FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

CITIZEN SCIENCE AS A GATEWAY FOR MOSQUITO MONITORING IN MIAMI-DADE COUNTY HOUSEHOLDS

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Helen Urpi Wagner Coello

2023

To: Dean Michael R. Heithaus
College of Arts, Sciences and Education

This dissertation, written by Helen Urpi Wagner Coello, and entitled Citizen Science as a Gateway for Mosquito Monitoring in Miami-Dade County Households, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: May 17, 2023

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Florida International University, 2023

DEDICATION

I would like to dedicate this dissertation to my loving parents Urpi Coello and Edwin Wagner who have given everything for me to pursue a better life. I am forever grateful for all your love and support. I also dedicate this dissertation to all the dreamers, working to build their own futures.

ABSTRACT OF THE DISSERTATION

CITIZEN SCIENCE AS A GATEWAY FOR MOSQUITO MONITORING IN MIAMI-DADE COUNTY HOUSEHOLDS

by

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Florida International University, 2023

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Mosquitoes can transmit deadly vector-borne viruses, causing substantial health risks. To be effective in their abatement, county control plans must understand urban habitats and the community factors that influence vector population growth. The primary objective of this dissertation is to understand mosquito infestation in Miami-Dade County and the extent that citizen science can be utilized for community-based mosquito surveillance programs. Mosquito infestation within Miami-Dade County showed infestation with mosquitoes year-round and a very pronounced difference between spring, summer, and fall ($p < 0.001$). Patterns in mosquito abundance were associated with meteorological factors: temperature, precipitation, and relative humidity ($p < 0.05$). A principal component analysis showed three distinct genetic populations separate the South Florida mosquitoes. Most mosquitoes were encompassed in one cluster and included samples from Miami-Dade, Broward, and Manatee County, suggesting the influence of genetic drift and natural selection where there is a long-term population history. Infestation information in this study was gathered through a citizen science program at Florida International University, Florida *Aedes* Genome Group (FLAGG). Citizen involvement in

FLAGG was analyzed to measure the impact of the program on students by comparing them to their peers. The FLAGG students scored significantly better than their peers when surveyed on mosquito abatement and were more likely to be willing to participate in mosquito control surveillance ($p < 0.001$). Furthermore, FLAGG intern counts and expert counts showed similar seasonal and site-based trends for mosquito abundance ($p < 0.001$). Programs like FLAGG can be used to improve mosquito control while also generating a benefit to the participant as it fosters a sense of empowerment and engagement.

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CHAPTER 1: Introduction

Mosquitoes can transmit deadly vector-borne viruses, parasites and bacteria, causing substantial health risks (Prudêncio, 2020; World Health Organization, 2020; Rhoden et al., 2021; Widmar et al., 2021). Mosquito-borne diseases contribute the most towards human vector-borne disease, threatening the health of 40% of the global population (Franklinos et al., 2019; Deng et al., 2023). Malaria alone has been reported to cause 619,000 deaths in 2021, and dengue epidemic areas have continued to increase dramatically with an estimated current annual infection rate of 100-400 million (World Health Organization, 2023a; World Health Organization, 2023; Deng et al., 2023). Out of the recognized 3,601 mosquito species, over one hundred are capable of spreading mosquito-borne viruses (Rueda, 2008; Azim et al., 2023; Wang & Zhiyuan, 2023).

Three commonly known prolific genera of mosquito vectors capable of carrying fast-spreading arboviruses are the *Anopheles, Aedes* and *Culex* mosquitoes (Trivedi et al., 2021; Allan et al., 2022). Species falling under these genera are known to thrive in urban habitats, adapt to human populations, and breed in high numbers in standing water (Wilke et al., 2019; Allan et al., 2022). Despite centuries of global health and economic decline as a result of mosquito-borne virus transmission, control efforts continue to be limited. Major limitations to control programs include long-term program sustainability, novel resistance to insecticide development, and lack of consistent and active community education (Tolle, 2009; Anoopkumar & Aneesh, 2022).

In Florida, anthropophilic mosquitoes are competent vectors, capable of transmitting viruses identified in recent outbreaks such as the Zika and West Nile viruses (Rhoden et al., 2021; Coatsworth et al., 2022). Year-round transmission of viruses such as the West Nile Virus (WNV), Eastern equine encephalomyelitis virus (EEEV) and St. Louis encephalitis virus (SLEV), have been commonly found to peak during the wet season (Blackmore et al., 2003; Giordano et al., 2021; Beeman et al., 2022). During the Zika outbreaks in 2016, Florida was unprepared. Insecticide resistance status was not known and the state lacked timely and efficient public education campaigns (Bond, 2016; McAllister et al., 2020; Dervisevic et al., 2021). It was after the Zika outbreak that programs heavily intensified and re-enforced their community education, effective spraying combinations, property inspections and emergency control methods (McAllister et al., 2020; Moise et al., 2021).

In south Florida, the urban dwelling *Ae. aegypti* vector is one of the predominant mosquito species thriving due to the state's ideal tropical environment (Rey et al., 2006; Wilke et al., 2019). Applying an area-wide approach in mosquito control would be effective to reduce *Ae. aegypti* mosquitoes, since integrated vector management (IVM) at the community level would prevent non-complying households from re-infesting their neighborhoods (Unlu et al., 2010). Integrated programs can include public outreach and education, community surveillance programs, or the use of novel genetic tools for control in addition to traditional insecticide usage (Fouet & Kamdem, 2019; Kondapaneni et al., 2021). Currently, labor-intensive traps capable of auto-disseminating pyriproxyfen have been deemed effective in substantially decreasing mosquito abundance within neighborhood areas (Buckner et al., 2021). However, IVM, which includes surveillance, educational campaigns and source reduction are more time and labor efficient and should be focused on as it provides similar reductions in mosquito density (Wilke et al., 2018;

Buckner et al., 2021). An example of sustainable community-wide efforts would be to have citizen-led reductions of household water-holding bromeliads which have been classified as a major contributor of *Ae. aegypti* proliferation within south Florida (Wilke et al., 2018; Brown, 2019; Wilke et al., 2020; Butterworth, 2022).

To be effective, county control plans should understand the needs of urban habitats and the community-based factors that could prevent vector population growth on both public and private landscapes (Yasuoka et al., 2006; Fouet et Kamdem, 2019; Sousa et al., 2022). Resident-managed property can accumulate containers in private areas unreachable to truck-based or aerial spraying, where only resident participation can prevent year-round mosquito development and population growth (LaDeau et al., 2013; Likos et al., 2016). In tourist-heavy south Florida, residents of tax-funded mosquito control districts have educated citizens that feel personally responsible for mosquito surveillance (Butterworth, 2022). This achievement of getting residents in surveying both private and public areas is complex. Socio-ecological factors involved such as skepticism, tension, and perceived regulations may be reversing the effects of community participation by holding *Ae. aegypti* abundance above the ideal 2% larval index required to reduce dengue health threats (Butterworth, 2022). If a sense of responsibility was coupled with early education that included mosquito control importance and district environmental regulation knowledge, it is possible that community participation would reduce the larval index (Foley et al., 2021).

Public engagement in mosquito surveillance is a relatively new field of study that has allowed for large-scale and real-time data collection of local abundance, diversity, and distribution (Abourashed et al., 2021; Sousa et al., 2022). Engaging through citizen science bridges education with outreach and professionals in academia with the local community

(Mahajan et al., 2020). This bridge works to empower students and communities to help control local populations of mosquitoes (Trout Fryxell et al., 2022). In citizen science mosquito surveillance programs, participants can be encouraged to inspect for and collect mosquito eggs, larvae, pupae, and adult stages, while accruing breeding site information (Montgomery et al., 2017; Montgomery, 2020; Parra et al., 2020; Sousa et al., 2020). Growing a low-cost, hands-on effective mosquito monitoring intervention program scales up data collection and has the potential to be used as community-built real-time outbreak warning systems (Sousa et al., 2022).

The primary objective of the proposed work is to understand the extent of citizen science benefits that can result from running the community-based mosquito surveillance program "Florida *Aedes* Genome Group (FLAGG)". This research is guided by the following questions:

Chapter 2

(1) *Can citizen-collected data help capture mosquito infestation fluctuation in* Miami-Dade County?

(2) What is driving the variation in mosquito infestation?

Chapter 3

(3) Can citizen collected data help capture the population structure within South Florida *Ae. aegypti* mosquitoes?

Chapter 4

(4) Can a college-based citizen-science internship increase participants' knowledge and efficacy towards mosquito surveillance?

(5) Can a college-based citizen-science internship increase participant confidence in science communication and career readiness?

(6) Can a college-based citizen-science internship increase participants' perceived sense of community engagement on or off campus?

(7) Are FLAGG participant mosquito egg counts reliable when compared to expert counts?

Preventing mosquito-borne disease is heavily influenced by health behaviors within a community. The FLAGG internship may serve as a sustainable model for mosquito education that can be implemented in other universities as a state-wide effort to improve knowledge, awareness, and prevention practices to help reduce the transmission of mosquito-borne diseases.

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CHAPTER 2: Understanding Mosquito Infestation within Residential Miami-Dade County using Community-driven Data

2.1 Background

With over half of the world's population at risk for vector-borne diseases, the geographical spread of mosquito vectors is of serious concern (World Health Organization, 2014). There are invasive hematophagous and anthropogenic mosquito vectors distributed worldwide that have expanded through passive vehicle, aircraft, and maritime sea transportation (Ibáñez-Justicia, 2020; Parry et al., 2021). Some of these vectors have expanded their geographical range and undergone urban habitat adaptation due to the availability of water-filled artificial man-made containers in urbanized areas that serve as oviposition sites (Afolabi et al., 2019; Agha &Tchouassi, 2022). A key component of mosquito control is identifying areas with abundant peridomestic mosquitoes and water reservoirs to target both immature and adult stages of mosquitoes (Fouet & Kamdem et al., 2019; Musah et al., 2019; Esmaili et al., 2021).

To successfully manage mosquito populations, there is a need for integrated multidisciplinary tactics for abatement. Traditional control includes surveillance and insecticide-usage (Moise et al., 2018; Dobson, 2021). The integration of citizen science has been used, in addition to traditional control, to enhance mosquito surveillance and to remove breeding habitats (Hoel at al., 2007; Fouet & Kamdem, 2019; Hribar et al., 2022). This integrative form of mosquito control increases community-involvement and sense of responsibility, where citizens proactively raised awareness on arbovirus transmission and reduced available mosquito breeding sites (Elsinga et al., 2017). Active surveillance by the community also allows for local mosquito control departments to address sites that would have otherwise gone undetected (Wilke et al., 2018; Wilke et al., 2022). This integrative tactic allows for the efficient targeting of residential areas with a high prevalence of mosquitoes (Bonds, 2012).

Three critical climate factors that influence mosquito population dynamics include regional rainfall, temperature, and humidity, all of which have been associated with oviposition prevalence in unmanaged water containers and mosquito abundance (Reiskind & Lounibos, 2013; Codeço et al., 2014; Reinhold et al., 2018; Santos et al., 2020). Seasonal patterns of the regional rainfall can generate mosquito breeding habitats within unattended containers while temperature can determine the survivability and rate of development of the immature stages of *Ae. aegypti* mosquitoes (Barrera et al., 2006; Serpa et al., 2006; Couret & Benedict, 2014). Both factors contribute to the development of humidity through evaporation, and their seasonal patterns have been used to predict mosquito fluctuations annually (Azil et al., 2010; Leisnham et al., 2014; Monaghan et al., 2019). Understanding regional seasonal vector population increase would allow for efficient surveillance, source reduction, and yard management at optimal time periods (Barrera et al., 2006).

Site-based factors within socioeconomic-classified neighborhoods can also influence mosquito population (LaDeau et al., 2013; Brown et al., 2021). Cases of neglected planters, birdbaths, garbage, and other unmanaged man-made containers that accumulate water generate mosquito breeding sites that could potentiate their abundance (LaDeau et al., 2013; Whiteman et al., 2019; de Jesus Crespo & Rogers, 2022). Within urbanized areas, socioeconomic variables related to site-based management of standing water include household income, employment, graduation rates, as well as housing age, density, and structure (Unlu et al., 2011; Dowling et al., 2013; Sallam et al., 2017;

Whiteman et al., 2019). Studies have found high levels of mosquito abundance in both low socioeconomic (Unlu et al., 2011; Dowling et al., 2013; Walker et al., 2018; Scavo et al., 2021) and high socioeconomic areas (Richards et al., 2008; Unlu et al., 2011; Dowling et al., 2013). High prevalence of trash such as discarded tires and containers, and poor neighborhood drainage leading to water accumulation has been associated with low socioeconomic areas and increased mosquito habitat suitability (Unlu et al., 2011; Dowling et al., 2013; Walker et al., 2018; Scavo et al., 2021). Meanwhile, in high socioeconomic areas, unmanaged functional containers such as planters, birdbaths, and garbage cans accumulating water have been associated with increased mosquito habitat suitability (Richards et al., 2008; Unlu et al., 2011; Dowling et al., 2013).

In some cases, high income inequality within neighborhoods may make it difficult to distinguish socioeconomic influence on mosquito population dynamics between neighborhoods (LaDeau et al., 2013; Florida et al., 2019; Brown et al., 2021). Household specific information in this case could be important to determine mosquito population dynamics. The use of window screens and air conditioning have been used to reduce the transmission of mosquito-borne disease (LaRocque & Ryan, 2016). Accessibility to air conditioning units, plot size, and distance to neighboring houses can be used to study within-neighborhood effects of socioeconomic factors on mosquito population dynamics (Reiter et al., 2003).

Historically, Miami-Dade County (MDC), Florida has been heavily affected by vector-borne diseases due to the subtropical climate, and its touristic and commercial qualities conducting an influx of over 120 million visitors annually (Pond et al., 1966; Patterson, 1992; Teets et al., 2014; Krisberg, 2014; Likos et al., 2016, Wilke et al., 2022).

The tropical climate and continuously expanding urbanization accommodate the proliferation of mosquitoes where 700,000 people are expected to immigrate to by 2030 (Wilke et al., 2021; United States Environmental Protection Agency, 2012). Tourists traveling from and to endemic areas are likely to introduce arboviruses potentiating outbreaks; combining this threat with the already-established abundance of mosquitoes in MDC, residents are at risk for an outbreak (Wilke et al., 2019; Wilke et al., 2022).

The primary objective of this project is to understand local residential mosquito infestation variability based on citizen collected data, in addition to environmental and socioeconomic factors that promote mosquito infestation in MDC. Using citizen science through the Florida *Aedes* Genome Group (FLAGG) internship enhanced the availability of collected data for this surveillance-based work. I measured the expansive presence of mosquito eggs within MDC residential sites and explored meteorological drivers of seasonal *Ae. aegypti* abundance, including temperature, precipitation, and relative humidity. Median income and house values were also analyzed as potential influencing factors of site-related mosquito abundance. The abundance project was inspired by two questions: (1) *Can citizen-collected data help capture mosquito infestation fluctuation in Miami-Dade County?,* and (2) *What is driving mosquito infestation?* I hypothesized that (H1) citizen collected data will reveal that mosquito infestation and infestation patterns are stable in MDC as increased mosquito control budget and measures within MDC are expected to prevent noticeable mosquito population increases (Philip et al., 2019; Moise et al., 2020). I also hypothesized (H2) that mosquito infestation in MDC is driven by meteorological factors due to mosquito sensitivity of seasonal shifts in a tropical climate (Zheng et al., 2020; Hermanns et al., 2021; Chen et al., 2023). The increase in precipitation within the wet summer season should generate more oviposition habitats from accumulated stagnant water and subsequently increase mosquito abundance (Sota et al., 1994; Maciel-de-Freitas & Lourenço-de-Oliveira, 2011).

2.2 Methods

2.2.1 Study site

Miami-Dade County, Florida is located between longitude 80° 11' 30.4368" W and latitude 25° 45' 42.0516" N. The county is a growing metropolis with over 2,000 square miles, of which about one one-third corresponds to the Everglades National Park (Alonso & Heinen., 2011). MDC also offers optimal tropical conditions for mosquitoes to survive and thrive. The average annual high and low temperatures are 91, and 60 degrees Fahrenheit, respectively. The average annual relative humidity is 84% in the morning and 63% in the afternoon, and the annual precipitation is 61.93 inches (Osborn, 2019).

2.2.2 Egg Collection and Mosquito Infestation indices

Mosquito eggs were collected throughout Miami-Dade County through a community-based student-focused internship program, FLAGG. The data were collected from 2019 to 2022 from ovicups (a trap using stagnant water to attract gravid females for oviposition) deployed in the backyards of Florida International University college student households. Within this program, each student was given a kit containing a black ovicup, a whirl-pack bag (Nasco Sampling/Whirl-Pak), and seed paper for egg collection, along with an instruction manual on sampling and surveillance methods needed for ovicup deployment. Ovicups are composed of black plastic drinking cups that have holes drilled on the side for water drainage. Students filled ovicups with 200 ml of tap water and lined the cup with seed paper (25.4 cm by 8.9 cm). Students placed ovicups outside their residence for three days in locations that are shaded and protected by rainfall and wind. At the end of the three days, students collected the seed paper, partially air dried it, and then placed the paper with the eggs into a whirl-pack bag. Collections were returned to the FLAGG team at Florida International University (FIU) monthly. Students deployed their ovicups for one semester (8 - 16 weeks). Collections were repeated for three semesters: Summer, Fall, and Spring for each year for a total of three consecutive years.

In the laboratory, eggs were collected from the ovicups and counted using a standard microscope, and sites were de-identified with a randomly generated ID using the python uuid module. The locations were de-identified to a 150 meter or less location from the actual collection site to protect the privacy of FLAGG participants. Randomization was done with the random module in python (Kuchling & Zadka, 2012). Eggs were counted each semester to obtain the total eggs recovered (TER), which was transformed into two main indices in order to measure mosquito productivity: Egg Density Index (EDI), and Ovitrap Positivity Index (OPI). The EDI is the amount of eggs divided by the total amount of positive traps set within a semester, while the OPI is a measure of the amount of positive traps divided by the total amount of traps for a certain location for the semester. A collection was deemed to be positive if there was at least one egg found within that collection.

2.2.3 Data Analysis: Can citizen collected data help capture mosquito infestation fluctuation in Miami-Dade County?

Mosquito infestation within Miami-Dade County was evaluated to determine if there were significant differences in the abundance of mosquitoes among varying time periods based on the semester (Fall, Spring, and Summer) and the year of egg collection (Year 1, Year 2, and Year 3). A non-parametric test, the Kruskal-Wallis, compared mosquito abundance indices with different sample sizes and non-normal distributions (McKnight & Najab, 2010). This test was done across semesters and across years for each mosquito abundance index (TER, OPI, and EDI) using the R package *ggstatsplot*. A p-value under 0.05 for the Kruskal-Wallis test would indicate no significant differences between groups. The ArcGIS Pro 3.1 World Imagery ESRI Basemap (Esri, 2021; Price, 2023) was used to generate maps for Miami-Dade County collections classified by semester. The weekly time series graphs were produced using Prism 9 and epidemiological indices and meteorological indices were graphed weekly for the relevant time periods of the study (GraphPad Software).

2.2.4 Data Analysis: What is driving the variation in mosquito infestation?

A Spearman correlation matrix was produced that included mosquito abundance indices and meteorological factors such as average (TAVE), minimum (TMIN), and maximum (TMAX) temperature, precipitation (PRCP), and relative humidity (RH). Meteorological data were obtained from the meteorological stations closest to the point of collection through the National Oceanic and Atmospheric Administration National Centers for Environmental Information (NOAA's, 2017). All weather stations within the county were queried and meteorological data were aggregated into average daily values. A correlation matrix was produced for the weekly meteorological data for the week of collection and 6 lag-times (1-6 weeks from collection week) using the R package *ggstatsplot* (Patil & Powell, 2018). Lag times represent weeks prior to oviposition activity. The lag times were chosen based on time periods in which meteorological variables can affect mosquito embryonic development, hatching time, pupation, and adult oogenesis (Clements, 1999; Hendy et al., 2021; Santos et al., 2020; Sasmita et al., 2021). Mosquito development in weeks prior to oviposition affects egg-based infestation levels observed and is essential to understanding what is driving the variation in mosquito infestation. A p-value higher than 0.05 would indicate a significant linear correlation between the egg counts and the meteorological data.

Median household values and income, obtained from the United States Census Bureau, were used to explore socioeconomic factors driving mosquito infestation (U.S. Census Bureau, 2021; U.S. Census Bureau 2021a). Each collection site was matched to its census tract and assigned the corresponding household and income values. To determine if there was a correlation between mosquito abundance and socioeconomic factors, a linear regression was run to predict abundance indices by socioeconomic factors using the R package *mgcv* (Wood & Wood, 2015). A generalized additive model (gam) was fitted to see if there were nonlinear effects, using a cubic spline as the basis.

2.3 Results

2.3.1 Can citizen collected data help capture mosquito infestation fluctuation in Miami-Dade County?

Mosquito eggs were detected in an estimated average of 77.28% (+/- 8.71, standard deviation: 7.69, at alpha: 0.05) of the surveyed residential households during the summer, 52.35% (+/- 10.22, standard deviation: 9.03, at alpha: 0.05) during the fall, and 37.47% (+/- 10.13, standard deviation: 8.95, at alpha: 0.05) during the Spring (Table 2.1). The results of the Kruskal-Wallis tests indicated a significant difference in mosquito abundance based on the Fall, Spring and Summer semesters, p-values < 0.001 (Figure 2.1), and most individual semesters, p-values < 0.05 (Figure 2.2) for every index analyzed. Kruskal-Wallis analysis based on years resulted in non-significant p-values > 0.05 between all years for every index analyzed.

Table 2.1. Summary of index data from collections performed by the FLAGG internship in Miami-Dade County

	Semester	Period - (From - To)	% deployed / recovered	Percent positive of sites (PPS)	Ovitrap Positivity Index % (OPI)	Total Eggs Recovered (TER)	Egg density index (EDI)
	Summer 2019	5/18/2019 to 07/20/2019	65.85	80.49	39.26	886	8.36
	Fall 2019	9/14/2019 to 12/7/2019	78.28	64.71	8.09	112	8.00
	Spring 2020	1/9/2020 to 4/18/2020	44.19	50.00	11.92	183	3.73
	Summer 2020	06/07/2020 to 7/26/2020	82.86	84.68	42.82	3041	8.64
	Fall 2020	9/20/2020 to 12/6/2020	57.99	48.98	17.74	594	4.91
	Spring 2021	2/28/2021 to 4/25/2021	64.47	29.63	8.72	331	8.07
	Summer 2021	5/30/2021 to 7/25/2021	57.52	66.67	41.29	2358	10.82
	Fall 2021	9/5/2021 to 12/5/2021	60.15	43.37	13.59	757	7.97
	Spring 2022	1/23/2022 to 5/1/2022	73.09	32.79	5.84	418	10.72
Sum	**All Semesters**	**5/18/2019 to 5/1/2022**				**8680**	
Average	**All Semesters**		**63.38**	**56.20**	**21.91**	**964.44**	**8.39**

Table 2.1. Summary of index data from collections performed by the FLAGG internship in Miami-Dade County

	Semester	No. of weeks	No. of sites	No. of positive sites (+)	No. chances to deploy/recover	No. recovered	No. of positive traps (+)
	Summer 2019	10	41	33	410	270	106
	Fall 2019	13	17	11	221	173	14
	Spring 2020	15	62	31	930	411	49
	Summer 2020	8	124	105	992	822	352
	Fall 2020	12	98	48	1176	682	121
	Spring 2021	9	81	24	729	470	41
	Summer 2021	9	102	68	918	528	218
	Fall 2021	14	83	36	1162	699	95
	Spring 2022	15	61	20	914	668	39
Sum	**All Semesters**	**105**	**669**	**376**	**7452**	**4723**	**1035**
Average	**All Semesters**	**11.67**	**74.33**	**41.78**	**828.00**	**524.78**	**115.00**

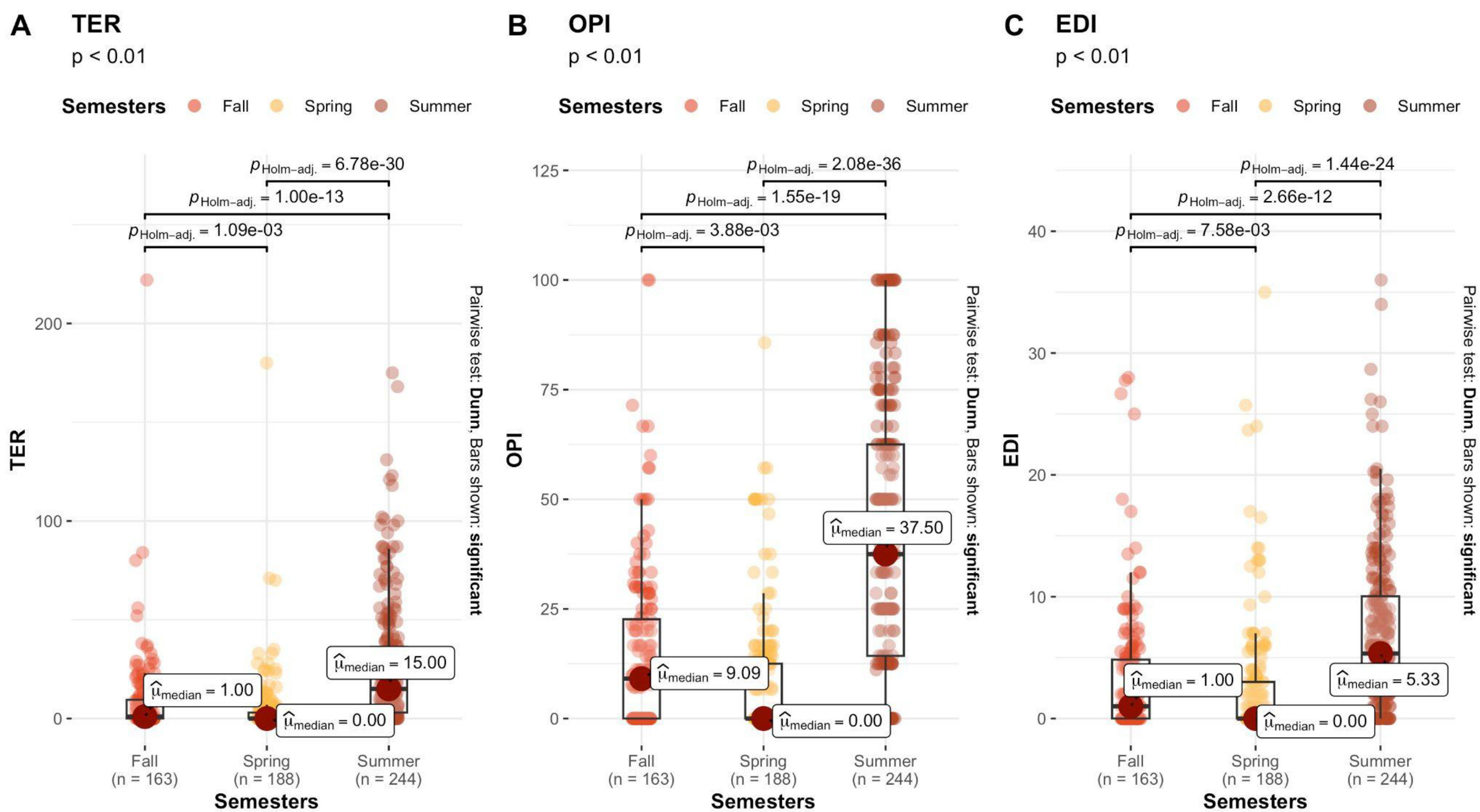


Figure 2.1. Comparison of weekly averages for (A) TER, (B) OPI, and (C) EDI between Fall, Spring and Summer semesters. All three indices show significantly higher infestation levels in MDC during the summer and significantly lower infestations during the spring.

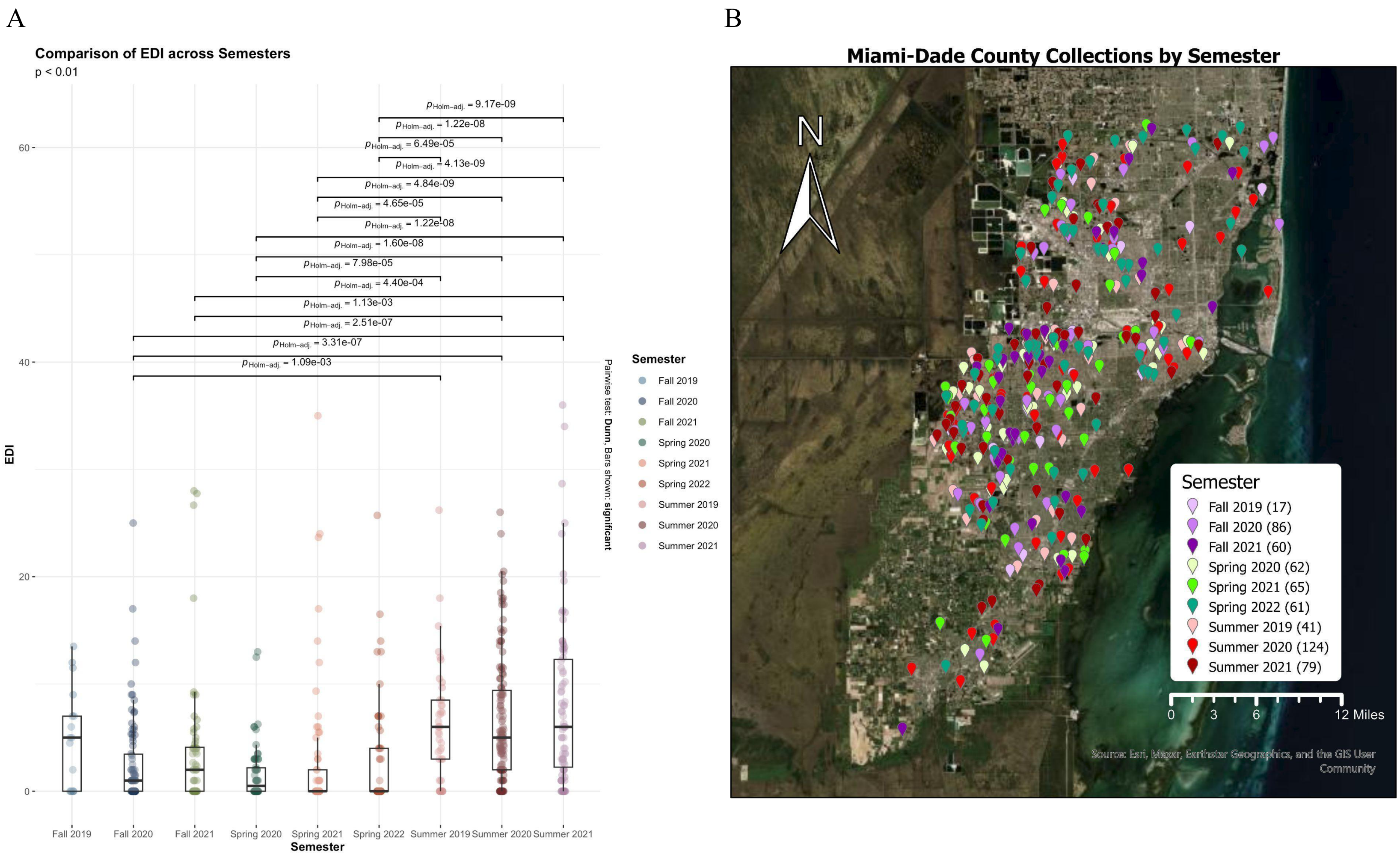


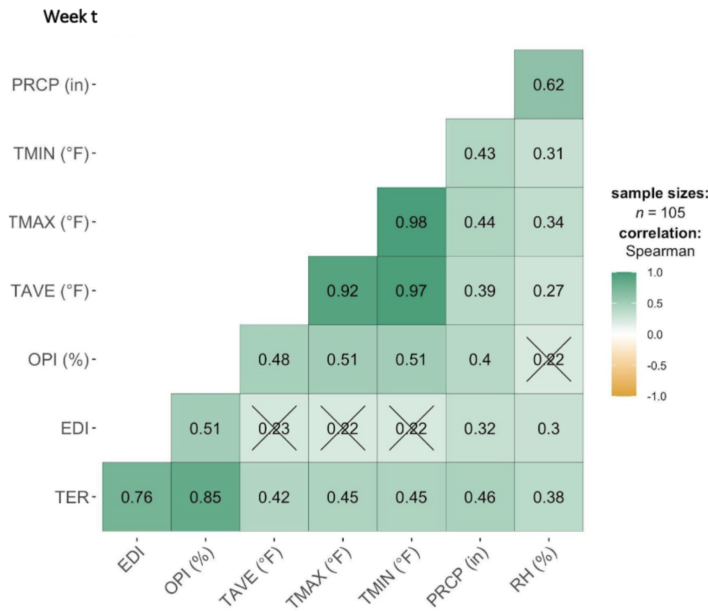
Figure 2.2. Comparison of weekly averages for (A) EDI between semesters using Kruskal Wallis showing significant differences between most semesters. (B) Map of collection sites throughout MDC showing that mosquito infestation is present throughout MDC households. Sites include student collections from Fall 2019 to Spring 2022.

2.3.2 What is driving the variation in mosquito infestation?

Meteorological factors were significantly correlated with mosquito abundance indices at p-value < 0.05 in week t, which is the week preceding the collection (Figure 2.3) and weeks t-1 through t-6 (Figure 2.4). The Spearman correlation matrix for week shows significant correlations at p-value < 0.05 for TER and the following meteorological factors in week t : TAVE, TMAX, TMIN, PRCP and RH, week t-1: TAVE, TMAX, TMIN, PRCP and RH, week t-2: TAVE, TMAX, TMIN, PRCP and RH, week t-3: TMAX and PRCP, week t-6: TMAX. Significant correlations were also found at p-value < 0.05 for EDI and the following meteorological factors in week t: PRCP and RH, and week t-2: RH. Lastly, significant correlations were found at p-value < 0.05 for OPI and the following meteorological factors in week t: TAVE, TMAX, TMIN, and PRCP, week t-1: TAVE, TMAX, TMIN, and PRCP, week t-2: TAVE, TMAX, TMIN, PRCP and RH, week t-3: TAVE, TMAX, TMIN, and PRCP, and week t-4 through week t-6: TMAX.

Socioeconomic factors did not have a significant effect on mosquito abundance indices. All three mosquito abundance indices (TER, OPI and EDI) were individually tested against household and income values, and the coefficients were not statistically significant for any test in either the linear regression or the gam model (p value > 0.05).

A



B

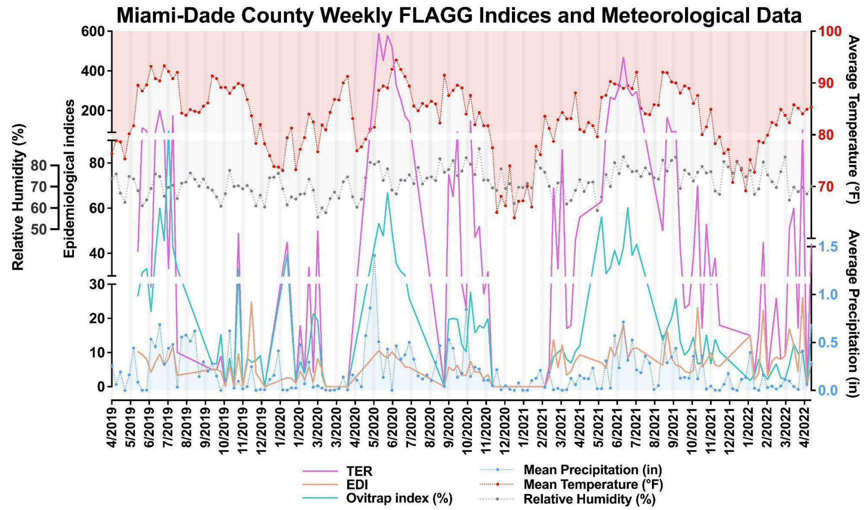


Figure 2.3. (A) Spearman correlation matrix of indices and meteorological variables for week t including average weekly precipitation (PRCP), minimum temperature (TMIN), maximum temperature (TMAX), average temperature (TAVE), and Relative humidity (RH). X within the correlation matrix boxes showcases a value that is non-significant at $p < 0.05$ (Adjustment: Holm). (B) Weekly distribution of total eggs recovered (TER), EDI and OPI compared to weekly mean precipitation, temperature and relative humidity for two years of collections.

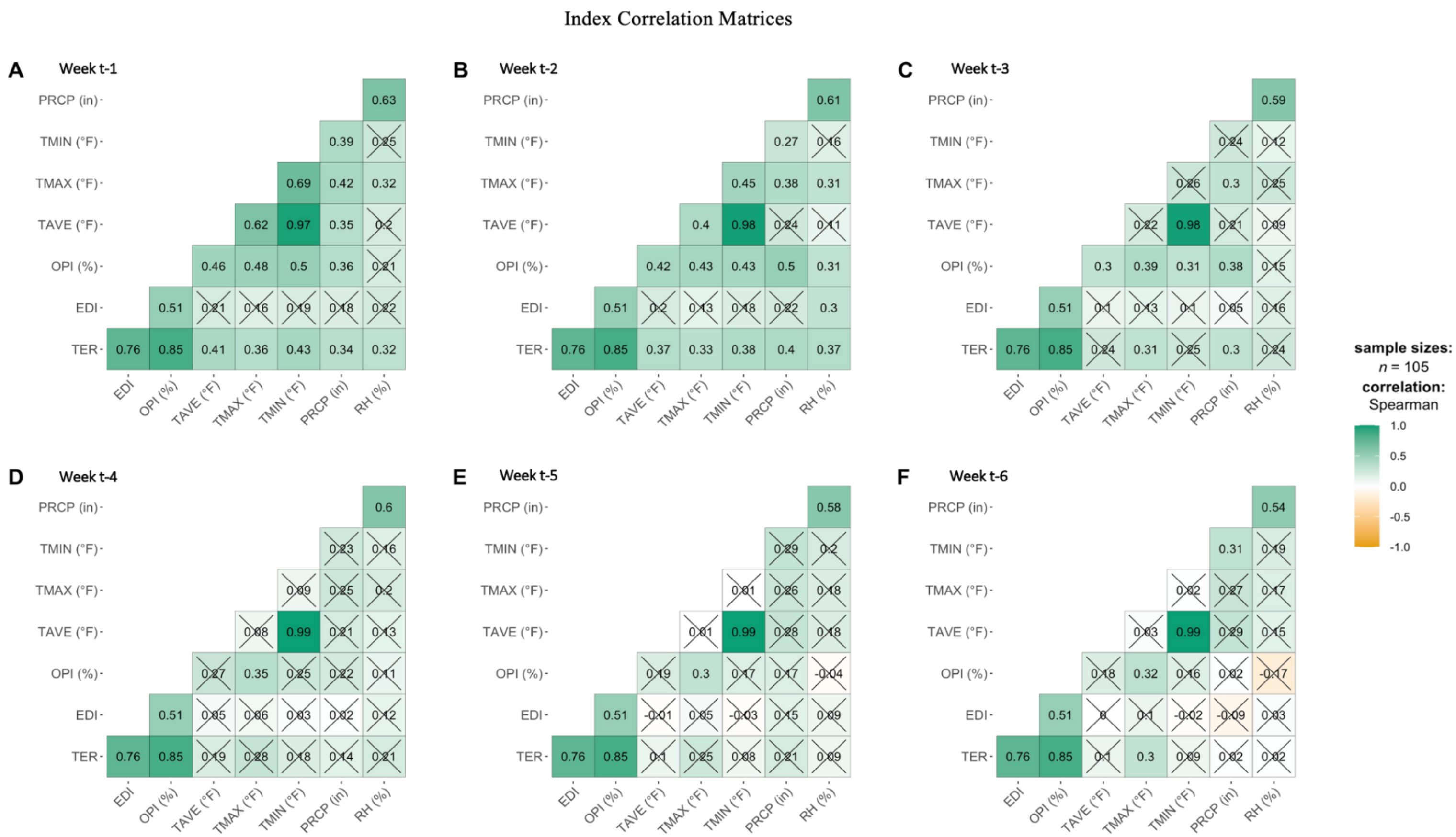


Figure 2.4. Spearman correlation matrices of indices and meteorological variables for weeks t-1 through t-6 (A-F, respectively). Matrices include average weekly precipitation (PRCP), minimum temperature (TMIN), maximum temperature (TMAX), average temperature (TAVE), and Relative humidity (RH). X within the correlation matrix boxes showcases a value that is non-significant at $p < 0.05$ (Adjustment: Holm).

2.4 Discussion

The primary objective of this project was to understand local residential mosquito infestation variability based on citizen collected data, and the environmental and socioeconomic factors that may promote mosquito infestation in Miami-Dade County. MDC is infested with mosquitoes year-round in a large percentage of residential sites. The mosquito abundance in MDC has a very pronounced difference between dry (spring), early-wet (Summer) and late-wet seasons (fall). This annual oscillation of mosquito abundance was associated with temperature, precipitation, and relative humidity. OPI was consistently associated with all three weather factors for the week of mosquito egg collections and up to two weeks prior to collection. These results show that the weather could impact the proliferation of mosquitoes in future generations, which has important implications for arbovirus risk in MDC residential households.

The correlation between meteorological variables and mosquito abundance indices in MDC can be explained by mosquito life cycle events that depend on temperature, precipitation, and relative humidity (Reiskind & Lounibos, 2013; Codeço et al., 2014; Reinhold et al., 2018; Santos et al., 2020). These three key weather variables can affect mosquito embryonic development, hatching time, pupation, and adult oogenesis and therefore, weather variables may have a lag time in their effects on the mosquito egg-based infestation levels observed (Clements, 1999; Hendy et al., 2021; Santos et al., 2020; Sasmita et al., 2021). These variables have been studied to designate meteorological lag times with the best correlation to mosquito infestation, cited as 2 (Azil et al, 2010; Stewart Ibarra et al., 2013), 3 (Dibo et al., 2008; Estallo et al., 2015; Stewart Ibarra et al., 2013), 4

(Dibo et al., 2008; Dominguez el al., 2000), and 6 (Estallo et al., 2008; Estallo et al., 2015; Stewart Ibarra et al., 2013) weeks prior to vector oviposition activity. Within MDC, the associated lag times correspond to weather impacting the mosquito development of at least one previous mosquito generation.

The seasonally oscillating patterns of mosquito abundance in MDC aligns with previous research (Chen et al., 2023). In MDC, the distinctive cyclic changes between the wet and dry seasons can be divided into three biologically meaningful seasons, the early rainy season, the late rainy season, and the dry season (Reiskind & Lounibos, 2013; Juliano et al., 2004). The trend of *Ae. aegypti* mosquito abundance has been documented for these seasons in MDC, where the biggest increase occurs from May to August, peaking in July (Leisnham & Juliano, 2009; Alexander et al., 2022). A lower relative abundance has also been found from September to December when compared to the time period of January to April (Leisnham & Juliano, 2009; Reiskind & Lounibos, 2013; Giordano et al., 2021). These trends are maintained within residential households in MDC.

Due to the subtropical climate in MDC, mosquito populations are largely present within residential sites during the spring season, potentially increasing arbovirus transmission during the coldest months (Monaghan et al., 2016; Roise & Wallace, 2022). Spring months have shown a simulated value near-zero for *Aedes aegypti* abundance, the primary priority vector in MDC, within most of the United States (Monaghan et al., 2016, Wilke et al., 2021). Meanwhile, MDC's favorable climate conditions support vector survival outdoors year-round within key habitats including tire shops, bromeliads, buckets, flower pots, storm drains and garbage cans (Wilke et al., 2018; Wilke et al., 2019; Wilke et al., 2020; Roise & Wallace, 2022). Currently, household inspections by MDC mosquito control are based on citizen complaint calls about adult mosquito presence (Wilke et al., 2020).

The surveyed MDC abundance suggests targeting the end of off-peak seasons to decrease the base populations that lead to peak-season abundance. Larger access to residential surveillance during the off-peak season could allow mosquito control to more efficiently target mosquitoes. This is because reductions made in the off-peak seasons can prevent the accumulation of large mosquito populations within peak season, reducing mosquito abundance and potentially arbovirus transmission (Leach et al., 2020). Targeting strategies can involve larvicides, adulticides, source reduction, and using traps such as ovitraps (used to collect mosquito eggs), sticky traps (used to collect adult mosquitoes), or CO_2 traps (used to collect female mosquitoes) (Kröckel et al., 2006; Baldacchino et al., 2015; Mains et al., 2019).

Implementation of management strategies would require careful mosquito surveillance and knowledge on their abundance drivers. Community-based surveillance programs and citizen science would greatly facilitate this initiative as they can be used to access private residential areas needed to help understand mosquito distribution across the community (Martinou et al., 2020). These programs could also bring forth consistent and systematic mosquito surveillance, providing timely information on mosquito abundance in the area (LaDeau et al., 2013; Likos et al., 2016). Community surveillance can also help identify potential breeding sites of importance and hotspots that can then be targeted by mosquito control. By having timely information, targeting methods can inform decisions on where, when, and how to target mosquitoes during off-peak seasons.

Understanding the distribution of vectors and how they are affected by seasonal change can inform evidence-based health policies to reduce the impacts of vector-borne disease prevalence (World Health Organization, 2014). This is of increasing importance with climate change, since temperature and precipitation increase relates to an increase in mosquito abundance (Reiskind & Lounibos, 2013; Codeço et al., 2014; Reinhold et al., 2018; Santos et al., 2020). As the MDC human population increases, we need more interventions to support human health (Al Rifat & Liu, 2022). Interventions would include access to residential sites which can be managed through the use of a community-based surveillance program like FLAGG.

2.5 Conclusion

Studying local residential mosquito infestation variability can help us understand the importance of household access for surveillance and mosquito oviposition site reduction. Mosquito oviposition sites are hard to find and eliminate, as private areas are difficult to access or are unreachable by truck spraying (Martinou et al., 2020). Residents from all socio-economic ranges within MDC are affected, and they can all be recruited to access sites that would have otherwise gone undetected. Programs like FLAGG can be used to reach a larger amount of residential sites during critical time periods to reduce peak season mosquito abundance. This in turn can better inform mosquito control of mosquito populations not covered by citizen complaint calls or routine surveillance.

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CHAPTER 3: Using Community-collected Population Genomics Field Data for Population Structure Analysis Within South Florida *Ae. aegypti* Mosquitoes

3.1 Background

An understanding of *Aedes aegypti* population genetics can lead to the ability to trace introductions and measure local health risk (Gloria-Soria et al. 2016; Garcia et al., 2019; Schmidt, 2019). The introduction and spread of mosquitoes across geographic regions can be assessed using genetic markers (Kamgang et al., 2011; Olanratmanee et al., 2013). When analyzing a large geographic range, fine-scale genomics on multiple individual mosquitoes can be analyzed to identify population structures potentially related to landscape variables (Kraemer et al., 2019; Schmidt et al., 2019; Schmidt et al., 2021a).

Invasion studies have begun using whole genomes, showing that invasions have often been found to have multiple origins (Schmidt et al, 2021a). In California, studies of *Ae. aegypti* population genomics found that mosquito invasions came from three different sources through the identification of three main genetic clusters (Lee et al., 2019). Invasions can lead to the adaptive introgression of alleles between spatially discrete populations and can intercede mosquito control strategies (Schmidt et al., 2021a; Schmidt et al., 2021b). This adaptation to the environmental conditions can then confer higher invasive potential to the local populations and potentially better survival and reproductive potential (Sherpa et al., 2019).

More recent local adaptive gene flow and selective sweeps may require population genomics since regions across the genome may present short genetic distances between populations (The *Anopheles gambiae* 1000 Genomes Consortium, 2017). Migration and demographics can be investigated by looking for linked selection within regions of low recombination and intraspecific inversion polymorphisms (Cheng et al, 2012; Schmidt et al, 2021a). Contiguous genome assemblies that are annotated would allow for this local adaptive evolution to be investigated at fine scales due to the increase in analytical options and power (Schmidt et al, 2021a).

The investigation of native and local populations is important as climatic fluctuations can affect both genetic and morphometric variations due geographic isolation driving genetic differentiation and environmental factors driving local adaptations (Roos et al., 2011; Nosil et al., 2012; Kramer et al., 2023). In urban areas, high spatial heterogeneity and low habitat connectivity can affect *Ae. aegypti* population dynamics and dispersal. This combination can lead to differences in genetic structure, typically in more recently established populations in highly urbanized areas (Maffey et al., 2022; Pless et al., 2021). As time passes within these highly fragmented urban regions, strong genetic drift can cause the progressive decrease in genetic structure (Fauvelot et al., 2009; Vandergast et al., 2009; Munshi-South et al., 2016). Understanding the population structure of mosquitoes is the first step to studying the origins and eventually the potential invasion success related to factors such as insecticide resistance, and cold, dry and saline environment adaptation (Schmidt et al, 2021a).

South Florida can act as a pathogen gateway for mosquito-borne diseases due to the high rates of urbanization and consistency of tourism, making genomic studies on wild *Ae. aegypti* genomes essential to understanding the spread of this species (Lee et al., 2019; Wilke et al., 2019). Whole genome samples of Florida-based wild *Ae. aegypti* samples have already been found to cluster with California-based populations, suggesting that Florida mosquitoes are the culprits of mosquito invasion and expansion within some Californian towns (Lee et al., 2019). As more genomic information becomes available, migration models and patterns can be analyzed based on source population and local adaptation identification (Savolainen et al., 2013; Flanagan et al., 2018).

The primary objective of this work on *Ae. aegypti* genetics is to identify genomic clusters in field *Ae. aegypti* mosquitoes throughout South Florida by using community-collected field mosquito sample data. This work addresses the following question: (3) *Can citizen collected data help capture the population structure within South Florida Ae. aegypti mosquitoes?* I hypothesize that the citizen collected data will show south Florida having a low number of genetically distinct populations (H3). Within urban areas, small fragmented populations have been reported in many species to lead to stronger genetic drift and a decrease in genetic diversity over time (Fauvelot et al., 2009; Vandergast et al., 2009; Munshi-South et al., 2016). Since *Ae. aegypti* mosquitoes have been well-established within South Florida, I would expect that genetic drift and natural selection have gradually led to decreased diversity on the genetic makeup of the population (Johnson et al., 2015; Johnson & Munshi-South, 2017).

3.2 Methods

3.2.1 Study sites and Collections

Ae. aegypti eggs were collected from six cities (Coral Gables, Miami Gardens, Homestead, Sunrise, Anna Maria Island, Myakka), and five census-designated places (CDP) (Kendall, Kendale Lakes, Westchester, Fontainebleau, Cortez) in South Florida (Table 3.1). The eggs were collected using black CDC ovitraps or ovijars for all south

Florida locations. Miami-Dade and Broward County collections were performed by Florida *Aedes aegypti* Genome Group (FLAGG) internship members in the same manner as described in Chapter 2. All egg collections made on private sites were conducted after acquiring resident or owner permission. The ovitraps and ovijars are cups that were filled up with 200 ml of tap water and lined with seed papers of approximately 25.4 cm by 8.9 cm to prepare for deployment. Ovijars were placed outside but near homes with water for a total of three days within a shaded area protected by rainfall and wind. Eggs were hatched in pans with 500 ml of hatching broth made from boiled water infused with two grounded Tetramin (catalog #16152, Tetra, Melle, Germany). Once in their pupal stage, males and females were separated into different cages. Two to three male biological replicates from each site were then individually identified and stored in 100% ethanol prior to undergoing the DNA extraction process.

Table 3.1. Study sites for south Florida *Ae. aegypti* mosquito samples and location attributes

Location	Coordinates	Human population Density (mi^2)	Number of Samples	Sample names	Subdivision	County
Coral Gables	25.7492° N, 80.2635° W	1,338	8	CG1-1, CG1-2, CG1-3, CG2-1, CG2-2, CG2-3, CG3-1, CG3-3,	City	Miami - Dade
Miami Gardens	25.9420° N, 80.2456° W	5,538	3	MG1-1, MG1-2, MG1-3,	City	Miami - Dade
Homestead	25.4687° N, 80.4776° W	4,424	3	HS1-1, HS1-2, HS1-3	City	Miami - Dade

Location	Coordinates	Human population Density (mi²)	Number of Samples	Sample names	Subdivision	County
Sunrise	26.1670° N, 80.2566° W	5,223	3	S1-1, S1-2, S1-3	City	Broward
Anna Maria Island	27.5041° N, 82.7145° W	2,101	3	H5-1, H5-2, H5-3	City	Manatee
Myakka	27°20′59″N 82°9′41″W	202	3	I8-1, I8-2, I8-3	City	Manatee
Kendall	25.6660° N, 80.3578° W	4,652	3	K1-1, K1-2, K1-3	Census Designated Place	Miami - Dade
Kendale Lakes	25.7082° N, 80.4070° W	6,472	3	KL1-1, KL1-2, KL1-3	Census Designated Place	Miami - Dade
Westchester	25.7548° N, 80.3273° W	6,724	3	W1-1, W1-2, W1-3	Census Designated Place	Miami - Dade
Fontainebleau	25.7729° N, 80.3478° W	14,258	5	FB1-1, FB1-2, FB1-3, FB2-2, FB2-3	Census Designated Place	Miami - Dade
Cortez	27.4692° N, 82.6862° W	995	2	D-1, D-3	Census Designated Place	Manatee

3.2.2 Sequence Preprocessing and Quality Control

Raw reads of the DNA were mapped to the *Aaeg*L5 mitochondrial reference genome with BWA-MEM version 0.7.17 (Li, 2013) prior to the mapping of the nuclear genomes. This follows mapping recommendations in Schmidt et al. and Lee et al. due to the potential confounding SNPs generated from mitochondrial pseudogene presence (Lee et al. 2019; Schmidt et al., 2019). Mapped and unmapped reads were extracted with Samtools version 1.9 (Li et al., 2009) and unmapped reads were converted from SAM to

BAM files using Samtools view. Unmapped reads were trimmed with Trimmomatic version 0.36 (Bolger et al., 2014) and mapped using *Aaeg*L5 as the reference genome and BWA-MEM. Mapped reads were converted from SAM to BAM files using Samtools view. The Picard version 2.18.3 (Picard Toolkit, 2019) tool AddOrReplaceReadGroups was used to assign the flow cell number and lane, library name, read name, and directory name to all reads in each sample. All BAM files for each individual sample were then merged using Picard MergeSamFiles, and duplicate reads in each DNA fragment were then identified and sorted using Picard MarkDuplicates and Picard SortSam.

Alignment statistics were generated using samtools flagstat to compute pass or fail Quality Check status for each sample, Samtools depth to generate the DNA read depth at each position, and mpileup to extract the number of reads, read bases, base qualities, and average coverage for a Binary Alignment Map (BAM) file. Further mapping statistics were performed with Qualimap version 2.2.1 (Okonechnikov et al., 20016) to evaluate the quality of the DNA alignment data. GATK (Van der Auwera & O'Connor, 2020) HaplotypeCaller was then used to convert the BAM file into a Genomic Virtual Contact File (GVCF) for each sample. GATK GenomicsDBImport was then used to consolidate all of the individual GVCFs into a database. Joint genotyping on all samples within the database was done using GATK GenotypeGVCFs, and it then produced a final VCF where all samples were genotyped jointly.

GATK SelectVariants was used on the final VCF to select for SNPs and GATK VariantFiltration applies multiple thresholds for hard filtering to the SNP VCF. Thresholds include QualByDepth (QD) < 2.0 to filter out the shoulder of variants with low unfiltered variant density and depth. The FisherStrand (FS) threshold was set to be > 60.0 to remove false positives without risking losing true positive variants. The threshold for StrandOddsRatio (SOR) was set to > 3.0 to remove the long tails of variants showing strand bias in read ratios that cover the alleles. The set RMSMappingQuality (MQ) < 40.0 allows for low mapping quality variants to be filtered out while still being lenient enough to keep potentially good quality variants. MappingQualityRankSumTest (MQRankSum) compares the reference and alternate allele mapping qualities and was set to < -12.5 in order to prevent passing quality variants. Lastly, the ReadPosRankSumTest (ReadPosRankSum) compares the reference and alternate allele position within the reads and the measure was set at < -8.0 in order to reduce the unfiltered variants with alleles at the ends of the reads without reducing variants that pass the variant criteria. Refer to supplementary table S3.1 for final mapping statistics for the wild *Ae. aegypti* whole genome sequence samples.

3.2.2 Data Analysis: Can citizen collected data help capture the population structure within South Florida *Ae. aegypti* mosquitoes?

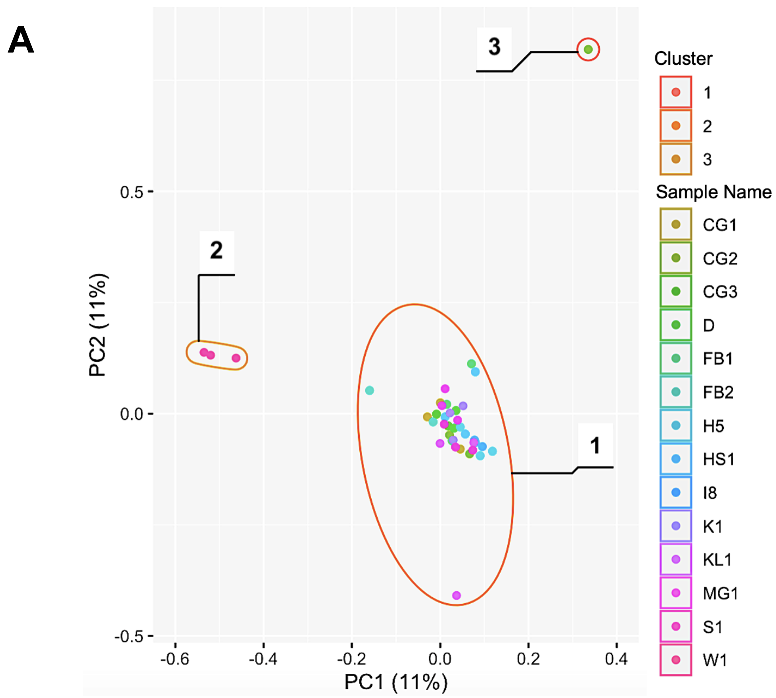
A principal component analysis (PCA) was used to identify the number of clusters that make up the population structure within South Florida *Ae. aegypti* mosquito samples. These clusters reveal genetically distinct populations through an estimated degree of genetic differentiation. If the PCA results in one major cluster encompassing the majority of individuals, this could mean there is one prevalent population, and other clusters may represent outliers with a distinct genetic makeup (Liu et al., 2013; Duforet-Frebourg et al., 2016). The optimal number of clusters was determined after running the elbow method, the average silhouette method, and the gap statistic method of cluster indication using the *factoextra* package in R (Kassambara & Mundt, 2017).

This method of computing k-means clustering was done for PCs 1 and 2, and PCs 3 and 4. PCs 1 through 4 represent the major sources of genetic data variation within the sample. PC 1 and 2 are used as they tend to be the most informative in explaining genetic variation, while PC3 and 4 are used to capture additional variation and identify subpopulations possibly separated in association with a geographic factor (Visscher et al., 2009; Radovanović et al., 2018). Clusters were mapped and cluster profiles were generated using R packages *factoextra*, *ggplot2* and *ggforce* (Wickham et al., 2016; Pedersen et al., 2020). Samples belonging to each cluster were identified using the R package *dplyr* (Wickham & Wickham, 2020).

3.3 Results

3.3.1 Can citizen collected data help capture the population structure within south Florida *Ae. aegypti* mosquitoes?

Each PCA resulted in three distinct genetic populations separating the mosquito samples, with one prevalent population. The first two principal components of the PCA each explained 11% of the total genetic variance. PC1 and PC2 divided the samples into three clusters: cluster 1 with 35 samples, cluster 2 with 3 samples, and cluster 3 with 1 sample (Figure 3.1). Additionally, the third and fourth principal components accounted for 11% and 10% of the total genetic variance, respectively. PC3 and PC4 divided the samples into three clusters: cluster 1 with 2 samples, cluster 2 with 29 samples, and cluster 3 with 8 samples (Figure 3.2). See Table 3.2 below for the sample names belonging to each cluster for each PCA.



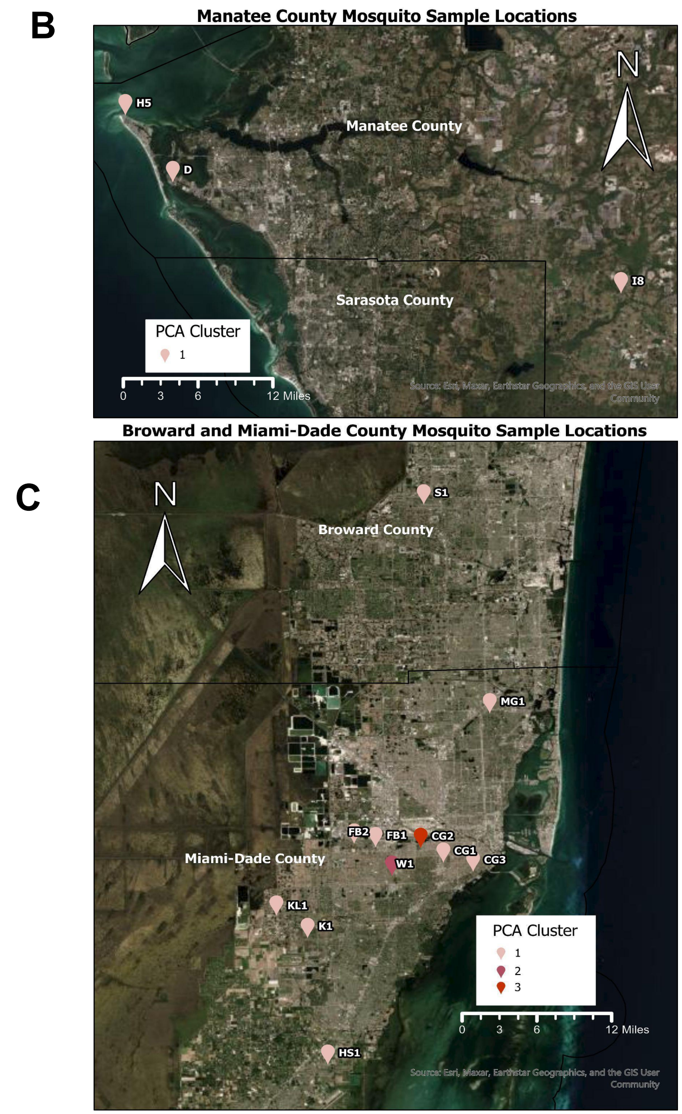


Figure 3.1. (A) PCA analysis showcasing clusters generated using PC1 and PC2 for mosquito samples. Maps of collection sites for the 3 sample locations in Manatee County (B) and for the 11 sample locations in Broward and Miami-Dade Counties (C). Sites include student collections from Spring 2019.

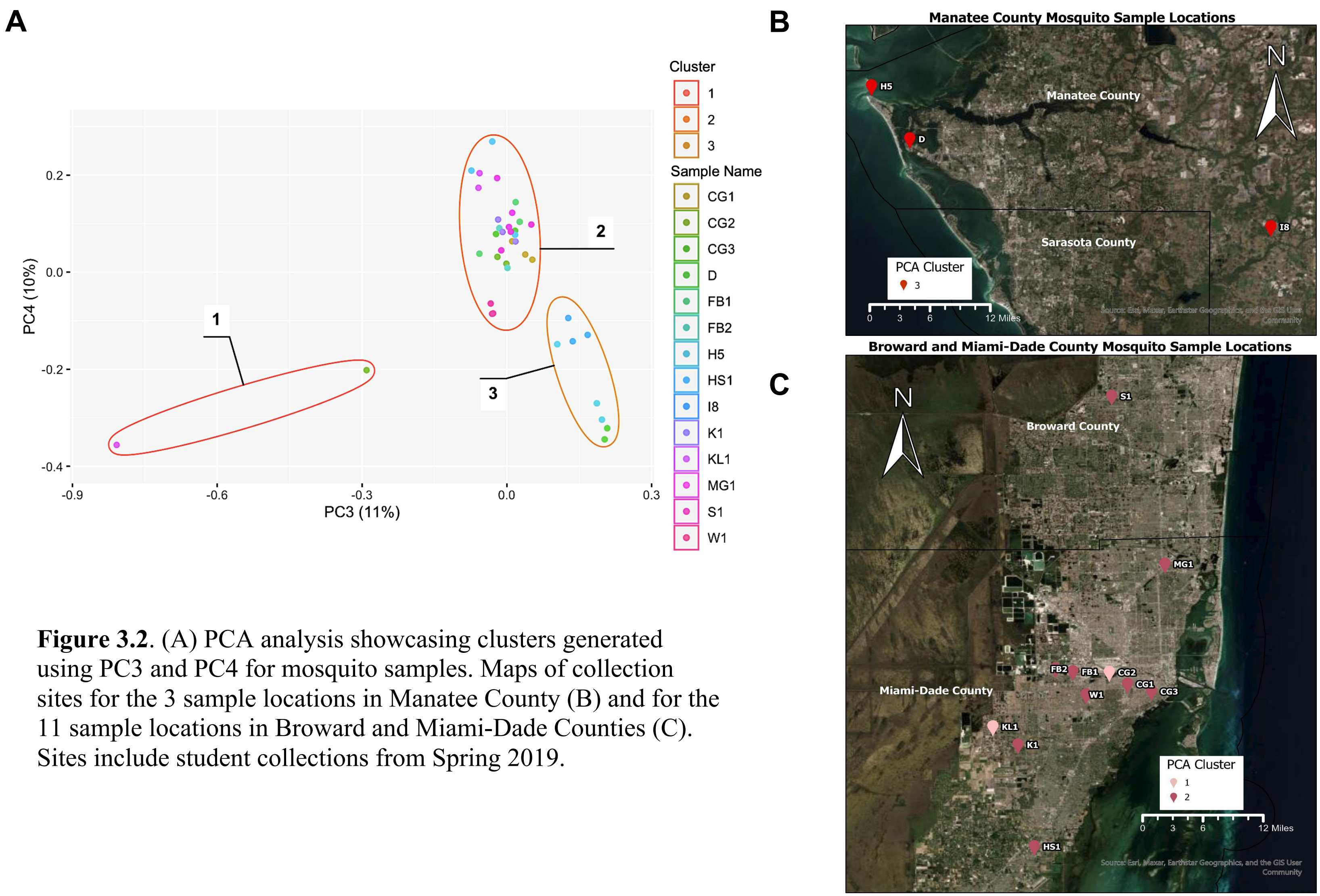


Figure 3.2. (A) PCA analysis showcasing clusters generated using PC3 and PC4 for mosquito samples. Maps of collection sites for the 3 sample locations in Manatee County (B) and for the 11 sample locations in Broward and Miami-Dade Counties (C). Sites include student collections from Spring 2019.

Table 3.2. List of *Ae. aegypti* mosquito sample names belonging to Figure 3.1 and Figure 3.2 clusters

PCA	Cluster	Sample names
PC1 and PC2	1	CG1-1, CG1-2, CG1-3, CG2-1, CG2-2, CG3-1, CG3-3, MG1-1, MG1-2, MG1-3, HS1-1, HS1-2, HS1-3, S1-1, S1-2, S1-3, H5-1, H5-2, H5-3, I8-1, I8-2, I8-3, K1-1, K1-2, K1-3, KL1-1, KL1-2, KL1-3, FB1-1, FB1-2, FB1-3, FB2-2, FB2-3, D-1, D-3
	2	W1-1, W1-2, W1-3
	3	CG2-3
PC3 and PC4	1	CG2-3, KL1-2
	2	CG1-1, CG1-2, CG1-3, CG2-1, CG2-2, CG3-1, CG3-3, MG1-1, MG1-2, MG1-3, HS1-1, HS1-2, HS1-3, S1-1, S1-2, S1-3, K1-1, K1-2, K1-3, KL1-1, KL1-3, FB1-1, FB1-2, FB1-3, FB2-2, FB2-3, W1-1, W1-2, W1-3
	3	D-1, D-3, H5-1, H5-2, H5-3, I8-1, I8-2, I8-3

3.4 Discussion

The primary objective of this work on *Ae. aegypti* genetics was to identify genomic clusters in field *Ae. aegypti* mosquitoes throughout south Florida by using community collected field mosquito sample data. We show that using citizen science through our FLAGG internship can facilitate the collection of population genomics field data. There were three distinct genetic populations separating the south Florida mosquito samples, however, most samples were encompassed in cluster 1. This suggests that the mosquitoes within cluster 1 share a large proportion of the genetic variation captured by PC1 and PC2. This cluster included samples from all three counties: Miami-Dade, Broward, and Manatee County. This suggests the influence of genetic drift and natural selection where there is a long-term population history. The genetic variation captured by PC3 and PC4 may be attributed to geographical pressures. Cluster 2 in this PCA contains samples from Miami-Dade and Broward County while Cluster 3 contains only samples from Manatee County. This shows valuable insights into individual variation influenced by location. In both PCAs, samples were detected to generate a small third cluster, potentially made up of outliers.

Cluster groupings along PCs can demonstrate population subdivisions relating to geographical groups, which can be seen in the South Florida *Ae. aegypti* mosquito samples (Clarkson et al., 2020; Yurchenko et al., 2020). Differences in mosquito clusters may be explained by population history, environmental conditions, landscape barriers, and migration patterns (Fonzi et al., 2015; Ravinet et al., 2017). Differences in ecological niches or different levels of insecticide resistance can also lead to clusters being genetically distinct (Strode et al., 2008). By understanding the formation of distinct subpopulations within an area, mosquito control can develop more targeted mosquito control strategies. Mosquito control programs can adjust where they spray insecticides, how much they spray, and what insecticides they use, generating a more effective approach to mosquito abatement.

Population differentiation between genomic clusters within PCAs, when used alongside other population structure analyses, have led to the attribution of lineage origin when conducting population analysis (Singh et al., 2019). In the future, other admixture, phylogenetic and demographic analyses can be used in conjunction with a PCA to understand genetic variation, identify individuals with mixed ancestry, and provide evolutionary relationships between populations and migration patterns (Manel et al., 2003; Lee et al., 2019; Rose et al., 2020). PCAs are only the beginning of a comprehensive population analysis of high-dimensional genomic data. By integrating multiple methods of analysis, other driving factors of population differentiation can be identified, such as geographic and environmental factors. Identifying drivers through genomic analysis can then provide a foundation to inform mosquito control management strategies, contributing to effective reduction of *Ae. aegypti* populations.

Developing control strategies based on regional population structure and mosquito adaptability depend on the availability of mosquito genomic exploration (Lee et al., 2019; Ruzzante et al., 2019). As genomic tools are used to help identify genetic markers associated with insecticide or vector competence, this information could aid in the development of new mosquito control tools and inform local interventions. With genomic tools that help identify areas of high genetic diversity and gene flow, we can better detect areas with increased risk of arbovirus transmission or development of insecticide resistance

(Steffler et al., 2016; Salgueiro et al., 2019). Interventions can then be tailored to certain residential neighborhoods or regions with distinct genetic profiles, leading to better health outcomes for local communities.

3.5 Conclusion

Understanding the population structure of *Ae. aegypti* mosquitoes within south Florida can provide important information for local mosquito control programs. Population genomic data are useful for the identification of genetic markers that can then be used for downstream applications. Using genomic data to monitor population structure can help us understand migration patterns, introductions, and genomic regions under selection. The population structure of wild mosquitoes from residential areas can eventually be used alongside field survey data and mosquito surveillance efforts to inform targeted mosquito management. This study provides a foundation for future research on *Ae. aegypti* population structure in South Florida. It highlights the potential utility of genomic tools that can be used in providing insight into the unique adaptability and dispersal patterns of a local population.

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Supplementary Materials

Supplementary Table S3.1. Mapping statistics for wild *Ae. aegypti* whole genome sequence samples

Sample	# reads	# mapped reads	% mapped reads	Mean mapping quality
CG1-1	179,892,788	164,279,536	91.32%	11.3949X
CG1-2	175,356,131	159,295,467	90.84%	11.541X
CG1-3	182,425,764	165,226,405	90.57%	11.2485X
CG2-1	170,971,384	156,782,452	91.70%	11.2791X
CG2-2	181,132,029	162,677,558	89.81%	11.0938X
CG2-3	165,736,666	151,764,256	91.57%	10.9741X
CG3-1	175,568,532	160,481,808	91.41%	11.4923X

Sample	# reads	# mapped reads	% mapped reads	Mean mapping quality
CG3-3	176,090,112	160,988,639	91.42%	11.3663X
D-1	177,413,214	161,273,533	90.90%	11.2985X
D-3	174,908,103	158,688,330	90.73%	11.2613X
FB1-1	182,327,448	163,591,372	89.72%	11.0715X
FB1-2	163,126,220	147,016,199	90.12%	11.0601X
FB1-3	163,629,040	148,894,263	91.00%	11.1804X
FB2-2	174,068,198	156,465,794	89.89%	11.2974X
FB2-3	181,915,250	165,613,847	91.04%	11.2244X
H5-1	177,924,498	161,706,212	90.88%	11.2402X
H5-2	171,237,358	153,229,285	89.48%	11.2536X
H5-3	176,745,325	159,639,346	90.32%	11.3495X
HS1-1	180,965,125	163,247,149	90.21%	11.1258X
HS1-2	181,670,334	165,969,851	91.36%	11.2461X
HS1-3	177,092,556	159,171,624	89.88%	11.1417X
I8-1	173,894,417	157,358,061	90.49%	11.1445X
I8-2	174,728,017	157,503,016	90.14%	11.3639X
I8-3	179,165,242	163,691,134	91.36%	11.2228X
K1-1	177,334,129	159,402,863	89.89%	11.2977X
K1-2	178,271,384	162,348,141	91.07%	11.2623X
K1-3	182,900,045	166,756,809	91.17%	11.1902X
KL1-1	175,568,361	160,878,839	91.63%	11.2915X
KL1-2	177,826,324	161,034,100	90.56%	11.0916X
KL1-3	182,965,854	166,059,992	90.76%	11.1778X
MG1-1	179,458,223	164,232,108	91.52%	11.4447X
MG1-2	177,305,582	161,038,315	90.83%	11.3496X
MG1-3	176,483,949	161,296,615	91.39%	11.2341X
S1-1	180,711,345	166,088,482	91.91%	11.3794X
S1-2	186,062,346	168,461,888	90.54%	11.1034X
S1-3	183,257,465	167,176,745	91.23%	11.3979X
W1-1	167,646,119	152,033,697	90.69%	11.1159X
W1-2	128,457,933	116,582,327	90.76%	11.233X
W1-3	179,288,290	164,037,539	91.49%	11.1797X

Github repository link with whole genome sequencing processing scripts: https://github.com/Hwagn008/FLAGG.git
Sequence Read Archive (SRA) Accession Number: PRJNA985220

CHAPTER 4: Evaluation of an Internship Program to Improve Mosquito Awareness and Prevention Practices Using Citizen Science Tactics

4.1 Background

The implementation of integrated tools into mosquito control has become increasingly important with the recent re-emergence of mosquito borne pathogen transmission (Schwab et al., 2018). Traditional mosquito control reliance on insecticide application within the last century has led to a pesticide treadmill. Within the treadmill, continuous increase of insecticides is used due to insecticide resistance, causing detrimental effects for ecosystems (Meier et al., 2022). Due partially to the lack of acknowledgement of insect movement, insecticide usage has also generated controversial, unsustainable, and un-coordinated insecticide applications (Kline, 2006; Dyck et al., 2021). Influenced by both ecological and political issues, mosquito control has expanded to include more community engagement and close coordination in introducing biological control, educational outreach, insecticide resistance training, environmental management, and surveillance (Dacko et al., 2020; Bueno-Marí et al., 2022; Javed et al., 2022).

Mosquito control programs need to be able to maintain a cost-efficient system for surveillance, especially in times where relocation of funds or reduced available funds are apparent (Moise et al., 2021). In the wake of the COVID-19 pandemic, multiple mosquito control programs were affected and unable to keep up with funding and personnel due to increased restrictions and health concerns leading to social distancing requirements (Grenadier, 2020). The ability to extend community engagement through the use of volunteer organizations that involve homeowners and residents can become critical to achieving a reduction in mosquito populations (Fouet et Kamdem, 2019). Citizen science can help reduce funding costs related to active field work and increase surveillance in private areas that are difficult to access or are unreachable by truck spraying (Martinou et al., 2020). Citizen science, engagement and education can even allow for mosquito surveillance to outperform traditional mosquito control methods as it is hands-on, low-cost, and highly scalable to expand to previously unmonitored jurisdictions (Palmer et al., 2017; Sousa et al., 2022).

Community engagement in mosquito control citizen science can increase residents' knowledge on how to reduce mosquito populations and therefore potentially reduce arbovirus transmission in their communities (Evans et al., 2021; Pernat et al., 2021a; Pernat et al., 2021b). A great example of integrated mosquito vector management implementation comes from the largest mosquito abatement program in the United States, in the Lee County Mosquito Control District (LCMCD). LCMCD has generated courses for local funded kindergartens through high schools to teach students basic mosquito biology knowledge and control techniques based on Florida standards of mosquito control (Foley et al., 2021). Increased knowledge would provide residents the ability to express their social concerns, increasing engagement time period and intensity, and improving participant confidence, sense of community and ability to participate. Using citizen science, participants can potentially collect specimens, report their findings, and help analyze the collected data (Sousa et al., 2022). To engage, participants need to learn about mosquito surveillance, mosquito stage and breeding spot identification, and accessible methods of control such as the use of ovitrap container reduction (Fonseca et al., 2013; Healy et al., 2014). This approach could be useful in Miami-Dade county where increased urbanization near natural areas has caused mosquito vectors to thrive and increase potential resident exposure to mosquito-borne viruses (Wilke et al., 2019).

The primary objective of this work was to understand the benefits of citizen involvement in the FLAGG community-based surveillance program. I worked with college-aged interns through the internship "Florida *Aedes* Genome Group" (FLAGG) in mosquito surveillance and control efforts, where interns were tested on knowledge and capability of mosquito surveillance. The interns participated in citizen science entomology practices, collected mosquito eggs in urban residential environments using ovitraps, and sent photographs and egg papers back to the surveillance team experts for analysis (Sousa et al., 2020; Cohnstaedt et al., 2016; Craig et al., 2021; Montgomery et al., 2017; Montgomery, 2020).

The impact of the internship was measured by exploring (4) *how college-based citizen-science internship increases participants' knowledge and efficacy towards mosquito surveillance,* (5) *increase participant confidence in science communication and career readiness,* (6) *perceived sense of community engagement on or off campus programs,* and (7) determined if *FLAGG participant mosquito egg counts are reliable when compared to expert counts?.* Given abundant information and cues to action on vector borne disease through the FLAGG internship, I hypothesized that (H4) FLAGG participants would be more willing and perceive themselves more capable of participating in mosquito control measures than their non-FLAGG intern and control group counterparts. I also hypothesized that (H5) FLAGG participants would be more confident in professional settings and that (H6) FLAGG participants would have an increased perceived sense of community engagement on campus. Lastly, I hypothesized that (H7) student egg count data would follow the same abundance patterns as expert egg count data.

4.2 Methods

4.2.1 Participants and data collection

Previous Florida *Aedes* Genome Group (FLAGG) participants were identified using contact information they previously provided to the FLAGG-internship program. The FLAGG program was a mosquito surveillance and control education internship at Florida International University. FLAGG focused on teaching college students and recent graduates basic mosquito knowledge such as how to differentiate mosquito life stages, how to identify breeding habitats, and were trained on how to use oviposition cups to collect mosquito eggs from their backyards. Other non-FLAGG participants were recruited through biology undergraduate courses and referrals from internship coordinators. Recruitment letters and flyers were distributed to students through email. Students were invited to participate through a Qualtrics link and offered either extra credit or community hours. The data collection and incentives protocol were approved by the FIU institutional review board (IRB # IRB-22-0068).

This research surveyed three internship experience groups of interest; FLAGG-interns (n=74), non-FLAGG interns (n=79), and non-interns (n=159). The FLAGG-interns represents a group of students which completed at least one semester of the FLAGG Internship, the non-FLAGG interns represent a group of students which completed at least one semester of an internship program not related to FLAGG at Florida International University, and the non-interns represents a group of students who have never participated in an internship program before. The survey was generated through Qualtrics

(Supplementary Materials), and students were recruited by email. Expected survey completion time is 5-10 minutes. This research evaluates the effectiveness of the FLAGG-internship at FIU which has served as a community mosquito surveillance program since 2018 and is rooted in south Florida, with most participants being located in the Miami-Dade County area.

4.2.2 Variables

Through comparisons of these three groups (FLAGG-interns, non-FLAGG interns, and non-interns), program effectiveness was analyzed by assessing differences in the following variables: (V1) *willingness to participate in mosquito control and exposure to mosquito control,* (V2) *knowledge on mosquito abatement,* (V3) *mosquito abatement behavior competence,* (V4) *career-related confidence,* (V5) *perceived academic confidence,* (V6) *self-esteem* and (V7) *sense of community engagement on and off campus*. The questionnaire focused on student accessibility to experiential education in the form of mosquito abatement internships and whether they help enhance specific student intended learning and personal outcomes. The FLAGG program participant surveys were analyzed to determine what communication goals have been met and which ones should be focused on in future semesters.

For the following variables:(V4) *career-related confidence,* (V5) *perceived academic confidence,* (V6) *self-esteem* and (V7) *sense of community engagement on and off campus*, the data were prepared where the respondent will answer whether they agree or disagree with a set of questions using a scale coded accordingly. Scales transformed the categorical data into quantifiable data in scales such as: strongly disagree = 1, disagree = 2, neutral = 3, agree = 4, strongly agree = 5 (Komorita, 1963).

4.2.3 Scale Reliability

The survey section for (4) *willingness to participate in mosquito control and exposure to mosquito control, knowledge on mosquito abatement,* and *mosquito abatement behavior competence,* were adapted from a mosquito awareness and prevention campaign in Western Australia (Potter et al., 2019). The (5) *career-related confidence* scale was adapted from the career decision profile revised using the vocational decision scale for college students (Jones, 1989). The (5) *perceived academic confidence* scale was adapted from an academic behavioral confidence study in the University of Wales Institute (Sanders & Sander, 2007). The (5) *self-esteem* section of the survey was adapted from the self-confidence scale from a labor market test in Hungary (Keller, 2010). Lastly, the student's (6) *sense of community engagement on and off campus* were adapted from the Sense of Community Index (SCI) (Chavis et al., 1986). All scales were analyzed for reliability using reliability statistics on SPSS, and all showed high reliability, with a Cronbach's Alpha coefficient greater than 0.6 to ensure the scale's reliability (Nunnally & Bernstein, 1994). The Self-Esteem scale, known to be difficult to define and measure, had the lowest reliability score (Cronbach's Alpha coefficient = 0.674) (Rubio, 2014). See Table 4.1 below for the individual scale reliabilities.

Table 4.1. Reliability Statistics for individual scales used

Scale	Cronbach's Alpha	N of Items
Academic Confidence	0.917	22
Campus Community	0.732	12
Neighborhood Community	0.826	12
Self-Esteem	0.674	6
Job Confidence	0.793	10
Mosquito Skills	0.943	8
Mosquito Behavior	0.883	16

4.2.4 Data Analysis: Does the FLAGG internship increase participants' knowledge and efficacy towards mosquito surveillance?

For the three groups of interest, the mean from every group of questions for each individual was used. A one-way Analysis of Variance (ANOVA) on SPSS was run to see if there are any statistical differences between the three internship groups (Park, 2009). In order to run an ANOVA, the distribution of the data was assessed based on the levene statistic of homogeneity of variance. The samples taken from the populations are independent of each other and do not depend on one another, hence the assumption of independence is met (St & Wold, 1989).

When the F statistic was significant, Tukey HSD Multiple comparisons analysis was used to compare differences between each pair of groups to detect the source of any statistically significant differences. When there was a significant finding under the test of homogeneity of variances with the Levene statistic, the Brown-Forsythe and Welch tests were then applied and multiple comparisons were tested using Games-Howell. These *post hoc* tests assessed the mean difference between the FLAGG-interns group and the non-FLAGG interns group, between the FLAGG-interns group and the non-interns group, and between the non-FLAGG interns group and the non-interns group. When p-values were lower than 0.05, then the conclusion signified a significant difference in the dependent variable between each group.

Questions which were grouped into "mosquito skills knowledge" included questions such as "How confident are you with identifying mosquito eggs?" and "How confident are you with understanding mosquito prevention measures?". The mosquito abatement behavior competence section included questions such as "On a scale of 1 (Never) to 5 (Daily), how often have you removed empty containers such as tires, flowerpots, birdbaths within the last 12 months to protect yourself and family from being bitten?" The same was asked for a set of 15 mosquito control behaviors and an average score was calculated for each participant.

4.2.5 Data Analysis: Does the FLAGG internship increase participant confidence in science communication and career readiness?

ANOVA and *post-hoc* tests were followed as described under section 4.2.4. Career confidence was measured using the Vocational Decision Scales that asked students to rate statements such as "My future career is not important to me right now". Academic confidence was established including questions such as their perceived ability to "give a presentation to a small group of fellow students" and "produce your best work under test conditions".

4.2.6 Data Analysis: Does the FLAGG internship increase participants' perceived sense of community engagement on or off-campus?

ANOVA and *post-hoc* tests were followed as described under section 4.2.4. The perceived sense of community engagement included questions where students evaluated circumstances from “strongly agree” to “strongly disagree” such as “people in this university/neighborhood don’t share the same values”. This set of questions were asked for on-campus engagement (within the university) and off-campus engagement (within their neighborhoods).

4.2.7 Data Analysis: Are FLAGG participant mosquito egg counts reliable when compared to expert counts?

The non-parametric Wilcoxon signed-rank test was used to compare the mosquito abundance indices (EDI, OPI and TER) between interns and FLAGG experts. This test compares two matched samples and assesses if the mean of the population ranks are significantly different. A p-value higher than 0.05 would indicate a significant difference in the egg counts between interns and FLAGG experts. The relationship between intern and FLAGG expert abundance indices (EDI, OPI and TER) was also assessed using a non-parametric Spearman ranked test, where a p-value higher than 0.05 would indicate a significant correlation between the egg counts. Both the Wilcoxon and Spearman tests were done between intern and FLAGG egg counts for both site-based and weekly-based data.

4.3 Results

4.3.1 Socio-demographic characteristics

A total of 312 Florida International University students participated. There were 312 total participants, 159 which were non-interns and 153 who were interns. The intern group was subdivided to include 74 FLAGG interns and 79 non-FLAGG interns. Most of the participants were female (60%), identified as Hispanic(a) or Latino(a) (80.2%), single (89.4%), spoke Spanish fluently (74.2%) as well English, were born in North America (61.7%), had some college experience but no degree (37.6%), majored in Biology (51.3%), aged 18 - 24 (79.5%), were employed part-time (35.7%), made less than $10,000 income through personal employment (24.7%), sometimes had difficulty meeting expenses (49.8%), and were not the primary income earners of their household (83.3%). Refer to Supplementary table S4.1 for full demographic description of the sample by internship category.

4.3.2 Does the FLAGG internship increase participants' knowledge and efficacy towards mosquito surveillance?

4.3.2A Willingness to participate in mosquito control and exposure to mosquito control

FLAGG-interns were the most likely to participate in mosquito control measures (mean = 4.05), compared to non-FLAGG interns (mean = 3.03) and non-Interns (mean = 3.06). The scale went from extremely unlikely (5) to extremely likely (1). The ANOVA analysis generated a p-value of < 0.001.

The majority of FLAGG-interns felt they either had "a lot" (21.9%) or "a great deal" (21.9%) of exposure on mosquito knowledge and education, while non–FLAGG-interns mostly felt they had “none at all” (38.8%) and non-interns felt they had “a little” (33.6%). Almost half of the FLAGG-interns (47.9%) knew all 4 mosquito life stages (egg, larvae, pupae, adult mosquito) while only 6.7% and 16% of non-FLAGG interns and non-interns did, respectively.

4.3.2B Knowledge on mosquito abatement

4.3.2B.1 Knowledge of breeding habitats

There was no significant difference in this variable between FLAGG and non-FLAGG participants, and the knowledge was high for all groups. When asked “What environment can become a mosquito breeding habitat?”, most students from the three groups answered with responses that included the following key words: water, hot/warm, household/man-made items holding water, humid, plants, and standing water. 100% of FLAGG students answered with at least one of the aforementioned key words, whereas only 94.9% and 91.2% of non-FLAGG interns and non-interns were able to answer with the aforementioned key words, the rest of the students answered with unknown. Refer to supplementary table S4.1 for individual survey components.

4.3.2B.2 Knowledge of mosquito control skills

No difference was observed between the non-interns (mean = 2.707) and non-FLAGG interns (mean = 2.575), based on the Brown-Forsythe and Welch tests. The ANOVA analysis generated a p-value of < 0.001, with the FLAGG interns scoring higher than their peers (mean = 4.283). This supports the hypothesis that mosquito abatement knowledge differs significantly between the FLAGG interns and all other participants.

4.3.2C Mosquito abatement behavior competence

Participants of the FLAGG-internship (mean = 2.977) on average scored higher than the non-interns (mean = 2.552) and non-FLAGG (mean = 2.302) interns, respectively. The ANOVA analysis generated a p-value <0.001, signifying a strong difference between the three groups. No difference was observed between the non-interns and non-FLAGG interns. This supports the hypothesis that mosquito abatement behavior differs between groups and is enhanced in the FLAGG group. Specifically, there were differences between FLAGG and non-FLAGG participant on their perceived ability to remove empty containers, use drainage systems for stormwater, use protective clothing, use insect repellent, use pesticides, clean gutters, avoid dusk or dawn hours, avoiding being outside in times of highest mosquito activity, Operating fans, and using automatic mosquito spray/mist. There was no significant difference between the three groups on their perceived ability to hire professional mosquito control services, use a mosquito zapper, use an insect screen/netting, and use mosquito coils.

4.3.3 Does the FLAGG internship increase participant confidence in science communication and career readiness?

4.3.3A Career-related confidence

Non-intern students generally scored lowest (mean = 3.270) when evaluated for job confidence when compared to the FLAGG (3.613) and non-FLAGG interns (3.611). The ANOVA analysis generated a p-value <0.001, signifying a strong difference between the three groups. Overall, the higher level of job-related confidence in both the FLAGG group and non-FLAGG interns suggest that job confidence is increased by the opportunity of being in an internship.

4.3.3B Perceived academic confidence

Although students within the FLAGG group showed the highest academic confidence throughout (mean = 3.996), the non-FLAGG interns scored second highest (mean = 3.936). Both significantly differed only against the non-interns group (mean = 3.692). The ANOVA analysis generated a p-value <0.001, signifying a strong difference between the three groups. The FLAGG group students had significantly higher scores (mean = 4.20) than the other two groups in their perceived ability to "plan appropriately to submit assignments" ($p<0.05$) (non-intern mean = 3.79, non-FLAGG intern mean = 3.78), but otherwise did not significantly differ from the non-FLAGG interns students in their perceived academic confidence. Overall, the higher level of academic confidence in both the FLAGG group and non-FLAGG interns suggest that job confidence is increased by the opportunity of being in an internship. Refer to supplementary table S4.1 for individual survey components.

4.4.3C Self-esteem

Within this question, students within the FLAGG group (mean = 4.31) and the non-FLAGG intern group (mean = 4.16) scored significantly higher than the non-interns group (mean = 3.97). Students from each group consistently scored high in this section, with mean scores above 3.5 for each group on each question. The ANOVA analysis for student levels of Self-esteem generated a p-value of 0.097, showing no difference between the three groups. Although overall Self-esteem did not significantly differ between students of each group, there was a significant difference in student's confidence to accomplish all their goals ($p<0.05$). The hypothesis that self-esteem differs between non-interns, FLAGG-interns and non-FLAGG interns, was not supported.

4.3.8 Does the FLAGG internship increase participants' perceived sense of community engagement on or off-campus?

The ANOVA analysis for student levels of sense of community on and off campus generated p-values of 0.095 and 0.306, respectively, suggesting there is no difference between the three groups. Although the hypothesis that sense of community on and off-campus differs between groups was not supported, some responses did significantly differ between groups. The students within the non-interns group scored significantly lower (mean = 4.42) than the other two groups (FLAGG-intern mean = 4.73, non-FLAGG intern mean = 4.78) ($p<0.001$) in their agreement with the following statement: "I expect to study in this university until I graduate." The following statement also differed between intern student groups in terms of their level of agreement ($p<0.05$): My classmates and I want the same thing from this university (FLAGG mean: 4.09, non-FLAGG interns mean: 3.62, non-intern mean = 3.87). Refer to supplementary table S4.1 for individual survey components.

4.3.9 Are FLAGG participant mosquito egg counts reliable when compared to expert counts?

The Wilcoxon test to determine the differences between student and FLAGG weekly counts for different mosquito abundance indices are grouped by index (EDI, OPI and TER). There was no significant difference between site-based EDI values between FLAGG and student interns ($p = 0.17$), and there was a significant difference between site-based OPI and TER values between FLAGG and student interns ($p < 0.001$ for both) (Figure 4.1). The Spearman test for site-based EDI, OPI and TER values between FLAGG and student intern counts were significantly correlated ($p < 0.001$ for all three correlations)

(Figure 4.2). There was a significant difference between weekly-based EDI, OPI and TER values between FLAGG and student interns based on the Wilcoxon test ($p < 0.001$ for all three) (Figure 4.3). The Spearman test for weekly-based EDI, OPI and TER values between FLAGG and student intern counts were significantly correlated ($p < 0.001$ for all three correlations) (Figure 4.4).

Table 4.2. Summary of Major Descriptive Results

Hypotheses	Non-Intern Mean	FLAGG Mean	Non-FLAGG Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	Results
Mosquito abatement knowledge differs between groups: non-interns, FLAGG-interns and non-FLAGG interns	2.707	4.283	2.575	3.036	**0.000**	**<.001**	**<.001**	**Supported**
Mosquito abatement behavior differs between groups: non-interns, FLAGG-interns and non-FLAGG interns	2.552	2.977	2.302	2.587	**0.000**	**<.001**	**<.001**	**Supported**
Lack of job confidence differs between groups: non-interns, FLAGG-interns and non-FLAGG interns	3.270	3.613	3.611	3.436	**0.000**	**<.001**	**<.001**	**Supported**
Academic confidence differs between groups: non-interns, FLAGG-interns and non-FLAGG interns	3.692	3.996	3.936	3.822	**0.000**	**<.001**	**<.001**	**Supported**
Self-esteem differs between groups: non-interns, FLAGG-interns and non-FLAGG interns	3.971	4.180	4.048	4.039	0.059	0.063	0.066	Not Supported
Sense of Community on-campus differs between groups: non-interns, FLAGG-interns and non-FLAGG interns	3.630	3.752	3.708	3.674	0.185	0.186	0.179	Not Supported
Sense of Community off-campus differs between groups: non-interns, FLAGG-interns and non-FLAGG interns	3.482	3.489	3.321	3.446	0.162	0.189	0.236	Not Supported

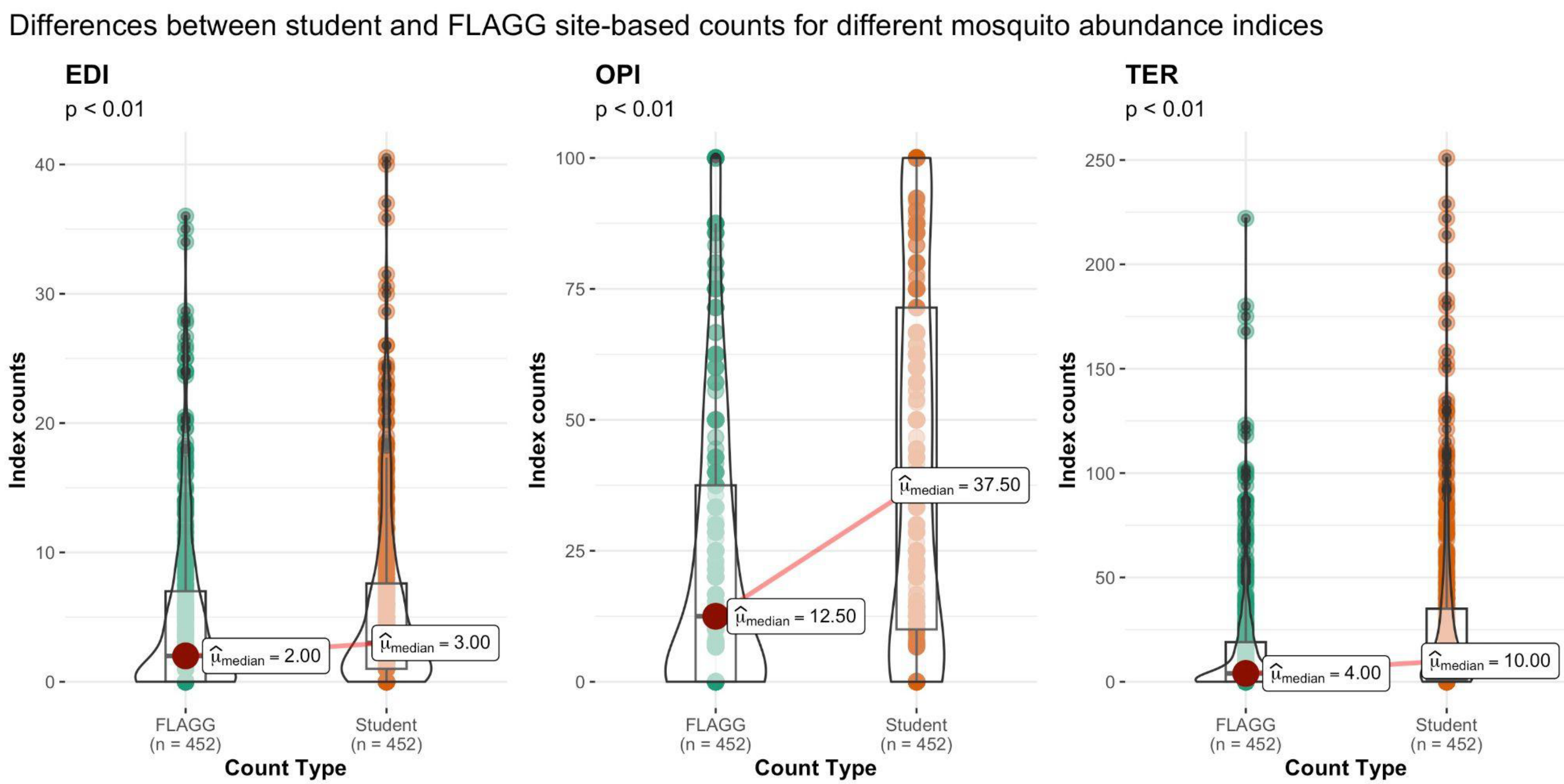

Figure 4.1. Comparison of weekly average EDI, OPI and TER between students and FLAGG data based on collection sites.

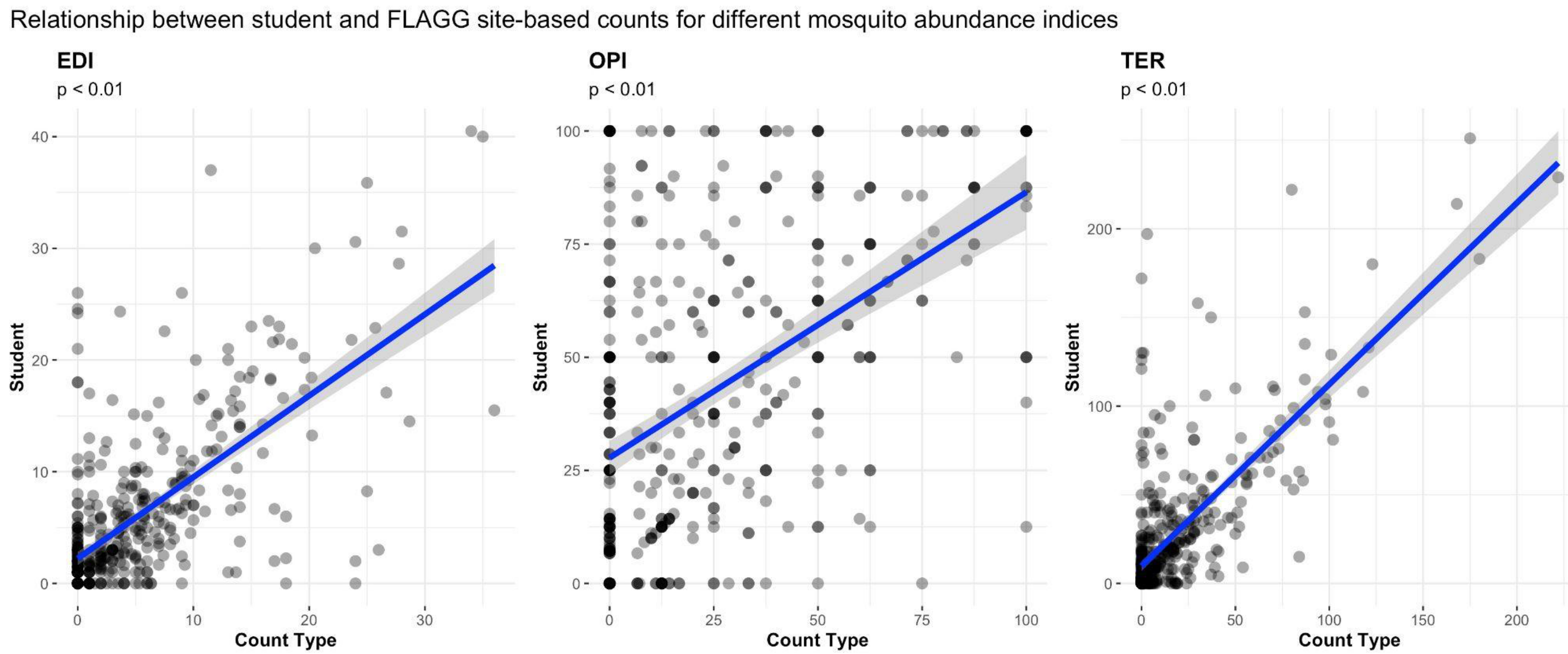

Figure 4.2. Relationship of weekly average EDI, OPI and TER between students and FLAGG data based on collection sites. The line of best fit based on the Spearman test is displayed with a 95% confidence interval.

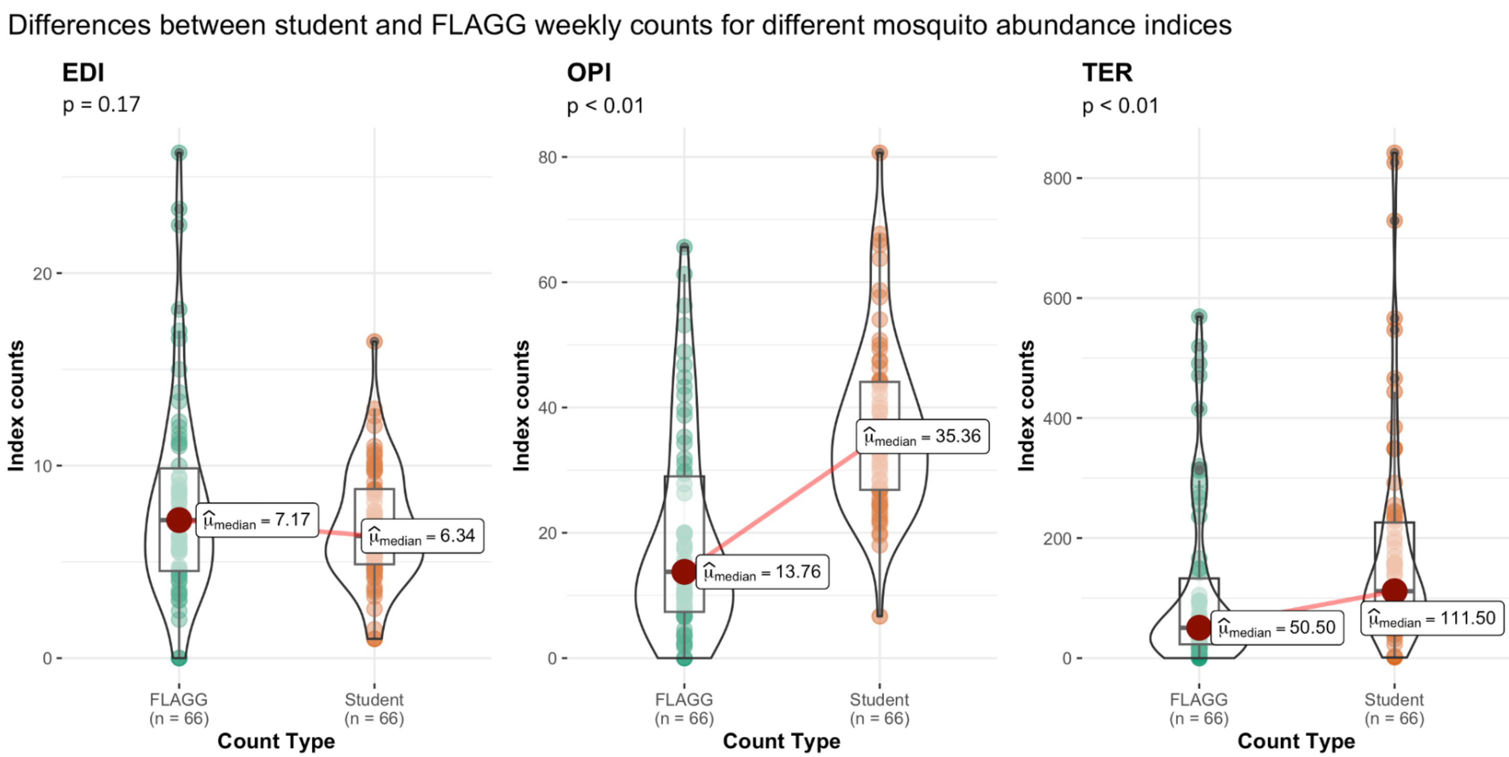

Figure 4.3. Comparison of weekly average EDI, OPI and TER between students and FLAGG data based on weekly counts.

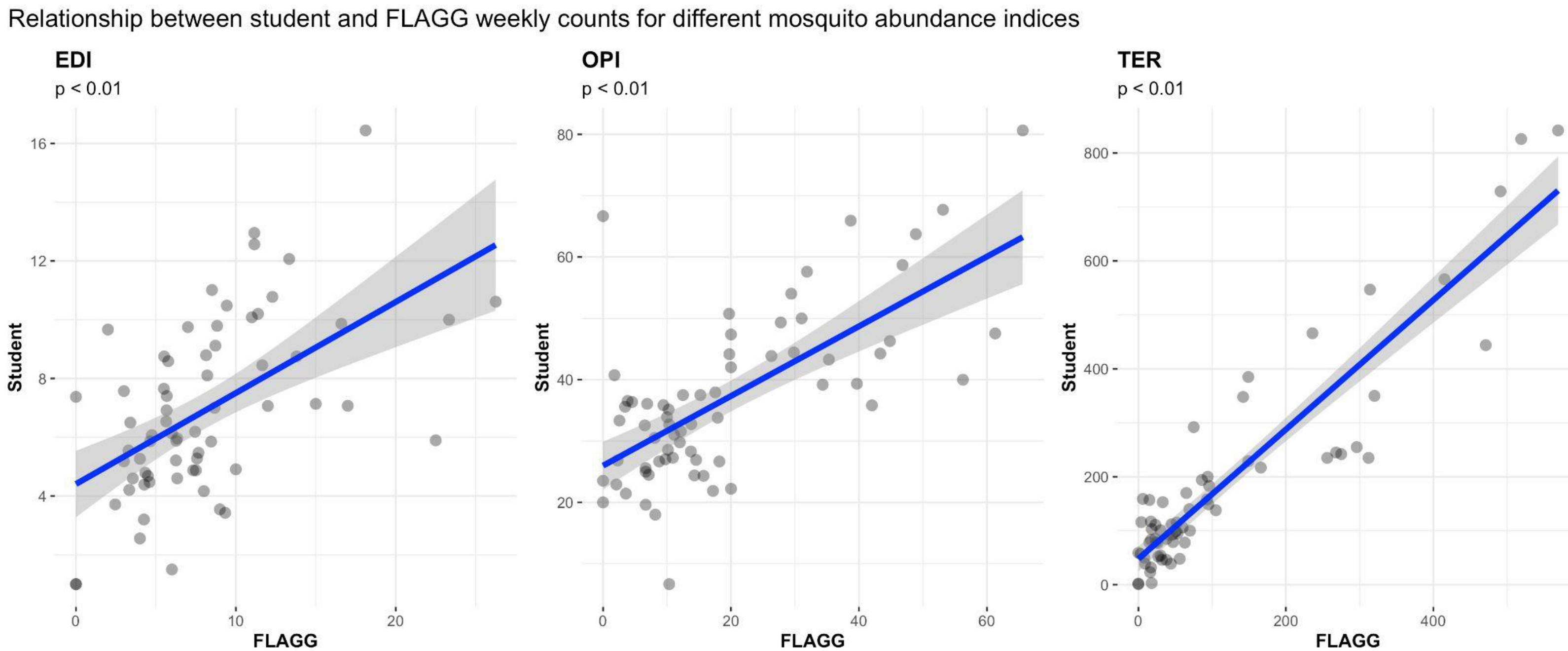

Figure 4.4. Relationship of weekly average EDI, OPI and TER between students and FLAGG data based on weekly counts. The line of best fit based on the Spearman test is displayed with a 95% confidence interval.

4.4 Discussion

The primary objective of this work was to understand the benefits of citizen involvement in the FLAGG community-based surveillance program. FLAGG students scored significantly better than their peers when asked about mosquito abatement and were more likely to be willing to participate in mosquito control surveillance. They also scored higher in job and academic related confidence, demonstrating the vast benefits FLAGG had on students. Furthermore, FLAGG intern counts and FLAGG expert counts showed similar seasonal and site-based trends for mosquito abundance, showing a level of preciseness to their work. These results show community engagement contributing to participant knowledge, which can have important implications on a major factor in implementing mosquito control and protective practices, the public perception of mosquito control.

FLAGG participants' increased willingness to participate in mosquito control when compared to their non-FLAGG intern counterparts allows for increased mosquito breeding habitat monitoring (Vásquez-Trujillo et al., 2021; Sousa et al., 2022). In order to efficiently engage in mosquito control activities, participants need to both learn and be capable of mosquito surveillance, identifying mosquito stages and breeding spots, and in container reduction (Fonseca et al., 2013; Healy et al., 2014). FLAGG participants excelled in all the aforementioned activities when compared to their peers. Through these backyard organization and water storage practices, community collaboration can give mosquito control access to private sites otherwise hard to access (Martinou et al., 2020). The increased surveillance would reduce mosquito population as breeding sites are reduced

(Vásquez-Trujillo et al., 2021). This cooperation between students and a mosquito internship gives them the opportunity to gain the hands-on experience of mosquito control practices they need to support mosquito control. Eventually, participants who are experienced and willing can extend their service further by collecting specimens, report their findings, and potentially help analyze collected data (Sousa et al., 2022).

When implemented effectively, a mosquito surveillance program can create an empowered community, as FLAGG has by demonstrating high career-related and perceived academic confidence in FLAGG interns when compared to their non-FLAGG intern peers. Enhancing participant personal development throughout an intern's education can lead to enhancing work-based motivation and reinforcing academic performance (Gracia & Jenkins, 2003; Surridge, 2009). An empowered community can then teach others within the community about the biology and behavior of mosquitoes and the diseases they transmit. Participants can also teach methods of surveillance and source reduction to reduce vector populations and increase disease prevention (Yasuoka et al., 2006).

As a long-term, hands-on, and low-cost citizen science opportunity, programs like FLAGG generate mobilization towards mosquito control (Sousa et al., 2022). A great example of this type of integrated and empowering mosquito vector management comes from the largest mosquito abatement program in the United States, in the Lee County Mosquito Control District (LCMCD). LCMCD has successfully generated courses for local funded kindergartens through high schools to teach students basic mosquito biology knowledge and control techniques based on Florida standards of mosquito control (Foley et al., 2021). Increasing the amount of community-based mosquito education and participation within MDC is highly scalable and can complement traditional mosquito control to reduce mosquito abundance (Palmer et al., 2017).

Citizen-involved programs inspire participants to feel comfortable teaching mosquito control practices, and prevent misinformation from disrupting public receptiveness of current or novel mosquito control tools (Likos et al., 2016). By integrating public input and educating citizens, local agencies could reorient the community to be receptive and even increase public engagement as well as transparency when it comes to mosquito control technologies and methods (Schairer et al., 2021). Future studies should assess public perception of mosquito control and compare it to the perception of individuals partaking in a community-based mosquito control surveillance program. Understanding perception is important, as it can impact the effectiveness of a program in implementing mosquito control practices. Perception of a mosquito program can persuade people to either support or oppose the control tools and strategies being used.

4.5 Conclusion

Studying programs like FLAGG can help us understand how an educational intervention can become a valuable opportunity for both students and professionals as they work together to reduce mosquito populations in peri-domestic habitats. The significant higher levels of knowledge on mosquito control achieved by participants in FLAGG led to both desired mosquito control goals and personal development. As programs like FLAGG are implemented, and citizens become more educated, the belief that they are capable of executing specific behaviors and making a difference will increase. This confidence can then translate to a public support of mosquito control measures. The approach used in this study can be implemented in different communities throughout MDC and by different university programs to expand the reduction of vector populations within residential households.

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Supplementary Materials

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
FIUCommunity - Please evaluate your FIU community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - I think my university is a good place for me to study.	4.49	4.57	4.49	4.51	0.82	0.80	0.78	0.79	0.83	1.00
REV_FIUCommunity - Please evaluate your FIU community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - People in this university don't share the same values.	3.33	3.43	3.33	3.35	0.76	0.77	0.76	0.76	0.84	1.00
FIUCommunity - Please evaluate your FIU community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - My classmates and I want the same thing from this university.	3.87	4.09	3.62	3.86	**0.01**	**0.01**	**0.01**	0.12	**0.01**	0.18
FIUCommunity - Please evaluate your FIU community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - I can recognize most of the people who study my major.	3.23	3.38	3.38	3.29	0.54	0.55	0.54	0.63	1.00	0.67

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
FIUCommunity - Please evaluate your FIU community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - I feel at home in this university.	4.06	4.22	4.01	4.08	0.34	0.37	0.35	0.40	0.41	0.94
REV_FIUCommunity - Please evaluate your FIU community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - Very few of my classmates know me.	2.56	2.77	3.14	2.74	**0.00**	**0.00**	**0.00**	0.42	0.16	**0.00**
FIUCommunity - Please evaluate your FIU community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - I care about what my classmates think of my actions.	3.22	3.18	3.30	3.23	0.79	0.78	0.77	0.95	0.76	0.85
REV_FIUCommunity - Please evaluate your FIU community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - I have no influence over what this university is like.	2.97	3.20	3.08	3.04	0.32	0.34	0.29	0.27	0.79	0.80

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
FIUCommunity - Please evaluate your FIU community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - If there is a problem in this university, people who study here will get it solved.	3.36	3.45	3.34	3.37	0.74	0.75	0.74	0.76	0.78	0.99
FIUCommunity - Please evaluate your FIU community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - It is very important to me to study in this university.	4.05	4.03	4.03	4.04	0.98	0.98	0.98	0.99	1.00	0.99
REV_FIUCommunity - Please evaluate your FIU community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - People in this university don't usually get along with one another.	4.01	3.99	3.99	4.00	0.98	0.98	0.98	0.99	1.00	0.99
FIUCommunity - Please evaluate your FIU community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - I expect to study in this university until I graduate.	4.42	4.73	4.78	4.57	**0.00**	**0.00**	**0.00**	**0.00**	0.83	**0.00**

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
NCommunity - Please evaluate your neighborhood community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - I think my neighborhood is a good place for me to live	4.32	4.41	4.28	4.33	0.71	0.71	0.70	0.79	0.70	0.94
REV_NCommunity - Please evaluate your neighborhood community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - People in this neighborhood don't share the same values.	3.21	3.26	3.11	3.20	0.69	0.70	0.71	0.94	0.70	0.79
NCommunity - Please evaluate your neighborhood community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - My neighbors and I want the same thing from this neighborhood.	3.68	3.66	3.56	3.65	0.61	0.61	0.64	0.99	0.77	0.62
NCommunity - Please evaluate your neighborhood community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - I can recognize most of the people who live in my neighborhood.	3.39	3.39	3.03	3.31	0.09	0.09	0.12	1.00	0.20	0.12

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
NCommunity - Please evaluate your neighborhood community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - I feel at home in this neighborhood.	4.15	4.15	3.97	4.11	0.35	0.38	0.39	1.00	0.54	0.37
REV_NCommunity - Please evaluate your neighborhood community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - Very few of my neighbors know me.	2.69	2.88	2.52	2.69	0.21	0.21	0.20	0.47	0.18	0.60
NCommunity - Please evaluate your neighborhood community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - I care about what my neighbors think of my actions.	3.10	2.93	2.90	3.02	0.38	0.37	0.38	0.59	0.98	0.43
REV_NCommunity - Please evaluate your neighborhood community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - I have no influence over what this neighborhood is like.	3.03	3.11	2.89	3.01	0.51	0.53	0.55	0.87	0.53	0.70

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
NCommunity - Please evaluate your neighborhood community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - If there is a problem in this neighborhood, people who live here will get it solved.	3.46	3.50	3.28	3.43	0.34	0.34	0.37	0.95	0.39	0.44
NCommunity - Please evaluate your neighborhood community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - It is very important to me to live in this neighborhood.	3.59	3.46	3.38	3.52	0.37	0.41	0.41	0.69	0.92	0.44
REV_NCommunity - Please evaluate your neighborhood community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - People in this neighborhood don't usually get along with one another.	3.79	3.69	3.66	3.74	0.56	0.58	0.57	0.75	0.98	0.62
NCommunity - Please evaluate your neighborhood community experience in the following circumstances from "Strongly disagree" to "Strongly agree". - I expect to live in this neighborhood for a long time.	3.36	3.43	3.28	3.36	0.78	0.79	0.79	0.92	0.77	0.90

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Give a presentation to a small group of fellow students.	3.43	3.78	3.96	3.64	**0.00**	**0.00**	**0.00**	0.05	0.53	**0.00**
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Produce your best work under test conditions.	3.20	3.69	3.39	3.36	**0.01**	**0.01**	**0.00**	**0.00**	0.22	0.47
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Plan appropriately to submit assignments.	3.79	4.20	3.78	3.88	**0.01**	**0.01**	**0.01**	**0.01**	**0.03**	1.00
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Remain adequately motivated throughout.	3.30	3.68	3.47	3.43	**0.04**	**0.04**	**0.04**	**0.03**	0.46	0.52
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Be on time for lectures.	3.98	4.14	4.08	4.04	0.51	0.51	0.51	0.51	0.93	0.78

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Attend most taught sessions.	4.34	4.46	4.32	4.36	0.50	0.49	0.47	0.53	0.51	0.98
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Respond to questions asked by a professor in front of a full lecture class.	3.08	3.32	3.66	3.28	**0.00**	**0.00**	**0.00**	0.37	0.24	**0.00**
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Write in an appropriate academic style.	3.86	4.23	4.01	3.98	**0.02**	**0.02**	**0.01**	**0.01**	0.36	0.50
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Manage your work load to meet coursework deadlines.	3.81	4.24	4.05	3.97	**0.00**	**0.00**	**0.00**	**0.00**	0.37	0.16
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Study effectively on your own in independent/private study.	3.74	4.12	3.97	3.88	**0.02**	**0.02**	**0.02**	**0.02**	0.65	0.22

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Pass tests and quizzes on the first try.	3.51	4.04	3.90	3.73	**0.00**	**0.00**	**0.00**	**0.00**	0.65	**0.03**
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Follow the themes and debates in lectures.	3.74	4.04	4.10	3.90	**0.01**	**0.00**	**0.01**	**0.04**	0.90	**0.01**
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Engage in academic debate with your peers.	3.36	3.64	3.92	3.56	**0.00**	**0.00**	**0.00**	0.23	0.25	**0.00**
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Understand the material outlined and discussed with you by lecturers.	3.85	4.24	4.04	3.98	**0.00**	**0.00**	**0.00**	**0.00**	0.26	0.22
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Attain good grades in your work.	4.07	4.32	4.14	4.14	0.08	0.08	0.06	**0.04**	0.34	0.81

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Make the most of the opportunity of studying for a degree at university.	4.04	4.27	4.32	4.16	**0.04**	**0.03**	**0.04**	0.14	0.94	0.06
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Read the recommended background material.	3.55	3.80	3.53	3.60	0.23	0.25	0.23	0.24	0.35	0.99
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Produce coursework at the required standard.	4.01	4.34	4.22	4.14	**0.01**	**0.01**	**0.01**	**0.01**	0.62	0.18
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Produce your best work in coursework assignments.	4.01	4.32	4.16	4.12	**0.03**	**0.02**	**0.02**	**0.01**	0.47	0.41
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Ask lecturers questions about the material they are teaching, in a one-to-one setting.	3.61	3.81	4.03	3.76	**0.02**	**0.01**	**0.01**	0.38	0.41	**0.01**

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Ask for help if you don't understand.	3.66	3.80	3.89	3.75	0.29	0.30	0.29	0.65	0.87	0.29
EduConfidence - Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident". - Ask lecturers questions about the material they are teaching, during a lecture.	3.28	3.43	3.66	3.41	0.08	0.09	0.09	0.65	0.52	0.08
REV_Self-Esteem - Please select how well these phrases describe you from "Does not describe me" to "Describes me extremely well". - I cannot solve my problems.	4.61	4.62	4.58	4.60	0.96	0.96	0.96	0.99	0.95	0.98
Self-Esteem - Please select how well these phrases describe you from "Does not describe me" to "Describes me extremely well". - I can accomplish all my goals.	3.97	4.31	4.16	4.10	**0.01**	**0.01**	**0.01**	**0.01**	0.48	0.24
REV_Self-Esteem - Please select how well these phrases describe you from "Does not describe me" to "Describes me extremely well". - It is difficult for me to control the turns my life takes.	3.71	3.78	3.78	3.74	0.83	0.84	0.84	0.89	1.00	0.87

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
Self-Esteem - Please select how well these phrases describe you from "Does not describe me" to "Describes me extremely well". - The shaping of my future depends primarily on me.	4.14	4.31	4.14	4.18	0.43	0.42	0.35	0.36	0.51	1.00
REV_Self-Esteem - Please select how well these phrases describe you from "Does not describe me" to "Describes me extremely well". - It is hard for me to relieve most of my problems.	3.55	3.85	3.77	3.67	0.13	0.14	0.13	0.16	0.92	0.38
Self-Esteem - Please select how well these phrases describe you from "Does not describe me" to "Describes me extremely well". - I trust my future.	3.85	4.20	3.85	3.93	0.05	0.06	**0.03**	**0.04**	0.12	1.00
REV_JobConfidence - The following questions are about your job and/or occupation in a scale from "Strongly disagree" to "Strongly agree". - I need information about educational programs I want to enter.	1.92	1.81	2.37	2.01	**0.00**	**0.00**	**0.01**	0.72	**0.01**	**0.02**

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
REV_JobConfidence - The following questions are about your job and/or occupation in a scale from "Strongly disagree" to "Strongly agree". - I know what my interests and abilities are, but I am unsure how to find occupations that match them.	2.61	3.01	3.14	2.84	**0.01**	**0.01**	**0.01**	0.07	0.84	**0.02**
REV_JobConfidence - The following questions are about your job and/or occupation in a scale from "Strongly disagree" to "Strongly agree". - I don't feel I know enough about the occupations that I am considering.	2.78	3.43	3.49	3.11	**0.00**	**0.00**	**0.00**	**0.00**	0.95	**0.00**
REV_JobConfidence - The following questions are about your job and/or occupation in a scale from "Strongly disagree" to "Strongly agree". - I am unclear about the education or training required for occupations in which I am interested.	3.04	3.77	3.63	3.36	**0.00**	**0.00**	**0.00**	**0.00**	0.80	**0.01**
REV_JobConfidence - The following questions are about your job and/or occupation in a scale from "Strongly disagree" to "Strongly agree". - I think there may be a number of occupations that I don't know about that I should consider.	2.30	2.45	2.67	2.43	0.11	0.12	0.13	0.69	0.55	0.11

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
REV_JobConfidence - The following questions are about your job and/or occupation in a scale from "Strongly disagree" to "Strongly agree". - Others are pressuring me to make a career choice.	3.50	3.81	3.58	3.59	0.26	0.28	0.27	0.24	0.58	0.90
REV_JobConfidence - The following questions are about your job and/or occupation in a scale from "Strongly disagree" to "Strongly agree". - My future work or career is not important to me right now.	4.24	4.47	4.53	4.37	0.09	0.07	0.08	0.26	0.93	0.09
REV_JobConfidence - The following questions are about your job and/or occupation in a scale from "Strongly disagree" to "Strongly agree". - I don't need to make a career choice at this time.	3.96	4.41	3.87	4.04	**0.01**	**0.01**	**0.00**	**0.01**	**0.01**	0.87
REV_JobConfidence - The following questions are about your job and/or occupation in a scale from "Strongly disagree" to "Strongly agree". - I don't have strong interests in any occupational field.	4.17	4.45	4.47	4.31	**0.05**	**0.04**	**0.04**	0.14	0.99	0.06

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
JobConfidence - The following questions are about your job and/or occupation in a scale from "Strongly disagree" to "Strongly agree". - I don't look forward to working in a specific career.	4.17	4.53	4.35	4.30	0.06	0.05	**0.05**	**0.04**	0.55	0.44
MosquitoSkills - How confident are you with the following skills? - Identifying mosquito eggs.	2.16	4.23	1.90	2.56	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.37
MosquitoSkills -How confident are you with the following skills? - Identifying mosquito larvae.	2.29	3.98	2.21	2.66	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.93
MosquitoSkills -How confident are you with the following skills? - Identifying hot spots for breeding sites around your home (standing water).	3.12	4.58	3.18	3.47	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.96
MosquitoSkills -How confident are you with the following skills? - Teaching friends/relatives/others how to identify hot spots for breeding sites (standing water).	2.79	4.55	2.75	3.18	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.98
MosquitoSkills -How confident are you with the following skills? - Understanding mosquito prevention measures.	3.19	4.40	3.00	3.42	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.64

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
MosquitoSkills -How confident are you with the following skills? - Understanding/Identifying the mosquito life cycle.	2.62	4.17	2.52	2.95	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.88
MosquitoSkills -How confident are you with the following skills? - Understanding diseases that *Aedes* mosquitoes may potentially carry.	2.73	4.28	2.52	3.03	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.62
MosquitoSkills -How confident are you with the following skills? - Doing field research.	2.72	4.02	2.61	2.99	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.88
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Removal of empty containers such as tires, flowerpots, birdbaths.	2.60	3.71	2.34	2.79	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.33
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Use of drainage system for storm water, such as ditches.	2.60	3.48	2.38	2.75	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.52

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Personal protection by wearing appropriate clothing.	3.49	4.11	3.32	3.59	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.63
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Personal protection by wearing insect repellent.	3.15	3.48	2.87	3.15	**0.02**	**0.02**	**0.02**	0.17	**0.02**	0.28
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Personal application of pesticides targeting mosquitoes.	2.42	2.78	2.10	2.43	**0.01**	**0.02**	**0.02**	0.22	**0.01**	0.20
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Hiring professional mosquito control services.	1.86	2.08	1.62	1.85	0.10	0.10	0.10	0.51	0.09	0.33

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Cleaning gutters of leaves, pine needles, and other debris.	2.44	3.25	2.03	2.52	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.08
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Avoiding being outside during dusk or dawn.	2.74	3.34	2.39	2.79	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.14
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Avoiding being outside in times of highest mosquito activity.	2.89	3.58	2.56	2.97	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.21
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Using a mosquito zapper.	2.09	2.45	2.18	2.20	0.20	0.21	0.22	0.19	0.51	0.88

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Using an insect screen/netting.	2.66	2.58	2.46	2.59	0.66	0.66	0.68	0.94	0.88	0.65
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Staying indoors.	3.37	3.68	3.23	3.40	0.07	0.06	**0.05**	0.12	0.05	0.69
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Using mosquito coils.	1.74	2.05	1.62	1.78	0.06	0.07	0.09	0.19	0.07	0.70
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Operating fans.	2.75	3.09	2.31	2.72	**0.00**	**0.00**	**0.00**	0.23	**0.00**	0.07

Supplementary Table S4.1. Summary of Individual Survey Components

	Non-Intern (NI) Mean	FLAGG (FI) Mean	Non-FLAGG (NF) Mean	Total Mean	ANOVA Sig.	B-F Sig.	W Sig.	G-H Sig (FI vs NI)	G-H Sig (FI vs NF)	G-H Sig (NF vs NI)
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Automatic mosquito spray/mist.	2.19	2.22	1.56	2.04	**0.00**	**0.00**	**0.00**	0.99	**0.01**	**0.00**
MosquitoBehavior - On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten? - Other.	1.85	1.77	1.85	1.83	0.89	0.89	0.90	0.89	0.92	1.00
How willing are you to participate in mosquito control measures?	3.06	4.05	3.03	3.28	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.98
How difficult do you think it is to participate in mosquito control measures?	2.93	3.54	2.92	3.07	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.99
How confident are you in your ability to attain an internship in your field?	3.82	4.11	4.34	4.02	**0.00**	**0.00**	**0.00**	0.08	0.28	**0.00**
How much exposure do you have on mosquito knowledge and education (includes previous social media exposure such as news articles)?	2.28	3.46	2.01	2.48	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.16

Internship Post-experience Survey

Sense of Community

Thank you for participating in this survey. This is part of a graduate student project. Your responses are anonymous unless you specifically ask to be identified, however your answers will never be linked to you in any way. Your participation is voluntary but it will help us improve internship programs. If you have any questions please contact: internshipsurvey2022@gmail.com

Please answer the following questions based on your community participation and self perception.

The next few questions are about your community involvement.

What type of community activities do you participate in on a regular basis? (Check all that apply)

- ☐ Religious or spiritual
- ☐ Volunteering
- ☐ Political
- ☐ Fund raising and charity
- ☐ Recreational (Family/Friend Events)
- ☐ None
- ☐ __________ Other

During your time at FIU how often have you regularly engaged in community activities at FIU? (e.g., volunteering, school clubs, sports, social events, etc.)

- ○ Never
- ○ Sometimes
- ○ Often
- ○ Always

How often do you regularly engage in community activities in your neighborhood? (e.g., volunteering, sports, social events, etc.)

- ○ Never
- ○ Sometimes
- ○ Often
- ○ Always

For this question please select the option marked "Strongly agree".

- Strongly agree
- Somewhat agree
- Neither agree nor disagree
- Somewhat disagree
- Strongly disagree

Please evaluate your **FIU community** experience in the following circumstances from "Strongly disagree" to "Strongly agree".

	Strongly disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Strongly agree
I think my university is a good place for me to study.					
People in this university don't share the same values.					
My classmates and I want the same thing from this university.					
I can recognize most of the people who study my major.					
I feel at home in this university.					
Very few of my classmates know me.					
I care about what my classmates think of my actions.					
I have no influence over what this university is like.					
If there is a problem in this university, people who study here will get it solved.					
It is very important to me to study in this university.					
People in this university don't usually get along with one another.					

	Strongly disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Strongly agree
I expect to study in this university until I graduate.	O	O	O	O	O

Please evaluate **your neighborhood** community experience in the following circumstances from "Strongly disagree" to "Strongly agree".

	Strongly disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Strongly agree
I think my neighborhood is a good place for me to live.	O	O	O	O	O
People in this neighborhood don't share the same values.	O	O	O	O	O
My neighbors and I want the same thing from this neighborhood.	O	O	O	O	O
I can recognize most of the people who live in my neighborhood.	O	O	O	O	O
I feel at home in this neighborhood.	O	O	O	O	O
Very few of my neighbors know me.	O	O	O	O	O
I care about what my neighbors think of my actions.	O	O	O	O	O
I have no influence over what this neighborhood is like.	O	O	O	O	O
If there is a problem in this neighborhood, people who live here will get it solved.	O	O	O	O	O
It is very important to me to live in this neighborhood.	O	O	O	O	O
People in this neighborhood don't usually get along with one another.	O	O	O	O	O
I expect to live in this neighborhood for a long time.	O	O	O	O	O

Confidence and Self-esteem

Please answer these questions based on how you self identify.

Evaluate your confidence in the following circumstances from "Not confident at all" to "Completely confident".

	Not confident at all	Slightly confident	Somewhat confident	Very confident	Completely confident
Give a presentation to a small group of fellow students.	○	○	○	○	○
Produce your best work under test conditions.	○	○	○	○	○
Plan appropriately to submit assignments.	○	○	○	○	○
Remain adequately motivated throughout.	○	○	○	○	○
Be on time for lectures.	○	○	○	○	○
Attend most taught sessions.	○	○	○	○	○
Respond to questions asked by a professor in front of a full lecture class.	○	○	○	○	○
Write in an appropriate academic style.	○	○	○	○	○
Manage your work load to meet coursework deadlines.	○	○	○	○	○
Study effectively on your own in independent/private study.	○	○	○	○	○
Pass tests and quizzes on the first try.	○	○	○	○	○
Follow the themes and debates in lectures.	○	○	○	○	○
Engage in academic debate with your peers.	○	○	○	○	○
Understand the material outlined and discussed with you by lecturers.	○	○	○	○	○
Attain good grades in your work.	○	○	○	○	○
Make the most of the opportunity of studying for a degree at university.	○	○	○	○	○

	Not confident at all	Slightly confident	Somewhat confident	Very confident	Completely confident
Read the recommended background material.	○	○	○	○	○
Produce coursework at the required standard.	○	○	○	○	○
Produce your best work in coursework assignments.	○	○	○	○	○
Ask lecturers questions about the material they are teaching, in a one-to-one setting.	○	○	○	○	○
Ask for help if you don't understand.	○	○	○	○	○
Ask lecturers questions about the material they are teaching, during a lecture.	○	○	○	○	○

Please select how well these phrases describe you from "Does not describe me" to "Describes me extremely well".

	Does not describe me	Describes me slightly well	Describes me moderately well	Describes me very well	Describes me extremely well
I cannot solve my problems.	○	○	○	○	○
I can accomplish all my goals.	○	○	○	○	○
It is difficult for me to control the turns my life takes.	○	○	○	○	○
The shaping of my future depends primarily on me.	○	○	○	○	○
It is hard for me to relieve most of my problems.	○	○	○	○	○
I trust my future.	○	○	○	○	○

Career confidence

The next few questions are about your career interests.

The following questions are about your job and/or occupation in a scale from "Strongly disagree" to "Strongly agree".

	Strongly Disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Strongly agree
I need information about educational programs I want to enter.	O	O	O	O	O
I know what my interests and abilities are, but I am unsure how to find occupations that match them.	O	O	O	O	O
I don't feel I know enough about the occupations that I am considering.	O	O	O	O	O
I am unclear about the education or training required for occupations in which I am interested.	O	O	O	O	O
I think there may be a number of occupations that I don't know about that I should consider.	O	O	O	O	O
Others are pressuring me to make a career choice.	O	O	O	O	O
My future work or career is not important to me right now.	O	O	O	O	O
I don't need to make a career choice at this time.	O	O	O	O	O
I don't have strong interests in any occupational field.	O	O	O	O	O
I don't look forward to working in a specific career.	O	O	O	O	O

Internship specific questions

How confident are you in your ability to attain an internship in your field?

- O Extremely unlikely
- O Somewhat unlikely
- O Neither likely nor unlikely
- O Somewhat likely
- O Extremely likely

Have you been a part of an internship(s)?

- ○ Yes
- ○ No

How many internships have you participated in?

- ○ 1
- ○ 2
- ○ 3
- ○ 4
- ○ 5+

During your time at FIU, to the best of your memory, what are the name(s) of the internship(s) you have been in? If no name, briefly describe.

[ ]

Would you consider yourself more engaged with your community after participating in your internship(s)?

- ○ Definitely yes
- ○ Probably yes
- ○ Might or might not
- ○ Probably not
- ○ Definitely not

While you were in an internship, how many hours a week did you spend on each of these activities in a typical week?

	0 hours	1 - 5 hours	6 - 10 hours	11 - 15 hours	16 - 20 hours	21 - 25 hours	25+ hours
Internship	○	○	○	○	○	○	○
Research	○	○	○	○	○	○	○
Other Employment	○	○	○	○	○	○	○
School clubs/sports	○	○	○	○	○	○	○
Volunteering	○	○	○	○	○	○	○
Academic classes	○	○	○	○	○	○	○

If you were enrolled at a university or college when you did your internship(s) what year were you in your program? If there were in multiple internships please select all the answers that are applicable.

- ☐ Freshman
- ☐ Sophomore
- ☐ Junior
- ☐ Senior
- ☐ Post-graduation

Did you participate in the Florida *Aedes aegypti* Genome Group (FLAGG)?

- ○ Yes
- ○ No

Thinking of your internship experiences, please evaluate your internship experience in the following circumstances on a scale from "Extremely" to "Not at all".

	Not at all	Slightly	Moderately	Very	Extremely
How useful was having an internship in helping you get into a research lab?	○	○	○	○	○
How useful was having an internship in helping you feel prepared to join a research lab?	○	○	○	○	○
How useful was having an internship in helping you understand research questions/papers/data?	○	○	○	○	○
How impactful do you think having an internship was/will be in your post-secondary school applications?	○	○	○	○	○
How beneficial do you think having an internship was/will be to your career development?	○	○	○	○	○

How satisfied were you with these aspects of your internship experience?

	Extremely dissatisfied	Somewhat dissatisfied	Neither satisfied nor dissatisfied	Somewhat satisfied	Extremely satisfied
Ease of registration	○	○	○	○	○
Length of program	○	○	○	○	○
Time requirements of program	○	○	○	○	○
Availability of feedback	○	○	○	○	○
Quality of program directions	○	○	○	○	○

The following questions are about your internship experience in a scale from "Strongly disagree" to "Strongly agree".

	Strongly disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Strongly agree
The program has a professional environment.	○	○	○	○	○
Students learned a lot.	○	○	○	○	○
The program provides interesting work.	○	○	○	○	○
The program has a good supervisor.	○	○	○	○	○
The program helped me make valuable contacts.	○	○	○	○	○
The program makes students felt like a part of the team.	○	○	○	○	○
The program has a flexible schedule.	○	○	○	○	○

The following questions are about your internship experience in a scale from "Strongly disagree" to "Strongly agree".

	Strongly disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Strongly agree
The program gives a meaningful task.	○	○	○	○	○
The program task is relevant to my studies.	○	○	○	○	○
The program task is relevant to my interest.	○	○	○	○	○
There is supervision toward students.	○	○	○	○	○
Staff of the program is always available for help.	○	○	○	○	○

	Strongly disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Strongly agree
The program teaches a new skill.	○	○	○	○	○
The program increases student self-knowledge.	○	○	○	○	○

The following questions are about your internship experience in a scale from "Strongly disagree" to "Strongly agree".

	Strongly disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Strongly agree
Students are satisfied with their internship program.	○	○	○	○	○
I am satisfied with the variety of activities my internship offers.	○	○	○	○	○
I am satisfied with the opportunities that my internship had given.	○	○	○	○	○
I am satisfied with the compensation I received from my internship (for example, volunteer hours or credit hours).	○	○	○	○	○

Were there significant barriers to your success during your internship experience?

○ Yes

○ No

What were some of the barriers to your success that you identified in the previous question?

Were there any negative outcomes related to your participation in an internship?

○ Yes

○ No

What were some of the negative outcomes you identified?

What was the strongest (best or most helpful) portion of your internship experience and why? Please consider the portion from which you learned the most and what will be most useful to you in the future.

How might the internship(s) you participated in be modified to make them more appealing to future students?

Mosquito Surveillance questions

The next few questions are about your knowledge on mosquito surveillance.

How much exposure do you have on mosquito knowledge and education (includes previous social media exposure such as news articles)?

- ○ None at all
- ○ A little
- ○ A moderate amount
- ○ A lot
- ○ A great deal

On a scale of 0 (not concerned) to 5 (most concerned), how concerned are you with diseases that mosquitoes may potentially carry?

	0	1	2	3	4	5
Level of concern						

How confident are you with the following skills?

	Extremely incompetent	Somewhat incompetent	Neither competent nor incompetent	Somewhat competent	Extremely competent
Identifying mosquito eggs.	○	○	○	○	○
Identifying mosquito larvae.	○	○	○	○	○

	Extremely incompetent	Somewhat incompetent	Neither competent nor incompetent	Somewhat competent	Extremely competent
Identifying hot spots for breeding sites around your home (standing water).	○	○	○	○	○
Teaching friends/relatives/others how to identify hot spots for breeding sites (standing water).	○	○	○	○	○
Understanding mosquito prevention measures.	○	○	○	○	○
Understanding/Identifying the mosquito life cycle.	○	○	○	○	○
Understanding diseases that Aedes mosquitoes may potentially carry.	○	○	○	○	○
Doing field research.	○	○	○	○	○

On a scale of 1 (Never) to 5 (Daily), how often have you taken these measures within the last 12 months to protect yourself and family from being bitten?

	Never	Rarely	Sometimes	Often	Always
Removal of empty containers such as tires, flowerpots, birdbaths.	○	○	○	○	○
Use of drainage system for storm water, such as ditches.	○	○	○	○	○
Personal protection by wearing appropriate clothing.	○	○	○	○	○
Personal protection by wearing insect repellent.	○	○	○	○	○
Personal application of pesticides targeting mosquitoes.	○	○	○	○	○
Hiring professional mosquito control services.	○	○	○	○	○
Cleaning gutters of leaves, pine needles, and other debris.	○	○	○	○	○
Avoiding being outside during dusk or dawn.	○	○	○	○	○

	Never	Rarely	Sometimes	Often	Always
Avoiding being outside in times of highest mosquito activity.	◯	◯	◯	◯	◯
Using a mosquito zapper.	◯	◯	◯	◯	◯
Using an insect screen/netting.	◯	◯	◯	◯	◯
Staying indoors.	◯	◯	◯	◯	◯
Using mosquito coils.	◯	◯	◯	◯	◯
Operating fans.	◯	◯	◯	◯	◯
Automatic mosquito spray/mist.	◯	◯	◯	◯	◯
Other.	◯	◯	◯	◯	◯

What environment can become a mosquito breeding habitat?

What are the mosquito life stages?

How willing are you to participate in mosquito control measures?

- ◯ Extremely unlikely
- ◯ Somewhat unlikely
- ◯ Neither likely nor unlikely
- ◯ Somewhat likely
- ◯ Extremely likely

How difficult do you think it is to participate in mosquito control measures?

- ◯ Extremely difficult
- ◯ Somewhat difficult
- ◯ Neither easy nor difficult
- ◯ Somewhat easy
- ◯ Extremely easy

Demographics

Please answer these questions based on how you self identify.

What gender do you identify as?

- ○ Male
- ○ Female
- ○ Third gender
- ○ Non-binary
- ○ Prefer not to say

Which of the following categories do you identify with? (Check all that apply)

- ☐ Hispanic(a) or Latino(a)
- ☐ White
- ☐ African-American or Black
- ☐ American Indian or Alaska Native
- ☐ Asian
- ☐ Native Hawaiian or Pacific Islander
- ☐ ________ Other
- ☐ Unknown
- ☐ Prefer not to say

For this question please select the option marked "Strongly agree".

- ○ Strongly agree
- ○ Somewhat agree
- ○ Neither agree nor disagree
- ○ Somewhat disagree
- ○ Strongly disagree

What is your marital status?

- ○ Married
- ○ Living with a Partner
- ○ Widowed
- ○ Divorced/Separated
- ○ Single

Which languages are you capable of speaking fluently?

- ☐ English

- ☐ Spanish
- ☐ Portuguese
- ☐ French
- ☐ Mandarin
- ☐ Korean
- ☐ Arabic
- ☐ ____________ Other
- ☐ Prefer not to say

Where were you born?

- ○ North America
- ○ Central America
- ○ South America
- ○ Caribbean Islands
- ○ Pacific Islander
- ○ Europe
- ○ Africa
- ○ Asia
- ○ Australia
- ○ Other
- ○ Prefer not to say

How many children under 18 live in your home?

- ○ None
- ○ 1
- ○ 2-4
- ○ More than 4
- ○ Prefer not to say

What is the highest degree or level of education you have completed as of completing this survey?

- ○ Some college, but no degree
- ○ Associates degree
- ○ Bachelor's degree
- ○ Graduate or professional degree (MA, MS, MBA, PhD, JD, MD, DDS etc.)
- ○ Prefer to not say

If you have attended college or university, what was your major?

- ☐ Biology
- ☐ Chemistry
- ☐ Biomedical engineering
- ☐ Computer science
- ☐ Physics
- ☐ Psychology
- ☐ Biochemistry
- ☐ Nature and applied sciences
- ☐ ________ If other please write your major below:

What is your age?

- ○ Under 18
- ○ 18 - 24
- ○ 25 - 34
- ○ 35 or older

What is your annual income based on your personal employment?

- ○ No income
- ○ Less than $10,000
- ○ $10,000 - $25,000
- ○ $25,000 - $49,999
- ○ $50,000 - $74,999
- ○ $75,000 - $99,999
- ○ $100,000 - $149,999
- ○ $150,000 or more
- ○ Prefer not to say

Did your family ever have difficulty meeting expenses?

- ○ Never
- ○ Sometimes
- ○ About half the time
- ○ Most of the time
- ○ Always

What is your current employment status?

- ☐ Employed full time
- ☐ Employed part time
- ☐ Unemployed looking for work
- ☐ Unemployed not looking for work
- ☐ Retired
- ☐ Student
- ☐ Disabled

Are you the primary income earner of your household?

- ○ Yes
- ○ No
- ○ Prefer not to say

This is an anonymous survey. However, if you want to receive class credit/volunteer hours for this survey or are okay with being identified for further evaluation, please answer "yes" to the question below. If you select "no" we will not be able to provide any form of credit.

- ○ Yes
- ○ No

Student Identification

Please answer the following questions to allow us to send your information to your professor to receive extra credit **or** to receive a community service certificate.

What is your full Name?

[ ]

What is your FIU panther ID

[ ]

If you are comfortable being contacted further about your experience in this program, please type your email below.

[ ]

FLAGG Experience Questions

If you have participated in a Florida *Aedes aegypti* Genome Group (FLAGG) collection semester, please answer these questions based around how you felt about your experiences after the collection semester(s) you have participated in concluded.

What year(s) did you participate in FLAGG? If there were in multiple internships please select all the answers that are applicable.

- ☐ Freshman
- ☐ Sophomore
- ☐ Junior
- ☐ Senior
- ☐ Post-graduation

During your time in FLAGG which cohort(s) were you involved in? Please select more than one if applicable?

- ☐ Dr. Andre de Silva's class
- ☐ FLAGG intern
- ☐ FLAGG volunteer

Please evaluate the FLAGG internship in the following circumstances from "Not at all" to "Extremely".

	Not at all	Slightly	Moderately	Very	Extremely
How influential was the internship on your knowledge of mosquito control?	○	○	○	○	○
How useful was the FLAGG internship in helping you get into a research lab?	○	○	○	○	○
How useful was the FLAGG internship in helping you feel prepared to join a research lab?	○	○	○	○	○
How useful was the FLAGG internship in helping you understand research questions/papers/data	○	○	○	○	○

	Not at all	Slightly	Moderately	Very	Extremely
How impactful do you think this internship was in your post-secondary school applications?	○	○	○	○	○
How beneficial do you think this internship was to your career development?	○	○	○	○	○

How satisfied were you with these aspects of your internship experience?

	Extremely dissatisfied	Somewhat dissatisfied	Neither satisfied nor dissatisfied	Somewhat satisfied	Extremely satisfied
Ease of registration	○	○	○	○	○
Length of program	○	○	○	○	○
Time requirements of program	○	○	○	○	○
Availability of feedback	○	○	○	○	○
Quality of program directions	○	○	○	○	○

The following questions are about your internship experience in a scale from "Strongly disagree" to "Strongly agree".

	Strongly disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Strongly agree
The program has a professional environment.	○	○	○	○	○
Students learned a lot.	○	○	○	○	○
The program provides interesting work.	○	○	○	○	○
The program has a good supervisor.	○	○	○	○	○
The program helped me make valuable contacts.	○	○	○	○	○
The program makes students felt like a part of the team.	○	○	○	○	○
The program has a flexible schedule.	○	○	○	○	○

The following questions are about your internship experience in a scale from "Strongly disagree" to "Strongly agree".

	Strongly disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Strongly agree
The program gives a meaningful task.	O	O	O	O	O
The program task is relevant to my studies.	O	O	O	O	O
The program task is relevant to my interest.	O	O	O	O	O
There is supervision toward students.	O	O	O	O	O
Staff of the program is always available for help.	O	O	O	O	O
The program teaches a new skill.	O	O	O	O	O
The program increases student self-knowledge.	O	O	O	O	O

The following questions are about your internship experience in a scale from "Strongly disagree" to "Strongly agree".

	Strongly disagree	Somewhat disagree	Neither agree nor disagree	Somewhat agree	Strongly agree
Students are satisfied with their internship program.	O	O	O	O	O
I am satisfied with the variety of activities my internship offers.	O	O	O	O	O
I am satisfied with the opportunities that my internship had given.	O	O	O	O	O
I am satisfied with the compensation I received from my internship (for example, volunteer hours or credit hours).	O	O	O	O	O

Were there significant barriers to your success during your FLAGG internship experience?

- O Yes
- O No

What were some of the barriers to your success that you identified in the previous question?

Were there any negative outcomes related to your participation in FLAGG?

- Yes
- No

What were some of the negative outcomes you identified?

What was the strongest (best or most helpful) portion of the FLAGG internship and why? Please consider the portion from which you learned the most and what will be most useful to you in the future.

How might this internship be modified to make it more appealing to future students?

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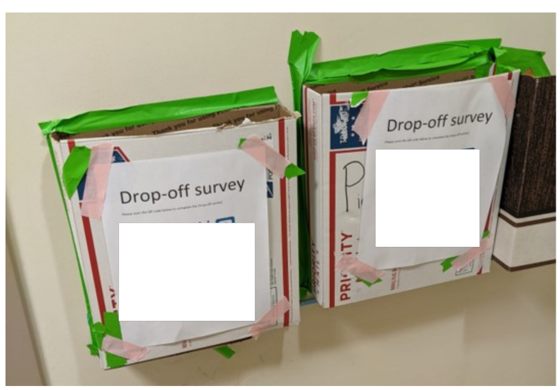
Picking up your materials

1. When you are preparing to begin collecting for the program, please first fill out the following survey to notify us when you will be stopping by:
   Doodle poll to schedule your pick-up time:
   https://doodle.com/poll/v2vm53bqmnb6rwpc?utm_source=poll&utm_medium
2. If an issue arises and you are not able to pick up materials at the time that you have specified in the previous survey, please notify the FLAGG team for an alternative pick up time.
3. After you have reserved a time to stop by the lab located at AHC 1 room 229 (https://m.fiu.edu/map/index.php?action=building&campus=1000&building=AHC1), you will be able to pick up your materials from the cardboard boxes located outside of the lab doors (these will later be the same boxes you drop off samples into).
4. Once you have picked up your materials please complete the pick up survey by scanning the QR code on the cardboard box.
5. After you have finished step 4 of this section please proceed to the next section.



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How to prepare your ovicup

1. Take the black ovicup that should be included within your materials, rinse it out, and add about 200ml of tap water.
2. Take the “seed paper” out from one of the plastic bags and place it comfortably within the cup.
3. If the seed paper was individually placed into different plastic bags, then place the now empty plastic bag somewhere for safe keeping.
4. An image of a properly set up cup is below for your reference.
5. Please make sure you have not set up the cup in a place where it is likely to get thrown away, and have notified all the residents of the house you are setting up at

Page 3

Where to set up your ovicup (Thursdays)

1. The following locations are generally the best for setting up an ovicup.

    a. An outdoor location.

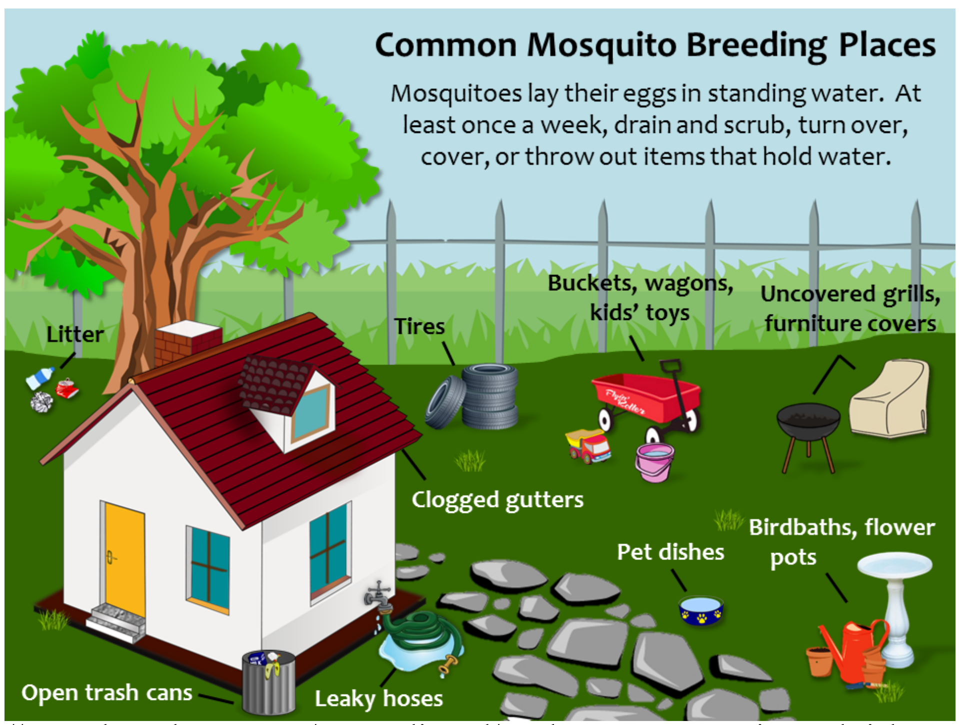
    b. A covered or shaded area.

    c. An area close to an indoor location.

    d. Backyard corners.

    e. An area protected by rainfall and wind

2. It is also recommended that you remove other potential breeding sites from your location, to ensure there is no competition between your ovicup and other sites. For your reference a picture of common breeding sites is below.



https://www.lawndoctor.com/annapolis-md/yard-armour-mosquito-and-tick-control/

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The survey for setting up

1. The survey for setting up can be reached at the following link: https://arcg.is/1qeO0r0
2. Please fill out this survey right after setting up, and in close proximity to your ovicup. This is necessary since one of the questions is used to grab your geolocation.
3. Please make sure that you are reading the questions for the survey with the week and the site of collection in mind.
4. If you have any questions about the survey or any technical issues, feel free to email the FLAGG email account: flagginternship@gmail.com

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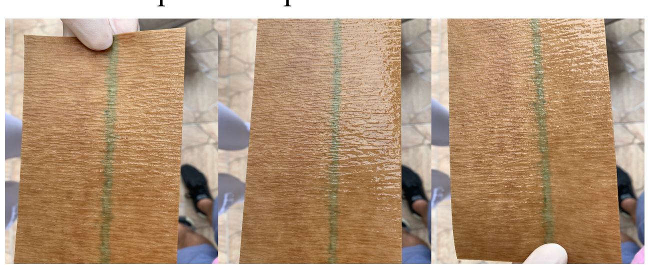
How to collect your sample from the ovicup (Sundays)

1. Place a clean paper towel onto a flat and clean surface.
2. Carefully remove the seed paper. Allow it to dry until slightly moist on the paper towel.
3. Throw remaining water from the ovicup onto a dry area of cement, to prevent accidental mosquito germination.
4. Once the seed paper is dried to the point of being only slightly moist, take three pictures of the front and the back of the paper.
5. These pictures must have the following qualities to be considered good quality
    a. They must cover the full length of the front and the back.
    b. They must be in focus.
    c. Each individual picture should just focus on a part of the "seed paper". This means the three pictures of the front should divide the paper into thirds. The same is true for the back.
    d. They should be taken as close to the paper as possible while remaining in focus.
    e. They can be taken from the phone, but any device that could provide a higher quality picture is preferred.
6. A reference picture is provided below.



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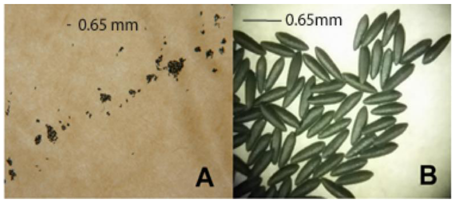
The survey for collecting

1. Fill out the collection survey right after setting up, and in close proximity to your trap. This is necessary since one of the questions grabs your geolocation.
2. Read the questions for the survey with the week and the site of collection in mind.
3. If you have any questions about the survey or any technical issues, feel free to email the FLAGG email account: flagginternship@gmail.com
4. After completing the survey, label the plastic bags in the ovicup kit with the following information. When you perform the egg count you may use your phone and zoom into objects on the paper to confirm if they are eggs.
    a. Your name and panther ID.
    b. The egg count.
    c. If you have multiple locations, number the location (i.e location 1, location 2, etc).
    d. The date of set-up and the date of collection.
5. Place the dry “seed paper” into the labeled plastic bag, and somewhere for safe keeping.
6. A reference of what mosquito eggs look like is included below.



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Dropping your samples off

1. Once per month you will need to drop off samples at AHC 1 room 229 (https://m.fiu.edu/map/index.php?action=building&campus=1000&building=AHC1). At this location there will be two cardboard boxes marked for dropping off samples outside the doors leading to the lab.
2. Make sure that before dropping off your samples all of them are clearly labeled in the same convention mentioned on page 7. Improperly labeled materials often have to either be discarded or require more time to process.
3. Once you have placed the samples to be dropped off in the cardboard boxes outside the lab, please scan the QR code located on the box and complete the survey that follows.
4. If you are lost please email this address: flagginternship@gmail.com or DM us through the FLAGG whatsapp group chat.
5. Please complete steps 1-4 of this section of the protocol until you have exhausted all the materials that were provided to you at the beginning of this internship.
6. After dropping off the last of your materials the ovicup is yours to keep, you do not need to attempt to return it.
7. Congratulations! You have completed the internship.

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CHAPTER 5: Conclusion and Future Directions

The primary objective of this dissertation was to understand the extent of citizen science benefits that can result from running the community-based mosquito surveillance program "Florida *Aedes* Genome Group (FLAGG)". The FLAGG program involved community participation in mosquito surveillance. Participants in FLAGG were educated on mosquito control and behaviors that helped reduce mosquito abundance in their community. They collected mosquito eggs to research local mosquito population dynamics, including understanding infestation seasonality, drivers of mosquito infestation, and population structure. Programs like FLAGG can be used to improve mosquito control while also generating a benefit to the participant as it fosters a sense of empowerment and engagement. Combining this educational platform with routine surveillance, and research that can help develop control strategies, can improve the current control program in Miami-Dade County (MDC) and help build the foundations for a feasible network between local mosquito programs.

Understanding factors affecting mosquito dynamics drivers in MDC is necessary to locally tailor surveillance and reduce infestation (Wilke et al., 2018). MDC mosquito control has used surveillance, immature and adult mosquito collections, insecticide interventions, release of *Wolbachia*-infected male mosquitoes, breeding source reductions, and use of educational brochures to control mosquito population abundance (Wilke et al., 2018; Mains et al., 2019; Wilke et al., 2021). Using citizen science through the FLAGG internship enhanced the availability of collected data for surveillance-based work in residential households. Considering that patterns were aligned with mosquito life cycle events, mosquito control can target household populations prior to peak seasons in the future, to affect abundance before weather drivers generate optimal conditions. Mosquito infestation and infestation patterns based on community collected data were found to be stable, with a very pronounced dry (spring), early-wet (Summer) and late-wet seasons (fall). Having access to a rich community-collected data set can be implemented by MDC mosquito control to understand when surveillance, collections, interventions, reductions and educational intervention is critical for reducing mosquito populations. Targeting off-peak seasons would be used to decrease the base populations that lead to peak-season abundance (Leach et al., 2020).

Accessing community driven data can help gain timely information on mosquito populations that need to be targeted (Fouet & Kamdem, 2019). To reinforce mosquito control competency, the development of real-time surveillance should be implemented using programs similar to FLAGG in South Florida. Community surveillance and citizen science would both yield household access for mosquito oviposition site reduction and real-time local surveillance, supporting targeted and effective mosquito control interventions (Low et al., 2021). The larger access to residential surveillance during the off-peak season would support a local targeted mosquito control method to reduce the foundational populations that contribute to high activity season abundance (Leach et al., 2020). By having timely information, targeting methods can inform decisions on where, when, and how to target mosquitoes during off-peak seasons. These populations can be tested for their susceptibility to insecticide, and environmental DNA surveillance. This tactic has been applied to get information about mosquito presence, abundance and distribution, improving the efficiency of mosquito surveillance and could be implemented in South Florida (schneider et al., 2016).

Incorporating a program, like FLAGG, to access mosquitoes from residential sites would also inform us of active genetic diversity and migration patterns to be used by mosquito control (Fouet & Kamdem, 2019; Gouagna et al., 2020). Using citizen science through FLAGG internship improved the feasibility of collecting population genomics field data. Next steps would be to use this genomics field data, alongside mosquito surveillance field survey data, to advise how mosquito control should adjust where and when they spray insecticides, and what insecticides they use (Maffey et al., 2022). The identification of one large cluster encompassing the majority of South Florida *Ae. aegypti* mosquitoes indicated low genetic differentiation between populations in Miami-Dade, Broward and Manatee County. This suggests gene flow, making the incorporation of a community program highly relevant to neighboring counties as it could potentially cause alterations to local vector competence (Caprio & Tabashnik, 1992; Hemme et al., 2010; Gauthier et al., 2014).

MDC year-round presence of mosquitoes also supports the need for a manageable communicative state program. Mosquito control in Florida has diverse region-based programs throughout the state (Moise et al., 2020; Kondapaneni et al., 2021). Sharing insights on mosquito population differences in dynamics, behavior, phenotype and genotype can therefore be a step taken to continue to improve the state's intervention efficiency (Kondapaneni et al., 2021). Since mosquito populations could migrate from district to district, re-establishing higher populations in counties with low mosquito abundance, there is a need to establish communication and collaborations between counties

(Kondapaneni et al., 2021). Knowledge on surveillance methods and local species distribution can be used to optimize control efforts (Peper et al., 2022). Volusia county, Florida, is gathering knowledge on presence, seasonal infestation patterns and abundance and using seasonal mosquito sampling with various traps to generate spatiotemporal prediction models to aid mosquito abatement programs (Rund et al., 2019; Giordano et al., 2020). Volusia found high infestation rates during the wet season and several year-round abundances that may be useful for neighboring states to know in real-time to actively implement abatement tactics (Giordano et al., 2020).

Engaging in community public meetings, educational campaigns, and citizen science initiatives should be implemented in the future as a way of expanding a community-based mosquito surveillance program in South Florida. Implementing these actions could increase in community surveillance, source reduction, and help generate a wider and more diverse sample of mosquito data to be collected and analyzed (Hribar et al., 2022; Khalighifar et al., 2022; Low et al., 2022). The FLAGG program benefits for both mosquito control and FLAGG participants included increased mosquito abatement behaviors and willingness to participate in mosquito control, as well as increased knowledge and confidence in participants. In the future, community participation in mosquito trapping, reporting, and providing information on breeding sites may be used to guide selection of locations for targeted mosquito trapping and genetic analysis (Low et al., 2022). Additionally, participants could help provide information on mosquito abundance, distribution, land use and water management practices, and human behavior patterns (Carney et al., 2022; Fraisl et al., 2022). Collected data can then be used to formulate a comprehensive understanding of regional population structure within MDC.

This project explored interdisciplinary approaches to improving mosquito control efforts with an implemented community-based surveillance program that promotes mosquito surveillance, education and engagement. Moving forward, future research should focus on expanding landscape ecology and genomic efforts to better understand local landscape factors and population dynamics influencing mosquito infestation. Future programs can expand to other communities within MDC, and to other universities to expand the reduction of vector populations within residential households. Expansion of the program can further aid in informing mosquito control of mosquito populations that may not be detected by MDC's current method of holding household inspections by citizen complaint calls or routine surveillance (Wilke et al., 2020). Collaborations with local schools and universities could recruit a diverse group of participants, engage young people in mosquito control and public health, and bring different perspectives to continue to improve a community-based surveillance program for MDC. Future work focusing on participant perception changes throughout time would also be a step forward to understanding how to efficiently use citizen science to promote consistent public engagement and support for mosquito control measures. Addressing these research gaps can strengthen mosquito control efforts towards reducing local mosquito populations.

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PUBLICATIONS AND PRESENTATIONS

H. Wagner, J. Quiñones, A.J. Bellantuono, M. DeGennaro. (April 2019) *Mapping genetic diversity and insecticide resistance of Aedes aegypti mosquitoes in Florida.* Annual Biomolecular Scientist Institute Research Symposium. Florida International University, Miami, Florida. (Poster)

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H. Wagner, J. Quinones, G. Perez, M. Ramon, K. Lopez, Dr. A. L. Costa-da-Silva, Dr. A.J. Bellantuono & Dr. M. DeGennaro (April 2023) *Addressing mosquito population dynamics in South Florida using a community-based mosquito surveillance program*. Graduate Student Appreciation Week 2023. Florida International University, Miami, Florida. (Poster)