5 in 2013, 5.1 to 24.0 in 2014, and 6.1 to 44.6 in 2015. Mosquito species identified were *Aedes aegypti, Culex quinquefasciatus* and *C. nigripalpus.* The most abundant container types ranked from greatest to least abundance were outdoor artificial containers, barrels/drums, and tires. Variability in mosquito density may reflect differences in population density, housing structures, water sources, and the presence of seasonally or permanently uninhabited homes. Localities with particularly high indices represent intervention targets such as container reduction and water treatment with larvicide.

ACKNOWLEDGMENTS

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INTRODUCTION

National vector control programs are a critical fixture in global public health, and their importance only grows with the continued emergence of vector-borne diseases. As these illnesses spread, maximizing the effectiveness of control programs is imperative. Over the past three years, Chikungunya and Zika viruses have spread through the Americas, and, by the nature of their most important vector, *Aedes aegypti*, have burdened primarily high-density, low-income regions [1,15]. Even in areas with lower disease incidence, exposure to domestically adapted mosquitoes remains high through unabated home infestations [3]. Montserrat, an active volcanic island in the Lesser Antilles of the southern Caribbean Sea, has seen reductions in a variety of local resources in the aftermath of a volcanic crisis spanning several years [10]. Two thirds of the population were displaced, and local infrastructure was heavily disrupted [10]. This has also influenced the cease of larger mosquito control initiatives as resources available for vector control have diminished [3,7].

The current epidemics of Chikungunya and Zika viruses in the New World began recently; Chikungunya was first reported in 2013 and Zika in 2015 [15,1]. Both infections are arboviral illnesses that are primarily transmitted by *Ae. aegypti*, with *Ae. albopictus* as a potential secondary vector [1,15]. Both viruses cause febrile illness along with myalgia and rash [1,15]. Long-term effects of other neurological sequelae associated with infection are still being investigated [15,9]. Chikungunya is known to be associated, in some cases, with encephalitis and other severe neurological outcomes in addition to chronic pain and joint inflammation, sometimes lasting months or years after the initial infection [8,9]. The spread of Zika in South America is also associated with an increase in newborn microcephaly cases resulting from infection of pregnant women [1].

The diagnosis and treatment of these infections remains challenging. Like dengue and yellow fever, no specific antiviral is approved for use in treatment of Chikungunya or Zika [1,15,13]. Instead, supportive therapy and measures to reduce exposure are the standard. No vaccine has been approved for these two viruses as well [15]. On the diagnosis front, the similarity in clinical illness is further complicated by the potential for cross-reactivity when testing for antibodies [1].

Chikungunya, Zika, and other mosquito-borne illnesses spread as their corresponding vector populations continue to proliferate. Mosquitoes such as *Ae. aegypti* that are well-adapted to live around human dwellings benefit from high population density and availability of standing water that urbanization provides [13]. Continued introduction into domestic environments has facilitated their habituation to artificial containers and anthropophagy [17]. The global distribution of *Ae. aegypti* has been facilitated historically by trade ships carrying infested water stores traveling from the African continent [16]. The spread and establishment is thought to have occurred from the African Continent to the New World and from the New World to Asia and Australia based on comparing genetic lineages of *Ae. aegypti* [17]. Globalization also has contributed to the more recent spread of emerging illness, in that infected travelers have been able to transport pathogens very rapidly around the world [13, 16]. In areas where both domestic and sylvan populations coexist and remote or island regions, some reversion to oviposition in natural habitats has been observed [17]. This reversion then creates a wider variety of oviposition sites, which necessitates greater source reduction purview for vector control programs.

The success of mosquito elimination programs has varied greatly by region and over time. In North and South America, large-scale yellow fever elimination programs coordinated by the Pan American Health Organization (PAHO) in the 1950s and 1960s eliminated or significantly reduced *Ae. aegypti* populations in Central and South America and the Caribbean Islands. As control programs subsided in the 1970s, *Ae. aegypti* re-infested the region and contributed to the spread of epidemic dengue fever [13,16].

As urbanization increased in resource-poor regions, crowding, poor sanitation, variable water availability, and open housing structures have offered a dense pool of human hosts surrounded by containers suitable for mosquito oviposition sites [2,13-14,16]. Whereas *Ae. aegypti* is perhaps the best known example of this behavior, other vector species such as *Culex quinquefasciatus* also oviposit in artificial containers [14]. In addition, a greater variety and abundance of human produced containers have become available as habitats for mosquito larva as a consequence of increased waste production and increasingly poor waste disposal. Examples of particularly troublesome waste products have been tires and old appliances that comprise many of the household containers that support mosquitoes such as *Ae. aegypti* [13]. Reliance on insecticides, which have variable efficacy due to insect resistance, imprecise application and overuse in agriculture, can pose risks to human and environmental health. Increased insecticide usage has also reduced the capital [monetary and otherwise] devoted to source reduction and other structural changes that have the potential for long-term efficacy [13, 2-4, 7].

Difficulty with vector control implementation is also associated with regional variability. Disease and vector distributions tend to be focal, and vary depending on land use, residential characteristics, topography and climate, and the sociocultural aspects that influence these factors [14]. Domestic mosquitoes are adapted to thrive focally due to the availability of microclimates in infested areas [16]. Container types that maintain mosquito infestations vary from place to place based on water collection strategies as well the ease of their removal [16]. Therefore, mosquito habitat reduction (source reduction) initiatives must be tailored to target regions [4-6]. Resource availability, social support, and accountability also influence the efficacy of these mosquito control initiatives [16]. For site-specific program development, the social history can be as critical as ecological trends. In Montserrat, this requires an understanding of the historical processes that have produced the current demography and population distribution.

Since 1995, Montserrat has experienced several volcanic eruptions of the Soufriere Hills volcano. The most devastating occurred in 1995 and caused the dislocation and redistribution of the population as two thirds of the original population of approximately 12,000 people. Most left the island [10], and the remaining 4,000 people were concentrated in the northwestern section of the island. The former capital of Plymouth has remained buried under volcanic ash. Many of the abandoned or ‘closed’ housing structures are uninhabitable but remain in close proximity to inhabited ones, and provide refuge for domestic vermin and mosquitoes. In addition, governmental operations and services were relocated, placing great stress on those who remained to rebuild and restructure neighborhoods, businesses, and facilities [10-11]. The health system in particular was critically burdened, as the crisis resulted in greater challenges to ensuring food safety and waste management [11]. Tourism in Montserrat has also suffered, although the history of the volcano and increased rarity of endemic species have become focal points of ecotourism along with educational tourism based in marine and terrestrial conservation efforts [10,12].

Current vector control programs in Montserrat are overseen by the Department of Environmental Health, which dispatches a team of inspectors to catalog containers and aquatic foci for mosquito larvae in residential areas. The inspections are undertaken primarily on foot. This allows for inspectors to engage with people and creates a comfortable avenue to discuss control strategies with homeowners, strategies such as properly covering water storage drums and keeping unused containers overturned. Recently, the size and purview of the program has decreased due to fewer staff which in turn has lengthened the time required to complete an inspection cycle.

Arboviral disease incidence in Montserrat remains low. Recent outbreaks have been self-limiting, likely due to a small population size that prevents the establishment of autochthonous transmission [6). However, Montserrat also receives a sizeable human traffic from other nations with arboviral epidemics, both from the nearby Caribbean islands states and the continent. The economic reliance of Monserrat on tourism and the dispersal of families and workers across multiple islands has resulted in a network of travel with multiple opportunities for disease importation, some of which have resulted in incidental cases of Chikungunya since 2014. With this potential present, establishing the characteristics of domestic mosquito infestation in Montserrat was necessary to provide a clearer picture of the circumstances that may lead to future outbreaks. The variability of neighborhood structure and topography indicated that locality-based evaluations can provide better insights on disease transmission risks than metrics based on national averages. Note that the latter were not available for Montserrat before 1995. The current study aimed to describe mosquito vector levels using index values based on the relative densities of mosquito populations in residential areas, the abundance of container types, and the species of mosquitoes present in each locality. Data were recovered from 2013-2015 inspections which reflected recent trends in mosquito population composition and were re-evaluated in the face of the emergence of two new arboviral pathogens.

MATERIALS AND METHODS

Specimen Collection and Identification

Mosquito larvae were collected during the summer residential inspections in the 2015 cycle conducted by author in association with the Monserrat Environmental Health Department Vector Control team. Inspections were carried out by locality, beginning with the northernmost region (St Johns and Lookout) and continued southward (Figure 1). Localities were divided into neighborhoods, with one inspector dispatched to cover a given block on foot. During the summer of 2015 between one to two dozen houses were examined by one inspector in one day. Blocks were inspected one house at a time. Inspections consisted of visual inspection of the home and surrounding area. The number and types of containers, and the presence of mosquito larvae were documented. Larvae were collected using pipetting and stored in vials for transportation. Larvae were identified to genus at time of collection, and infested containers were treated with insecticide or were emptied. Inspection records and collected specimens were returned to the Environmental Health Offices at the end of each day. In each form, the following data were recorded: location of larval focus, date, time of collection, and locality code. Specimens were preserved in alcohol as larvae or reared to adults in the laboratory; the latter were sight identified without microscopy. The preserved larvae were later identified to species off-site with microscopy.

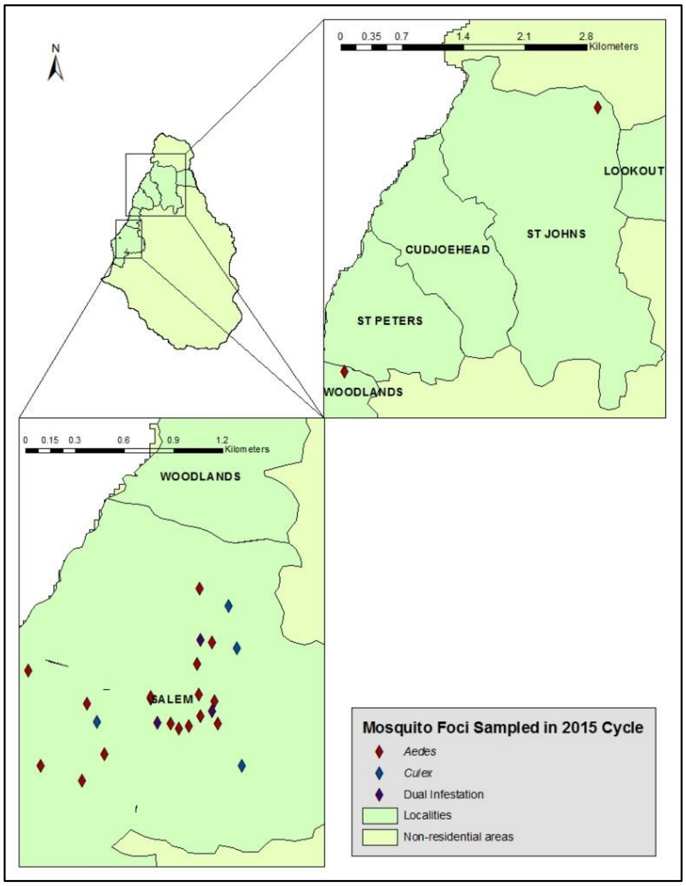

Figure 1 –Documented mosquito foci sites during the 2015 summer inspection cycle.

Historical Data

Inspection data from 2013-2015 were extracted from available records of the Montserrat Environmental Health Department. Regions were referred to as localities, which were further subdivided into sections. The extracted data for each inspection site included numbers of containers (twelve types), number of larval habitats categorized by genus (*Aedes*, *Culex*, or mixed) as well as the date of inspection, locality, and section. Genus identification was provided for all recorded infestations, and species identification occurred only for samples taken in 2015.

Records were not uniformly available for all localities, and for the locality of Woodlands, data were only available for the 2015 cycle.

Quantitative and Spatial Analysis

Infestation indices were calculated per locality based on inspection records from 2013 to 2015. Indices chosen were the House Index--the proportion of infested homes, the Container Index--the proportion of infested containers, and the Breteau Index--the number of infested containers per 100 houses. All collection sites were georeferenced by degrees-minute-seconds coordinates using GPS and GIS. Maps for comparing index values by locality were produced using regional infestation data (Google Maps, ArcMap 10.2.2).

Container data were characterized by relative abundance. Container type descriptions were based on an existing classification used in all inspection forms. Twelve categories were used, and counts were recorded for each inspection site. In the current study, the three most frequently noted categories comprised roughly 90% of all containers. The twelve categories noted in the inspection records were classified as barrels/drums, tires, overhead tanks, ground level tanks, clay containers, roof gutters, trees/plants, drains, wells/cisterns, and special artificial containers (interior), special artificial containers (exterior), or other containers. The special artificial container category referred to artificial containers that did not fall into any of the other defined categories and were classified based as to whether they were kept outdoors (exterior) or indoors (interior).

RESULTS

Infestation inspections were performed on 2,411 houses in 2013, 2,027 houses in 2014, and 2,857 houses in 2015. Over the period of these inspections, 446 larval habitats in 5,256 containers were documented in 2013, 239 in 4,283 containers were documented in 2014, and 337 in 4,981 containers were documented in 2015. Infestation measures showed fluctuation over the 2013-2015 period among localities and over time (Tables 1-4). The Woodlands district consistently had the highest index values for the year 2015. Note that this was the only year for which data were available in Woodlands.

Table 1 shows the House Index for each locality in each year during the 2013-2015 study period. Values for the House Index (Table 1) per locality ranged from 2.5% (St Peters) to 11.4% (St Johns) in 2013, 5.1% (St Peters) to 11.55 (Salem) in 2014, and 3.9% (Lookout) to 18.5% (Woodlands) in 2015. No consistent decreases were seen from year to year, although St Johns and Salem showed the greatest level of decrease over this period. Tables 2 and 3 show the Container Index values. These have a similar pattern to the House Index of overall decrease. For all mosquito species recorded, the percentages ranged from 4.2% (Lookout) to 11.7% (St Johns) in 2013, 2.4% (St Peters) to 9.2% (Salem) in 2014, and 3.7% (Cudjoehead) to 20.6% (Woodlands) in 2015. For *Aedes* species Container Index values range from 2.9% (Cudjoehead) to 0.0% (Salem) in 2013, 2.4% (St Peters) to 9.2% (Salem) in 2014, and 3.6% (Cudjoehead) to 16.6% (Woodlands) in 2015. Breteau Index values are shown in Table 4. These range from 7.66 (Lookout) to 25.51 (Salem) in 2013, 5.13 (St Peters) to 23.96 (Salem) in 2014, and 6.13 (Lookout) to 44.57 (Woodlands) in 2015. Figures 1-4 show these changes spatially in order to highlight the extent of regional variation in infestation variation over time.

Table 1 – House Index by locality from 2013-2015 (all mosquito species)

LOCALITY	2013 INDEX	2014 INDEX	2015 INDEX
St. Johns	11.4%	5.9%	7.3%
Lookout	4.6%	6.9%	3.9%
Cudjoehead	5.7%	7.4%	5.1%
St. Peters	2.5%	5.1%	4.1%
Salem	11.3%	11.5%	8.1%
Woodlands	---	---	18.5%

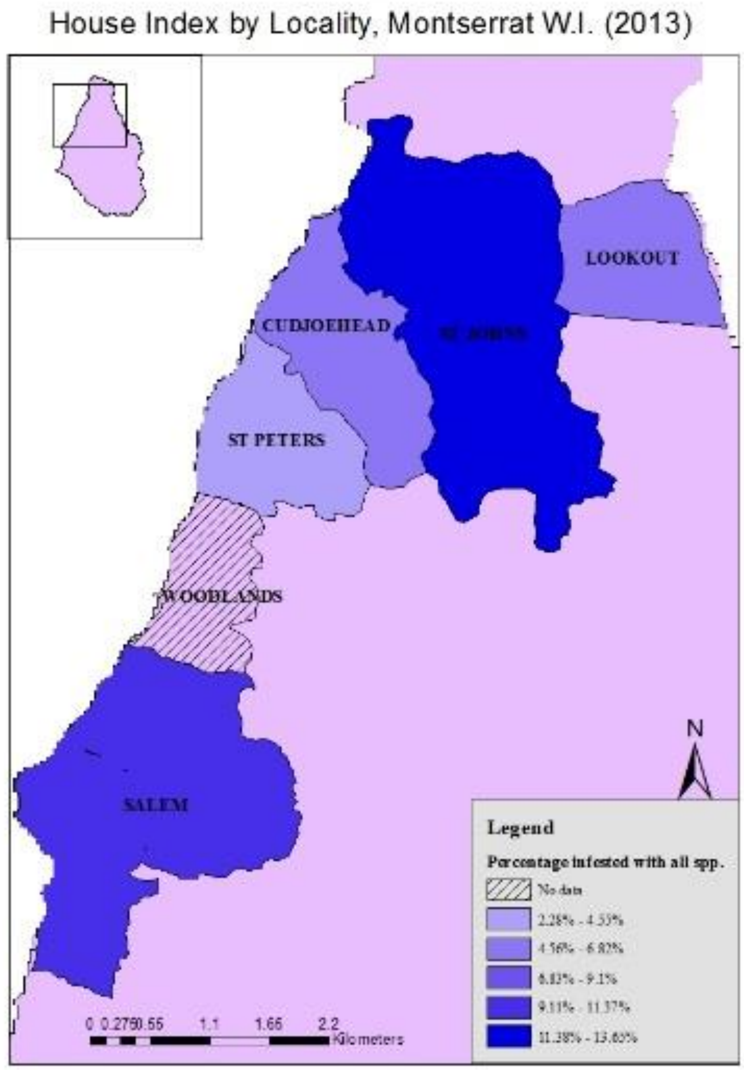

Figure 2A - Changes in House Index by locality in 2013 (all mosquito species)

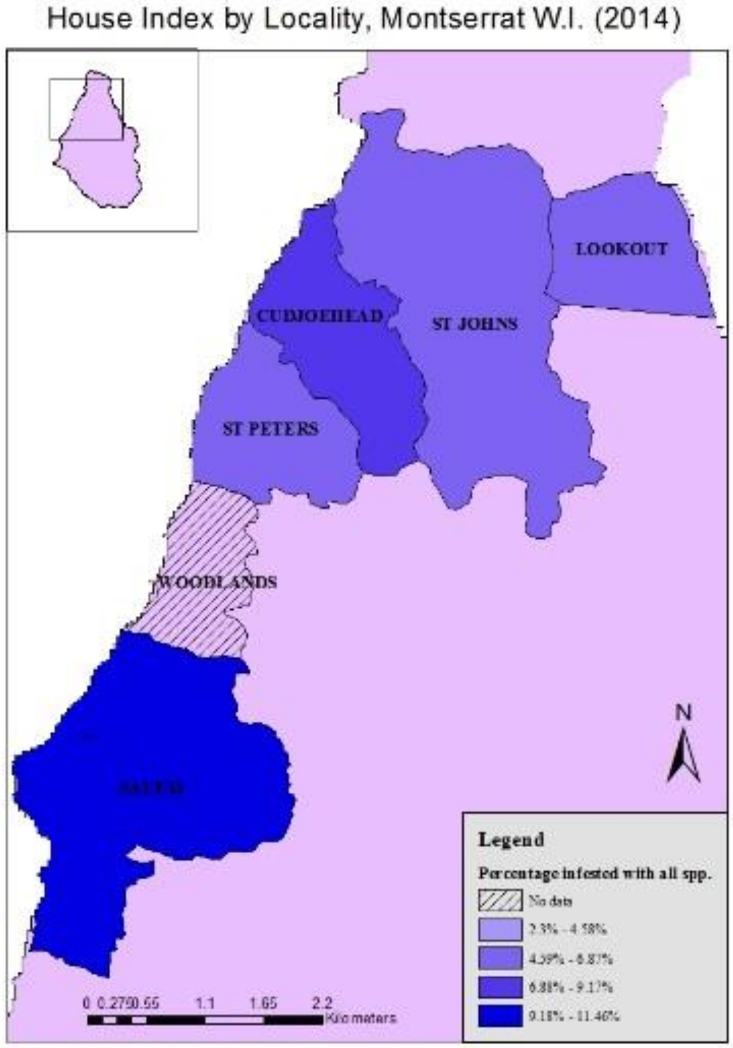

Figure 2B – Changes in House Index by locality in 2014 (all mosquito species)

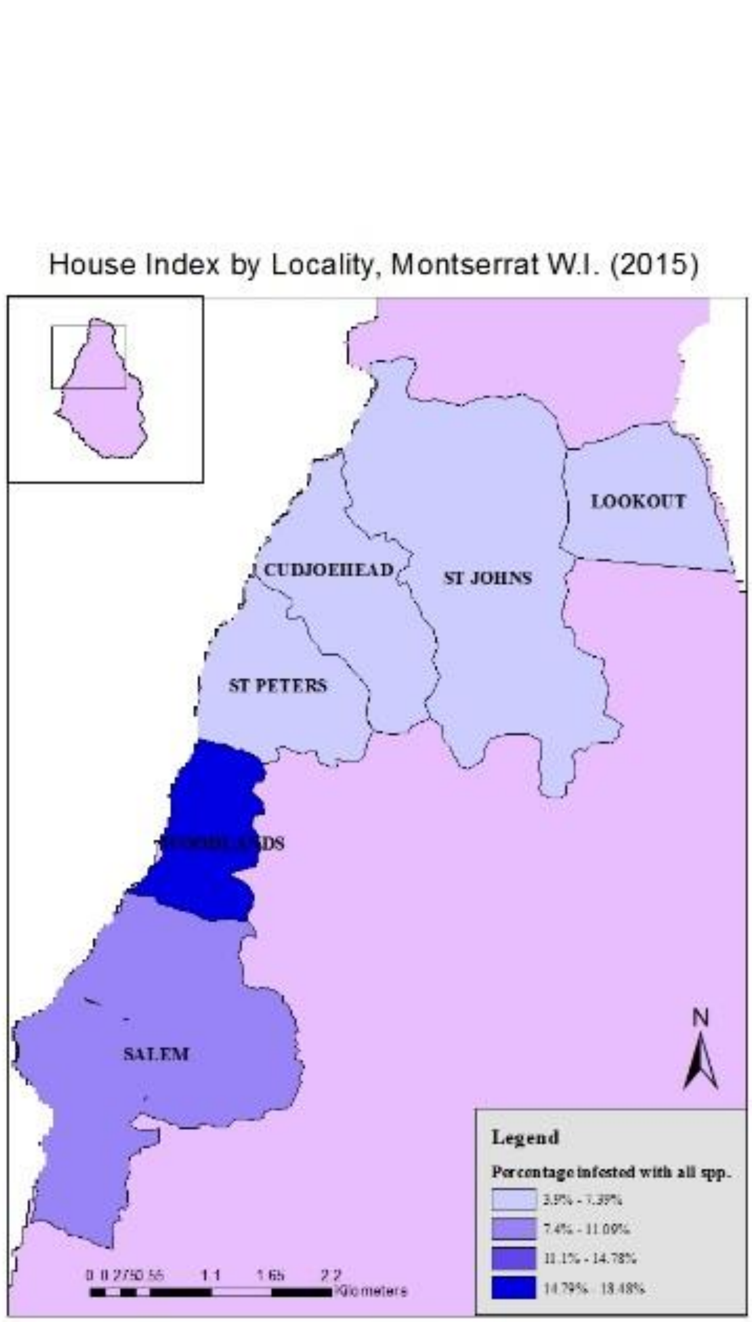

Figure 2C – Changes in House Index by locality in 2015 (all mosquito species)

Table 2 – Container Index by locality from 2013-2015 (all mosquito species)

LOCALITY	2013 INDEX	2014 INDEX	2015 INDEX
St. Johns	11.7%	5.0%	6.5%
Lookout	4.2%	5.6%	5.1%
Cudjoehead	18.7%	6.3%	3.7%
St. Peters	6.7%	2.4%	4.3%
Salem	11.4%	9.2%	9.1%
Woodlands	---	---	20.6%

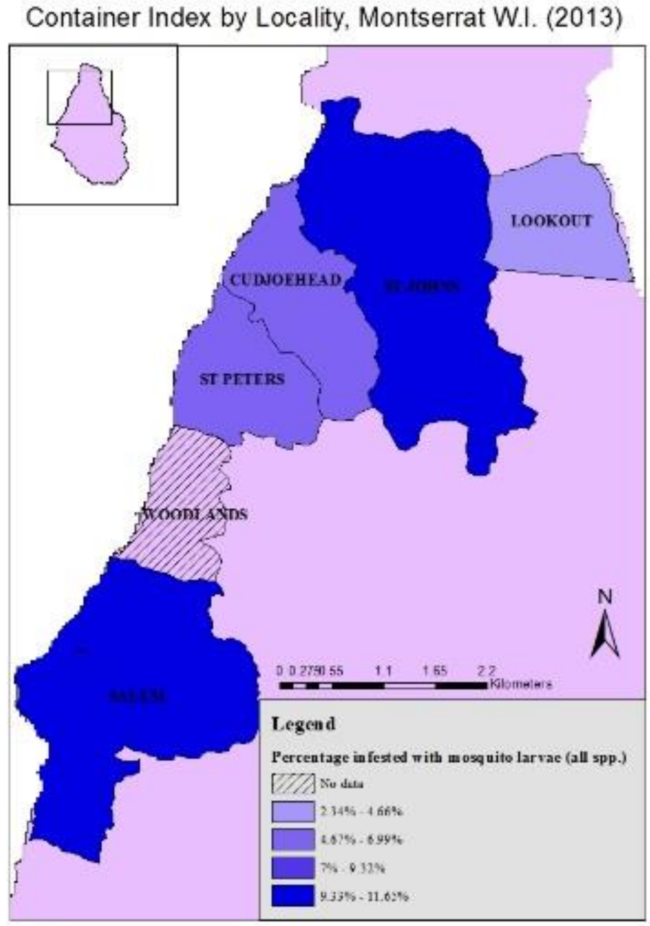

Figure 3A – Changes in Container Index by locality in 2013 (all mosquito species)

Container Index by Locality, Montserrat W.I. (2014)

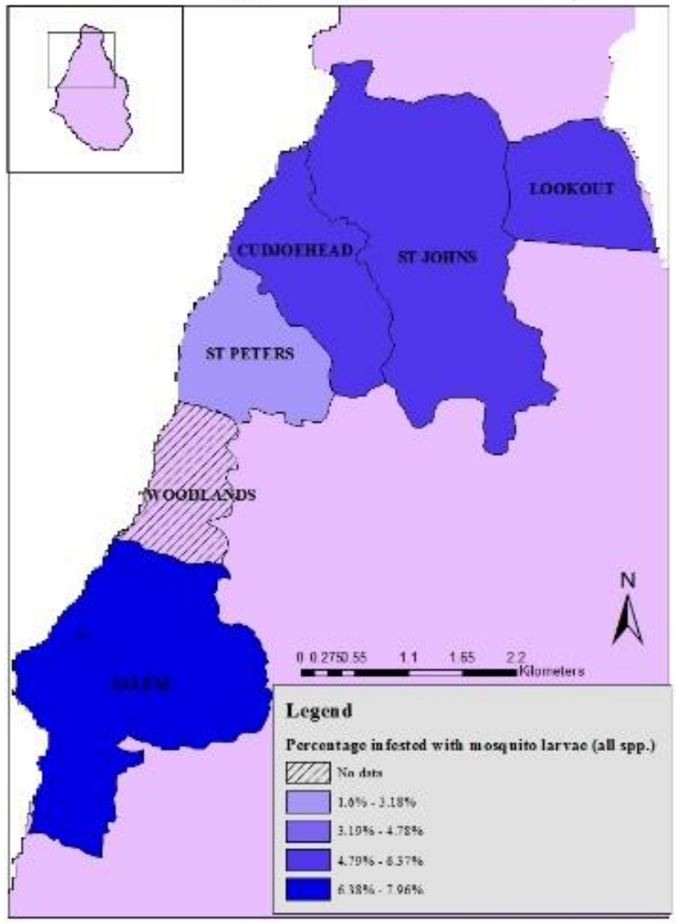

Figure 3B – Changes in Container Index by locality in 2014 (all mosquito species)

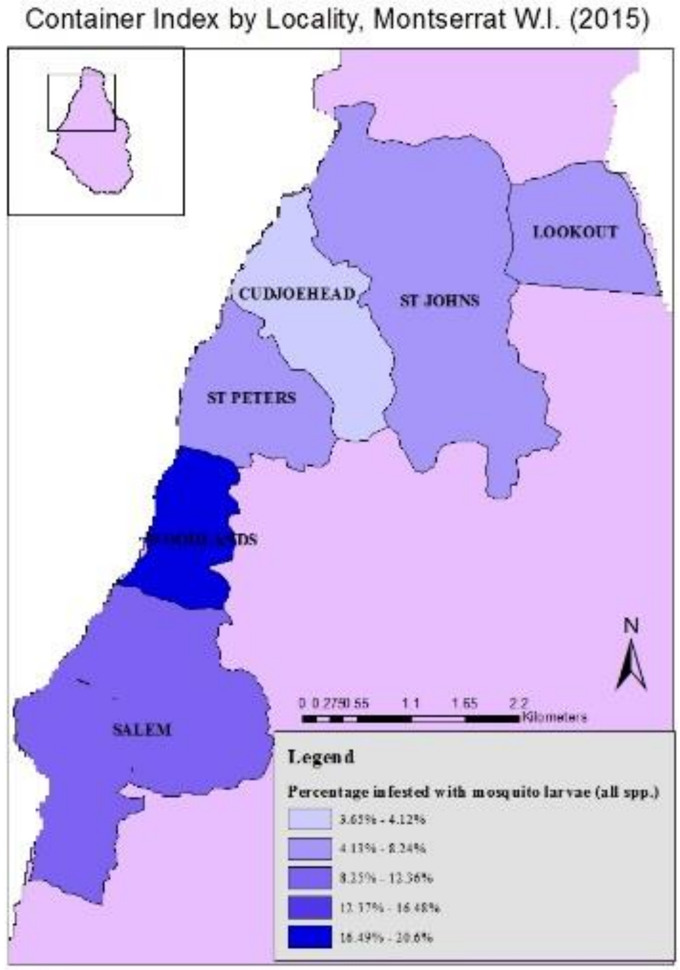

Figure 3C – Changes in Container Index by locality in 2015 (all mosquito species)

Table 3 – Container Index by locality from 2013-2015 (*Aedes* species)

LOCALITY	2013 INDEX	2014 INDEX	2015 INDEX
St. Johns	9.3%	3.9%	6.2%
Lookout	3.7%	5.1%	5.1%
Cudjoehead	2.9%	5.4%	3.6%
St. Peters	6.3%	2.4%	4.0%
Salem	9.9%	9.2%	6.4%
Woodlands	---	---	16.6%

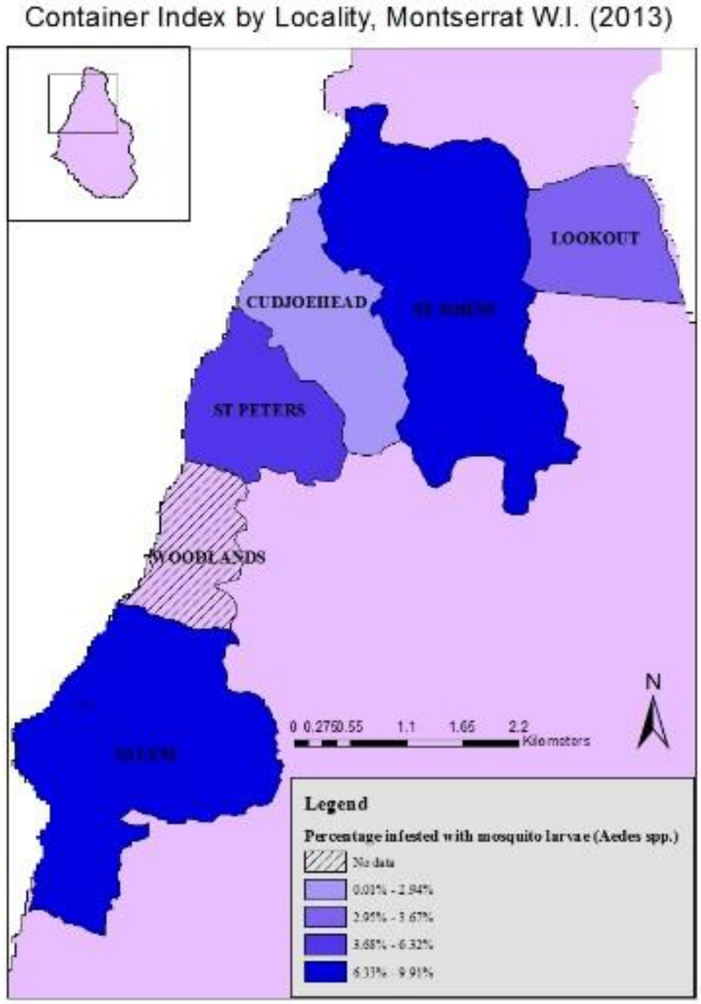

Figure A – Changes in Container Index by locality in 2013 (*Aedes* species)

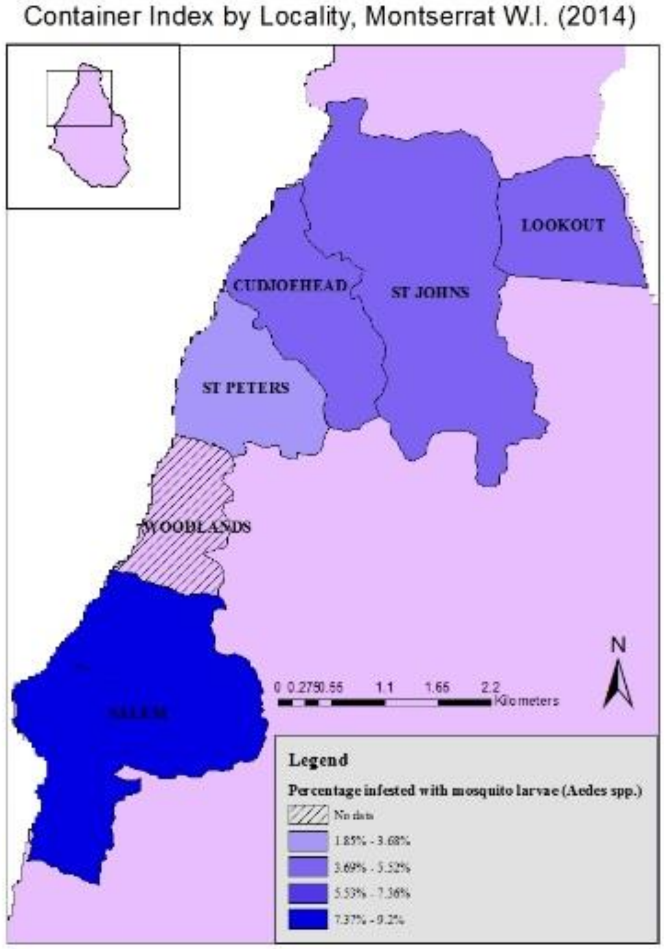

Figure 4B – Changes in Container Index by locality in 2014 (*Aedes* species)

Container Index by Locality, Montserrat W.I. (2015)

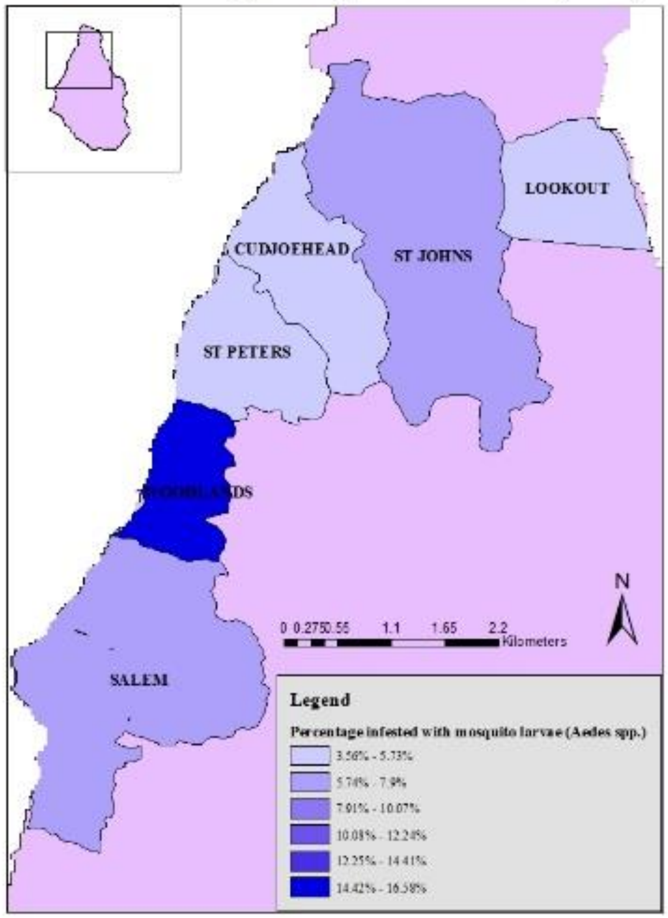

Figure 4C – Changes in Container Index by locality in 2015 (*Aedes* species)

Table 4 – Breteau Index by locality from 2013-2015 (all mosquito species)

LOCALITY	2013 INDEX	2014 INDEX	2015 INDEX
St. Johns	24.2	10.3	13.0
Lookout	7.7	9.6	6.1
Cudjoehead	11.7	14.6	6.2
St. Peters	18.3	5.1	7.0
Salem	25.5	24.0	15.6
Woodlands	---	---	44.6

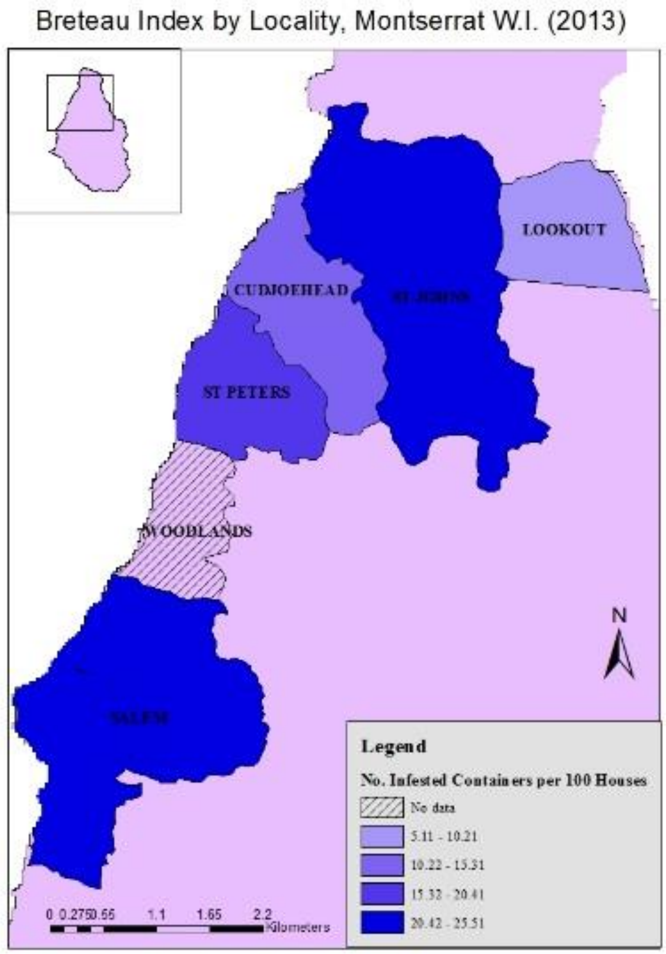

Figure 5A – Changes in Breteau Index by locality in 2013 (all mosquito species)

Breteau Index by Locality, Montserrat W.I. (2014)

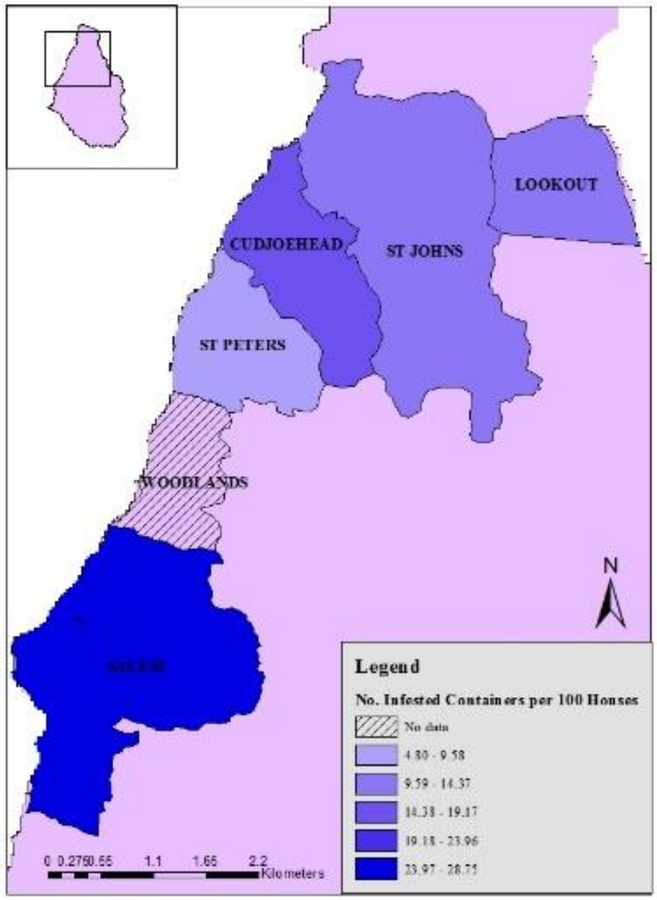

Figure 5B – Changes in Breteau Index by locality in 2014 (all mosquito species)

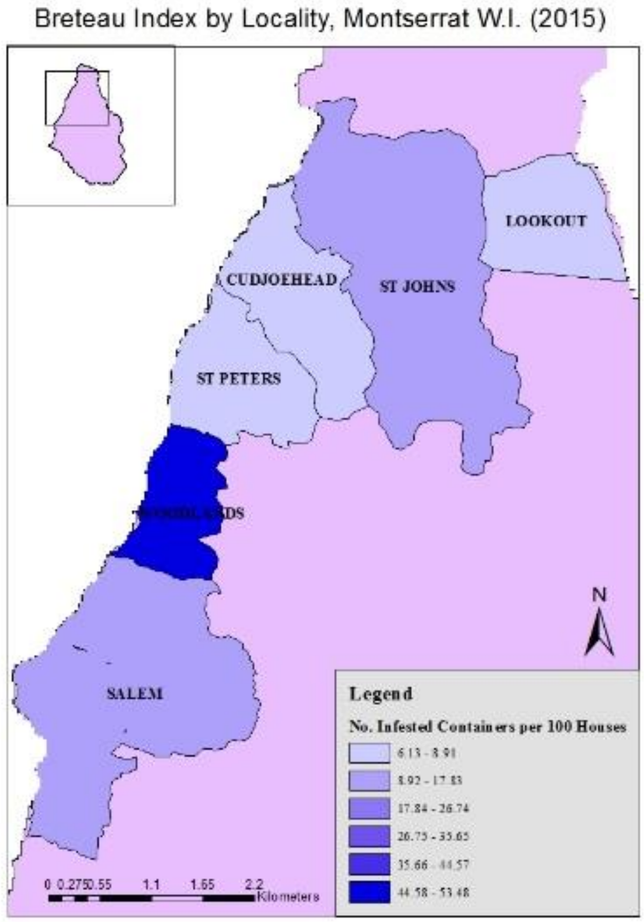

Figure 5C – Changes in Breteau Index by locality in 2015 (all mosquito species)

Ranking container types by frequency found that the same types were consistently the most abundant across the study period. Of the twelve types of containers, outdoor artificial containers, barrels/drums, and tires were the most abundant, as shown below in Table 5. The remaining 10-11% comprised of overhead tanks, ground level tanks, clay containers, artificial containers (indoors), roof gutters, trees/plants, drains, wells/cisterns, and other containers.

Table 5 – Relative abundance (%) of three most frequent container types by inspection year

Container Type	2013	2014	2015
Outdoor artificial containers	54.7	52.9	53.0
Barrels/drums	21.3	23.3	24.8
Tires	12.5	12.7	11.8

Specimens collected during the 2015 inspection cycle were identified to species level. The list of species of positively identified specimens are given below in Table 6.

Table 6 – List of mosquito species identified during 2015 cycle

Localities	Identification
Salem	*Ae. aegypti*
	Culex quinquefasciatus
	C. nigripalpus
St. Johns	*Ae. aegypti*
St. Peters	*Ae. aegypti*

DISCUSSION

Variation in mosquito infestation levels may be attributed to a variety of factors including environmental characteristics, neighborhood structure, sanitation, and control practices regarding containers. Although complete records were not available for all localities over the 2013-2015 period, the available data described the regional variability of mosquito infestations in

Montserrat in general terms. Despite the small size of Monserrat (roughly 102 km^2, less than half of which now hosts human habitation), differences in topography and housing types vary considerably between localities [12]. Index values for the four indicators decreased across some localities during the study period. This lead to some optimism for the outlook of vector control program efficacy. Earlier reports published a house index of 16.0% and Breteau index of 50.4 for Montserrat in 1990 [3,7]. Although index values of the 2013-2015 period were smaller than the earlier nationally reported values, this is probably related to the drastic reduction and rearrangement of the human population in the years following those reports [3,7,10]. Furthermore, mosquito population sizes and distributions were undoubtedly disrupted in late 2014 during the outbreak of Chikungunya when extensive insecticide fogging occurred.

St Johns and Salem showed the greatest extent of change in infestation levels during the study period. St Johns hosts many businesses and facilities as well as some residential areas, and many buildings are quite close together. The northern section of the island (St Johns] is also much drier, which necessitates a greater emphasis on the need for water storage containers. Salem is further south and closer to the coastline; it is a sufficiently large locality to contain some smaller, more densely placed homes, as well as larger vacation homes or villas. Some sections of Salem are part of the former exclusion zone, *i.e.*, sections that were previously uninhabitable (due to the volcanic eruption and indicate a status of conditional evacuation based on hazard levels. Many abandoned or closed homes are sufficiently near to occupied residences that they can provide refuge for *Ae. aegypti* populations. Although Woodlands consistently had the highest index values of any locality, data from previous years are necessary before determining whether these levels are increasing or decreasing. Nonetheless, the high values point to an interesting facet of the locality. It is comprised primarily of large homes on widely spaced properties, many of which are inhabited only seasonally, and are further isolated by steep and long driveways. Most of these homes have swimming pools, which, without proper maintenance, collect rainwater and which were frequently noted as larval sources. This large source was the second most common container type in Woodlands.

Relative container abundance also remained fairly consistent among the three top categories that altogether comprised nearly 90% of the containers tabulated. The lack of variability provides a clear set of targets for larval habitat reduction. In 2014, the Environmental Health Department introduced a clean-up initiative where it offered to remove from residential areas the more cumbersome containers such as tires and old appliances. This offer relieved the burden for property owners for transporting large and unwieldy discards. Outdoor artificial containers were often discarded appliances and larger containers that had been used as planters, but left in yards and outside homes. This category also included other unspecified outdoor containers. Altogether, this “special container” category consisted of more than half of the containers recorded during inspections. Tires were approximately one-tenth of the container records. They were mostly in large quantities around construction sites and automobile yards; here, the accountability for their removal was less straightforward. Finally, the elimination of barrels and drums was not possible because they were necessary for home and shop owners for water storage. Promoting and ensuring proper coverage of these containers was a topic often discussed with homeowners during inspections.

Of the specimens identified from the 2015 summer inspection cycle, the most were attributable to *Aedes* spp., and later identified as *Aedes aegypti*. Other foci yielded *Culex* spp., later identified as either *C. quinquefasciatus* or *C. nigripalpus*. Few foci had dual infestations; however, the competitive dynamics between *Culex* and *Aedes* spp. were not well characterized in this setting. Because larvae were only collected and identified to species in 2015, long-term trends of species abundance cannot be described. Previous records identified infestations to genus, and microscopy was not consistently available to further identification to species. In continuing to describe mosquito populations in Montserrat, the question of species abundance requires further exploration. Other future investigations may address the efficacy of source reduction interventions, local attitudes toward and knowledge of mosquito-borne illness, and the potential presence of mosquito refugia in areas that are not inspected as part of the annual cycle.

Although all the identified species have vector potential, the lack of identifiable arboviral illness (or other vector-borne infections) during this period was a reflection of the low-risk status of mosquito-transmitted infections in Montserrat over the past several years. Although Chikungunya was introduced in 2014, the outbreak was short-lived. The low incidence of mosquito-borne illness in Montserrat makes quantifying risk difficult; however, understanding the potential for vector exposure allows for proactive measures to be taken. For example, in addition to continuing inspections and promoting source reduction initiatives, in the event of an outbreak, localities with greater infestation burdens can be prioritized for more aggressive interventions. Increased source reduction can be accomplished by conducting multiple clean-up programs like the initiative of 2014 and be implemented by more frequent inspections in high-infestation areas associated with spot applications of larvicide. The significance of source reduction for mosquito infestations may also inform other programs under the purview of Environmental Health Department, such as waste management and land use, to further promote responsible and sustainable practices.

Chikungunya and Zika viruses represent but two emerging pathogens. With the continued proliferation of urban sprawl and the concomitant demographic and ecological changes associated with economic development and social expansion, the importance of describing vector patterns only grows. In order to conduct long-term vector control programs, effectively directing resources and community engagement requires an effective surveillance program directed to the targeted vector populations.

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APPENDIX

DEPARTMENT OF ENVIRONMENT

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Ref.: DOE 2/2

07 August 2015

To Whom It May Concern

Please be advised that Ms Sarah Abusaa has been granted permission to take 34 individual samples of mosquitoes from Montserrat into the United States of America, on or before **Wednesday 12 August 2015**, as follows:

1. Thirteen (13) samples in a dry preservative (chlorocresol), 10 of which are identified to Genus.

2. Twenty-one (21) samples in alcohol, 17 of which are identified to Genus.

All of the mosquitoes have been killed. The samples are for scientific purposes and are of no commercial value.

Sincerely,

DEPARTMENT OF ENVIRONMENT
AUG 07 2015
MONTSERRAT

Gerard A L Gray
Director of Environment

GALG/galg