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Morphological Comparison of Fourth-Instar Larvae from Various Populations of Wyeomyia smithii

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Morphological Comparison of Fourth-Instar Larvae from Various Populations of *Wyeomyia smithii*

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Department of Biology.

By
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Under the mentorship of Dr. William S. Irby

ABSTRACT

Populations of pitcher plant mosquitoes, Wyeomyia smithii, have exhibited recent shifts in blood-feeding behavior, likely in response to changes in climate and food availability. A comparison of various morphological structures in fourth-instar larvae was conducted to investigate the hypothesis that geographic and temporal dietary differences are reflected in changes in organismal size.

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INTRODUCTION

Wyeomyia smithii, commonly referred to as pitcher plant mosquitoes (PPM), were once characterized by their inability to blood feed. Housed in the water provided by the leaves of the pitcher plant, *Sarracenia purpurea*, larvae of *W. smithii* obtain their nutrients for survival from this fluid environment. The water stored in the leaflets of *S. purpurea* contains microorganisms and protozoa - the primary food sources for these mosquitoes (Zilic, 2013). In North America, the range of the mosquitoes extends from the Gulf Coast up to the north-central part of Canada (Lair, Bradshaw & Holzapfel, 1997) (Figure 1).

Recently, there has been a divergence among the populations of *W. smithii* in regards to feeding patterns. Northern populations of *W. smithii* exhibit obligate non-blood feeding patterns while the more Southern populations show signs of increased blood feeding. (Bradshaw, et al., 2017; Irby, Personal Communication). Northern populations are referred to as the more alpine populations whereas Southern populations fall along the Gulf Coast near North Carolina (Lair, Bradshaw & Holzapfel, 1997). The blood-feeding mosquitoes are multivoltine, and the non-biters are univoltine (Zilic, 2013) i.e., blood-feeding populations produce multiple broods per year, whereas non-biters only produce one.

This behavioral separation likely is due to differences in environmental stress, as measured by autogenic reproductive output and hexamerin (larval storage protein) accumulation (Irby, Personal Communication). Climate changes and food levels are two types of environmental factors that can cause stress on mosquito populations. Within the next 100 years, climate change will increase the global mean temperature by 1º - 4.5º C.

W. smithii are capable of living in the thermal environment that coincides with their location (Bradshaw, Fujiyama & Holzapfel, 2000). The mosquitoes must adapt to the temperatures and consequent water availability of the pitcher plants in order to survive. The number of existing *S. purpurea* populations in the Southern range has diminished over the years, largely due to habitat modification, and the distance between locations has increased making it more difficult for the mosquitoes to migrate to new locations, as demonstrated by inbreeding in Georgia populations (Irby, Personal Communication). Therefore, adaptations will play a pivotal role in the success of these mosquitoes. When comparing populations of *W. smithii* reared in nearly-optimal conditions versus environmentally stressed conditions, there was a 54% decrease in the fitness of the mosquitoes under stressed conditions (Bradshaw, Steiner & Holzapfel, 1999). In a 1999 study, the relationship between adaptation, or the mosquito's ability to adjust to its environment in present-day time, and persistence, or the mosquito's longevity over the long run, were compared (Bradshaw, Steiner & Holzapfel, 1999). The mosquitoes proved to adapt quite well to heat shock, but the persistence of these same mosquitoes was very poor. These findings showed that adaptations to the environment does not, necessarily, equate to an improvement in fitness in the long-run (Bradshaw, Steiner & Holzapfel, 1999).

Fluctuating food levels is a stressor to *W. smithii* populations since these mosquitoes are weak fliers and rarely travel far from their immediate *S. purpurea* population (Zilic, 2013). Consequently, adaptation or competition occurs in order for the mosquitoes to survive under food-stressed conditions. Under normal conditions, these mosquitoes eat bacteria, protozoans, and decaying matter housed within the pitcher plant.

A relationship between *W. smithii* and *Metriocnemus knabi*, the pitcher plant midges, has been established in which the number and food-processing efficiency of *M. knabi* impacts the food levels of the mosquitoes. The midges produce bacteria as a by-product after food consumption, and bacteria is the direct food source of pitcher plant mosquitoes (Heard, 1994). When this relationship is in balance, the mosquitoes are in a more favorable environment, but adaptations begin to emerge when food levels are low. Blood-feeding in *W. smithii* was found to be a heritable trait which may be due in part to limited food sources (Bradshaw, et al., 2017). Obtaining nutrients from a host is an opportunistic lifestyle that is energetically costly, yet it provides the mosquitoes with the nutrients that are no longer available from the pitcher plant (and representing an evolutionary shift in blood feeding). Competition is another result of limited food sources. Going southward towards the Southern populations of *W. smithii*, there are fewer *S. purpurea* populations causing an increase in competition for food sources. There are also more blood-feeding populations of *W. smithii* as one goes further south (Irby, Personal Communication). This could represent the adaptation for the mosquitoes to combat competition and a lack of natural food sources.

This change in thermoregulation and competition for nutrients could have an impact on the morphological characteristics of *W. smithii*, especially in the larval form. Insects are well documented to vary in size based on both the quality and quantity of dietary resources (Aparna & Wells 2004). The sclerotized portions of the fourth-instar larvae – the head and siphon – are standard measures for the overall size of the mosquitoes. Proper diet and environmental conditions positively correlate with larval size. With there being a decrease in the competition for resources as one continues to go northward, I hypothesize that fourth-instar larval size will increase as one goes northward.

METHODS AND MATERIALS

Collection of mosquito larvae

To test this hypothesis, three locations were chosen for mosquito larvae collection. The locations were as follows from the most northern to the most southern population: Highlands, North Carolina (NC); Tattnall County, Georgia (GA); and Apalachicola, Florida (FL) (Figure 1).

Figure 1. Diagram depicting the range of *W. smithii*. Arrows indicate the locations where the mosquitoes were collected from in 2012 and 2017.

The northern mosquitoes were collected on November 20, 2017 at the Highlands Biological Station in North Carolina (30.0998ºN, 84.7316ºW). The NC location functioned as the Northern population for this study, representing the most southerly expression of that population. The Georgia population represents an intermediate location between Northern and Southern (Florida) populations, and is isolated by at least 300 kilometers from the next nearest populations. The Tattnall County, Georgia mosquitoes were collected on February 10, 2017 with the GPS coordinates of 32.0635ºN, 82.0843ºW. The southern mosquitoes were collected from the Apalachicola National Forest in Florida at 30.0539ºN, 93.1894ºW on September 2, 2017.

Mosquito larvae were collected from visually healthy-looking pitcher plants with the use of a disposable 3 mL plastic pipette. This pipette was inserted into the pitcher plants until it reached the liquid enclosed within the pitcher plant. Samples were only retrieved from plants that did not have liquid that displayed a yellow or orange color. This tint is possibly indicative of a pathogenic bacterial presence in the liquid solution. Once retrieved from the plant, the liquid was placed in a 50 mL plastic centrifuge tube. Enough samples to fill ten tubes were collected per location, with each tube containing 200 or more larvae. After transport back to the laboratory, the contents of each tube were placed in individual glass bowls with tap water added to the bowls as needed. Larvae were kept at room temperature (˜20^{o} C) and under ambient photoperiods. Mosquitoes were fed regularly (every 2-3 days) with TetraMin Tropical Fish Flakes. Care was taken to not overfeed larvae, as indicated by increased turbidity of larval water due to bacterial growth. If this occurred, larvae were filtered from the water in the bowl, and placed in clean water.

Preservation

W. smithii larvae from 2017 were reared in the lab until the fourth-instar larval stage was reached. Upon achieving the desired maturation size, the larvae were preserved in 1.5 mL centrifuge tubes with 400 µL of 95% ethanol. The tubes were labeled based on their population and the date of collection and preserved in a freezer at $^{-}$4^{o} C. The 2012 mosquitoes (courtesy R. Morreale) were heated at almost boiling temperature for 30-60 seconds and preserved in vials that contained 70% ethanol at room temperature. The 1940s mosquitoes (courtesy Harry S. Pratt Mosquito Collection, donated by the CDC, Atlanta, Georgia) were mounted on glass slides with the siphon detached from the body for better visualization of siphon characteristics and likely were preserved in the same fashion as the 2012 mosquitoes.

Measurements

Each mosquito was placed on a glass depression slide and covered with a glass cover slip and observed under a compound microscope using the 10x objective lens. The ocular micrometer in the eyepiece of the right lens was used to measure each specimen in micrometers (µm). The longitudinal measurement of the head at the longest point (the midline) was measured as the head length (HL). The horizontal measurement of the head at the widest point was measured as the head width (HW) (Figure 2). The siphon length (SL) was the vertical measurement down the center of the siphon. The siphon width (SW) was measured as the widest part of the siphon (the base) of the mosquito (Figure 3).

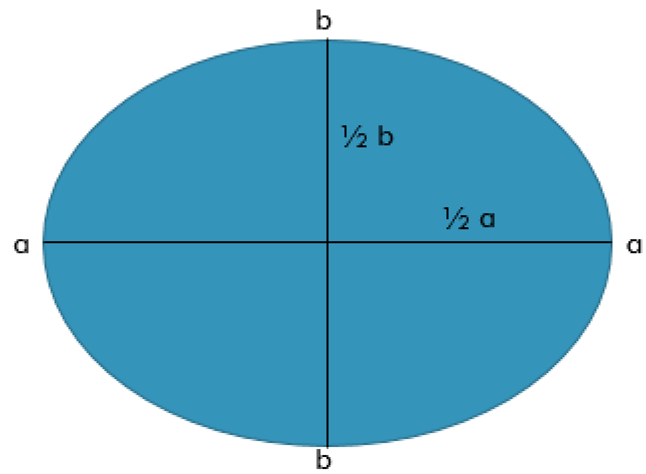

Figure 2. Diagram depicting the shape used to calculate the head area of the mosquitoes where “a” stands for head width and “b” stands for head length.

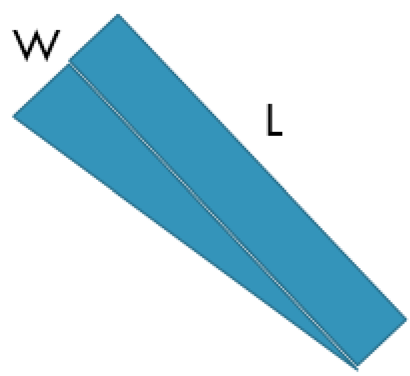

Figure 3. Diagram depicting the shapes used for a more precise measurement of the siphon area of the mosquitoes.

The total area of the mosquito was calculated by using these aforementioned measurements of the sclerotized parts of the mosquito – the head and siphon. For measurement purposes, the larvae heads were assumed to be circular and symmetrical although this assumption is an estimate. An ellipse was used to calculate the area of the head through the use of the following equation:

$$\textbf{Head Area (HA)} = (\textbf{0.5HL} * \textbf{0.5HW} * \Pi)$$

Siphon area was measured by dividing the siphon in half width-wise. This division gives rise to two different shapes – a rectangle and a triangle. The area of the siphon was computed by the addition of the area of the rectangle and the area of the triangle as follows, respectively:

$$\textbf{Siphon Area (SA)} = ((0.5SW * SL) + ((0.5SL * SW)/2)$$

After the computation of the HA and the SA, these measurements were combined to produce the estimated total area of the fourth-instar mosquito larvae:

$$\textbf{Total Area (TA)} = HA + SA$$

Statistics

Analysis of variance (ANOVA) testing was completed to determine the variance between and among the populations. One-way ANOVA analyzes the mean data of three or more independent groups of data to test for the presence of variance. This test was used to compare a single measurement, such as head width, from independent groups (different populations) from a single year. Two-way ANOVA analyzes the mean data of independent groups for two separate variables. In this experiment, the two variables were the year of the mosquito population and the location of the population. ANOVA testing revealed the p-value for that set of data. The p-value states the level of significance within a data set, and denotes the probability that the given data set occurred by chance. If the p-value indicates that the given data were significantly different, the Tukey HSD test reports which means differ.

RESULTS

Overall, measurements of heads and siphons of PPM larvae varied between populations, but not in a consistent fashion. For the 2017 locations, the largest average measurements for HL, HW, SL, and SW varied with no population consistently having the largest values for all measurements. Although, the NC population averaged the largest HL and SL, there was still variation among the other measurements (Table 1, Figure 4).

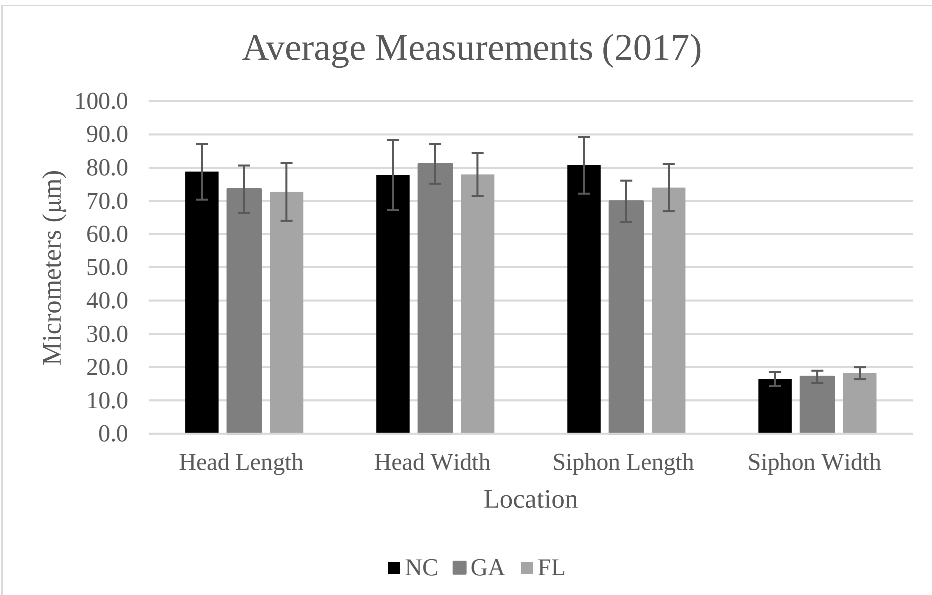

Figure 4. Average measurements, in micrometers (µm), of head length, head width, siphon length, and siphon width per location in 2017.

Table 1. Average measurements from the NC, GA, and FL locations from 2017.

	Average Measurements			
	Head Length (µm)	Head Width (µm)	Siphon Length (µm)	Siphon Width (µm)
NC	78.8	77.8	80.7	16.4
GA	73.5	81.1	69.9	17.1
FL	72.7	77.9	74.0	18.2

The head area was the larger value out of the two components of the total area, and, thus had the larger effect on the total area of the larvae. The mean head area of the mosquito populations decreased as the location was further south (Table 2). A one-way analysis of variance (ANOVA) test comparing head areas of larvae from each population, showed that across all three populations, there was a significant difference in size(p = 0.00581). Tukey's HSD test showed there was no significant difference between the head areas of the NC and GA populations, but there was a significant difference ($p < .01$) between the NC and FL populations. The mean head areas of the GA and FL populations also were significantly different at the .05 level ($p < .05$). ANOVA testing followed by Tukey's HSD test revealed that there was no significant different between the total areas of the NC and GA populations. The total areas of NC and FL were significantly different ($p < .01$). There was no significant difference between the GA and FL population total areas. There is a decrease in total areas as the populations continue further south (Figure 5).

Table 2. Average area measurements from the NC, GA, and FL locations from 2017.

	Average Measurements		
	Head Area (μm^2)	Siphon Area (μm^2)	Total Area (μm^2)
NC	4831.3	995.7	5827.0
GA	4681.2	895.1	5576.2
FL	4446.7	1009.4	5456.1

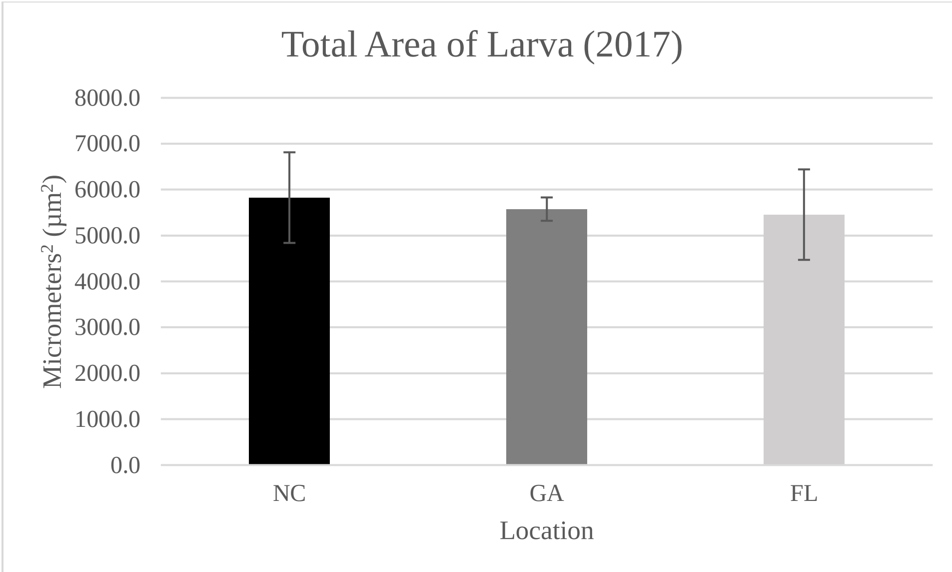

Figure 5. Comparison of the average total areas (µm^2) from each location in 2017.

As with the 2017 populations, there was variation for each of the average measurements for HL, HW, SL, and SW in the 2012 populations. For 2012, ANOVA showed that the NC population had the largest average HL and SL (Table 3). Among all three populations, there was a significant difference ($p = 0.0079$) for the head area measurements. The Tukey's HSD test indicated that there was no significant difference for the head areas of the NC and the GA populations. There is a significant difference ($p < .01$) between the NC and FL populations. The GA and FL populations were not significantly different. The total areas of the NC and GA populations were non-significant. There was a significant difference ($p < .01$) between the total areas of the NC and FL populations. There was no significant difference between the total areas of the GA and FL populations.

Table 3. Average measurements for each of the three locations in 2012.

Average Measurements (2012)				
	Head Length (μm)	Head Width (μm)	Siphon Length (μm)	Siphon Width (μm)
NC	74.5	81.7	83.4	16.5
GA	69.9	83.4	73.6	16.7
FL	65.4	79.5	77.5	15.9

Table 4. Average area measurements for NC, GA, and FL for 2012.

Average Measurements (2012)			
	Head Area (μm^2)	Siphon Area (μm^2)	Total Area (μm^2)
NC	9560.3	1030.7	10590.9
GA	9159.5	921.3	10080.8
FL	8157.0	925.2	9082.2

Since the values for the samples for the 1940s mosquitoes were rather limited, the averages for each location were not calculated. The values were, instead, used for ANOVA testing. The Rhode Island (RI) location is comparable to the NC location from the 2012 and 2017 locations and will signify the "northern" population for this study. The South Carolina (SC) population will be considered as the middle population as is GA for the 2017 and 2012 populations. The two FL populations, although from different locations within FL, will both be considered the "southern" populations. ANOVA testing revealed that there was a significant difference of 0.0004 among the head areas of the RI, SC, and FL populations. There was a significant difference at the $p = .01$ level between the RI and SC as well the same level of significance between the RI and FL populations. The SC and FL locations exhibited no significant difference. The total area among the three populations was significantly different ($p = 0.0072$). There was no significant difference between the RI and SC populations or between the SC and FL population total areas. However, the RI and FL populations were significantly different ($p < .01$).

A two-way independent sample ANOVA was performed for the total area measurements from the 3 population locations – NC, GA, and FL - in 2017 and the 3 population locations – NC, GA, and FL – in 2012. There was a significant difference ($p < 0.001$) between the whole data set from each year, so the 2017 data was variable in comparison to the 2012 data. There, also, was a significant difference ($p = 0.0005$) between each population. For example, there was variance between the data from the GA location for both years. When comparing both year and location as the ANOVA parameters, there was a p-value of 0.018 thus indicating that both year and location contributed to differences.

DISCUSSION

The mosquitoes collected from the NC, GA, and FL populations in 2017 support my hypothesis. There was an apparent decline in the total area of sclerotized regions of the mosquito larvae from the NC to the FL location. From 2012 to 2017, there has been a vast decrease in larval size, however, this may largely be attributable to differences in preservation techniques used. Average larval size decreased by 44.9% in the NC populations, 44.7% in the GA populations, and 39.9% in the FL populations. This same trend of increasing larval size when going northward was expressed in the 2012 NC, GA, and FL populations. From the measurements of the total area size from the 1940s, the northern population, RI, had the smallest mosquito larvae. Meanwhile, the FL population had the largest mosquitoes. The data retrieved from the 1940s mosquitoes does not support my hypothesis, but is based on a small sample size that is not amenable to statistical analysis. This 1940s trend is opposite from the trend that is evident in the mosquito populations now. Ironically, there was a dramatic increase in size from the total area of the larvae from the 1940s to 2012.

Improper nutrition and lack of living space can result in a decrease in larval size and longevity (Klowden, Blackmer & Chambers, 1988). Increased growth rates and decreased habitat per pitcher because of temperature and lowered water availability, largely from drought, likely is the cause of increased competition as you go south (Morreale, 2014). In a 2017 study, blood-feeding was shown to be a heritable trait that became apparent over time (Bradshaw, et al., 2017). This increase in blood-flooding is reflects an increasing reversion to ancestral blood-feeding behavior where increasing larval competition inhibits larval consumption of an adequate diet to forego blood-feeding for egg production. Sub-optimal larval nutrition also contributes to lowered adult success (Klowden, Blackmer & Chambers, 1988). Not only has climate change affected the diet and feeding patterns of the mosquitoes, but it has had an effect on growth periods for the mosquitoes. The increase in temperature has altered the diapause schedules of *W. smithii*. Diapause is the stage of life in which the mosquitoes avoid unfavorable conditions. These mosquitoes have shifted to shorter critical photoperiods, resulting in mosquitoes becoming active earlier in the Spring and remaining active later into the Fall than previously (Bradshaw & Holzapfel, 2001). This behavioral shift in critical photoperiods is heritable, marking the first evidence of global warming driving evolutionary change. (Bradshaw & Holzapfel, 2001). Although clinal change in size of larvae is a more plastic trait, with diet, both quantity and quality, clearly having an effect on outcome, it is an additional example of the effect of climate change on organismal development and success. Additional studies on the critical quantities and qualities of diet relative to likelihood of reversion to blood-feeding behavior in these mosquitoes are strongly recommended.

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Appendix: Additional figures and complete data for measurements.

Table 5. Measurements of one-hundred mosquitoes collected from the Highlands, NC location in 2017.

	Highlands, NC (2017)			
	Head Length (µm)	Head Width (µm)	Siphon Length (µm)	Siphon Width (µm)
1	80.0	89.0	77.0	15.5
2	70.0	80.0	79.0	19.0
3	66.0	71.0	83.0	19.0
4	71.0	78.5	78.0	16.0
5	60.0	76.0	78.0	15.0
6	79.0	90.0	89.0	15.0
7	86.0	78.0	83.0	18.5
8	85.0	79.0	91.0	17.0
9	89.0	81.0	82.0	17.0
10	77.0	80.0	82.0	18.0
11	69.0	81.0	81.5	14.0
12	86.0	83.0	84.0	17.0
13	57.0	80.0	80.0	16.5
14	68.0	93.0	85.0	19.0
15	83.0	71.0	78.0	14.0
16	89.0	82.0	93.0	16.0
17	69.0	78.0	79.0	17.0
18	87.0	78.0	92.0	15.5
19	67.0	83.0	77.5	15.0
20	88.0	69.0	88.0	16.5
21	82.0	80.5	86.0	14.0
22	94.0	81.0	89.0	17.5
23	85.5	82.0	87.0	19.0
24	59.0	54.0	61.0	17.0
25	81.0	54.0	50.0	14.0
26	62.0	56.0	56.0	12.0
27	84.5	74.0	84.0	15.0
28	66.0	82.0	82.0	19.5
29	79.0	79.0	87.0	15.0
30	84.0	80.0	81.0	11.0
31	80.0	71.5	78.0	14.0

32	69.0	69.0	52.0	16.0
33	82.0	76.0	85.0	19.0
34	81.0	83.0	82.5	19.0
35	86.0	89.0	87.0	18.0
36	84.0	86.5	89.0	13.5
37	85.0	81.0	76.0	18.0
38	74.0	96.7	64.0	13.0
39	86.0	86.5	85.0	20.0
40	53.0	75.0	83.5	12.5
41	83.0	88.0	82.0	16.0
42	79.0	74.0	70.0	17.0
43	80.0	75.0	83.0	17.0
44	77.5	80.0	70.0	17.0
45	87.0	65.0	62.0	16.0
46	82.0	76.0	82.0	13.0
47	84.0	67.0	82.0	14.0
48	73.5	66.0	59.0	13.0
49	73.0	8.0	85.0	17.0
50	87.0	85.0	87.0	19.0
51	78.0	75.0	82.5	19.0
52	77.0	73.0	89.0	16.0
53	84.0	76.0	90.0	17.5
54	80.0	72.0	76.0	14.0
55	77.0	78.0	92.0	17.0
56	59.0	64.0	65.0	10.0
57	83.5	80.5	86.0	14.5
58	89.0	80.0	84.5	19.0
59	72.0	82.0	84.5	14.0
60	75.0	85.5	77.5	16.0
61	86.0	79.5	86.0	18.0
62	73.0	82.0	75.5	17.0
63	77.0	81.0	87.0	16.0
64	87.0	84.0	88.0	18.0
65	84.0	80.5	82.0	16.0
66	86.0	83.0	83.0	14.0
67	75.0	76.0	74.0	16.0
68	78.0	80.0	83.0	17.0
69	88.0	84.0	87.0	18.0
70	75.0	80.0	87.0	15.0
71	79.0	80.5	86.5	16.0
72	74.0	74.0	75.0	16.5

73	83.0	83.0	83.5	18.0
74	88.0	86.0	84.0	17.0
75	72.5	83.0	85.5	15.0
76	73.0	75.0	76.0	16.5
77	66.0	87.5	79.0	14.5
78	77.0	77.5	82.5	18.0
79	77.0	79.0	77.0	17.0
80	85.0	82.5	92.0	15.0
81	92.5	91.0	89.0	18.5
82	81.0	76.0	85.0	19.0
83	71.0	87.0	86.0	18.0
84	80.0	89.0	84.0	15.0
85	86.0	85.0	81.0	15.0
86	71.5	87.0	87.0	18.0
87	84.0	65.0	91.0	21.0
88	80.0	82.0	83.0	18.0
89	83.0	81.5	84.0	16.0
90	86.0	78.0	82.0	17.0
91	73.0	59.0	62.5	11.0
92	86.0	65.0	77.0	18.0
93	82.0	82.0	81.0	17.0
94	81.0	75.0	71.0	19.0
95	75.0	72.5	77.0	17.0
96	88.0	78.0	75.0	17.0
97	72.0	79.0	77.0	15.5
98	80.0	79.0	88.0	18.5
99	98.5	90.0	86.5	19.0
100	79.0	78.0	76.0	17.5
AVG	78.8	77.8	80.7	16.4
SD	8.4	10.5	8.5	2.1
SE	0.8	1.1	0.9	0.2

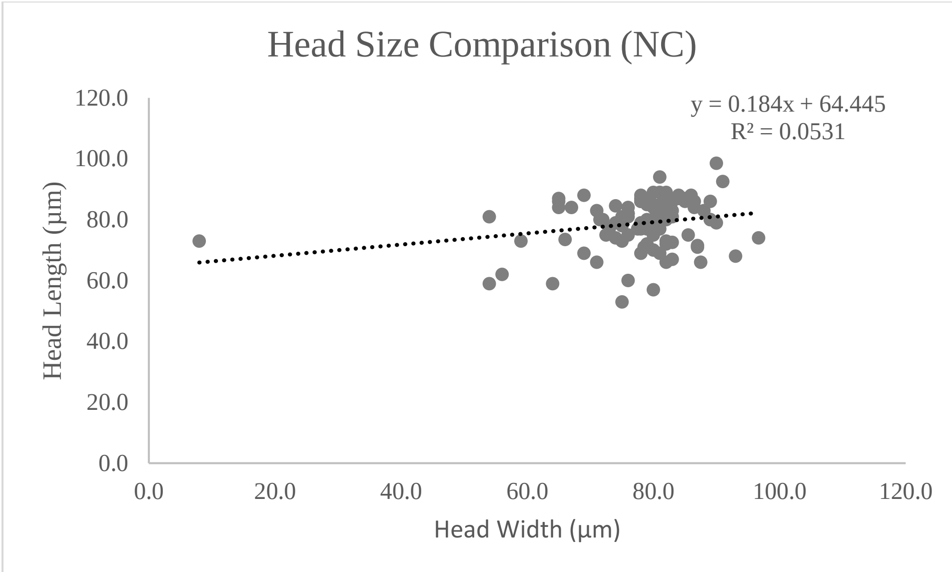

Figure 6. Comparison of head width (µm) and head length (µm) of the mosquitoes collected from Highlands, NC in 2017.

Table 6. Calculated areas for the one-hundred mosquitoes collected from the Highlands, NC location in 2017.

Highlands, NC (2017)			
	Head Area (µm2)	Siphon Area (µm2)	Total Area (µm2)
1	5592.0	895.1	6487.2
2	4398.2	1125.8	5524.0
3	3680.4	1182.8	4863.1
4	4377.4	936.0	5313.4
5	3581.4	877.5	4458.9
6	5584.2	1001.3	6585.4
7	5268.4	1151.6	6420.1
8	5273.9	1160.3	6434.2
9	5661.9	1045.5	6707.4
10	4838.0	1107.0	5945.0
11	4389.6	855.8	5245.3
12	5606.2	1071.0	6677.2
13	3581.4	990.0	4571.4
14	4966.9	1211.3	6178.1

15	4628.3	819.0	5447.3
16	5731.8	1116.0	6847.8
17	4227.0	1007.3	5234.3
18	5329.7	1069.5	6399.2
19	4367.6	871.9	5239.5
20	4768.9	1089.0	5857.9
21	5184.4	903.0	6087.4
22	5980.0	1168.1	7148.1
23	5506.4	1239.8	6746.2
24	2502.3	777.8	3280.0
25	3435.3	525.0	3960.3
26	2726.9	504.0	3230.9
27	4911.1	945.0	5856.1
28	4250.6	1199.3	5449.8
29	4901.7	978.8	5880.4
30	5277.9	668.3	5946.1
31	4492.5	819.0	5311.5
32	3739.3	624.0	4363.3
33	4894.6	1211.3	6105.8
34	5280.2	1175.6	6455.9
35	6011.4	1174.5	7185.9
36	5706.7	901.1	6607.8
37	5407.5	1026.0	6433.5
38	5620.1	624.0	6244.1
39	5842.6	1275.0	7117.6
40	3122.0	782.8	3904.8
41	5736.5	984.0	6720.5
42	4591.4	892.5	5483.9
43	4712.4	1058.3	5770.6
44	4869.5	892.5	5762.0
45	4441.4	744.0	5185.4
46	4894.6	799.5	5694.1
47	4420.2	861.0	5281.2
48	3810.0	575.3	4385.2
49	458.7	1083.8	1542.4
50	5808.0	1239.8	7047.8
51	4594.6	1175.6	5770.2
52	4414.7	1068.0	5482.7
53	5014.0	1181.3	6195.2
54	4523.9	798.0	5321.9
55	4717.1	1173.0	5890.1

56	2965.7	487.5	3453.2
57	5279.2	935.3	6214.5
58	5592.0	1204.1	6796.2
59	4637.0	887.3	5524.2
60	5036.4	930.0	5966.4
61	5369.8	1161.0	6530.8
62	4701.4	962.6	5664.0
63	4898.5	1044.0	5942.5
64	5739.7	1188.0	6927.7
65	5310.9	984.0	6294.9
66	5606.2	871.5	6477.7
67	4476.8	888.0	5364.8
68	4900.9	1058.3	5959.1
69	5805.7	1174.5	6980.2
70	4712.4	978.8	5691.1
71	4994.7	1038.0	6032.7
72	4300.8	928.1	5229.0
73	5410.6	1127.3	6537.9
74	5943.9	1071.0	7014.9
75	4726.1	961.9	5688.0
76	4300.1	940.5	5240.6
77	4535.7	859.1	5394.8
78	4686.9	1113.8	5800.6
79	4777.6	981.8	5759.3
80	5507.6	1035.0	6542.6
81	6611.1	1234.9	7846.0
82	4834.9	1211.3	6046.2
83	4851.4	1161.0	6012.4
84	5592.0	945.0	6537.0
85	5741.3	911.3	6652.5
86	4885.6	1174.5	6060.1
87	4288.3	1433.3	5721.5
88	5152.2	1120.5	6272.7
89	5312.8	1008.0	6320.8
90	5268.4	1045.5	6313.9
91	3382.7	515.6	3898.3
92	4390.4	1039.5	5429.9
93	5281.0	1032.8	6313.8
94	4771.3	1011.8	5783.0
95	4270.6	981.8	5252.3
96	5391.0	956.3	6347.2

97	4467.3	895.1	5362.5
98	4963.7	1221.0	6184.7
99	6962.5	1232.6	8195.2
100	4839.6	997.5	5837.1
AVG	4831.3	995.7	5827.0
SD	890.2	185.1	987.1
SE	89.0	18.5	98.7

Table 7. Measurements of one-hundred mosquitoes collected from the Tattnall County, GA location in 2017.

	Tattnall County, GA (2017)			
	Head Length (μm)	Head Width (μm)	Siphon Length (μm)	Siphon Width (μm)
1	83.0	78.0	65.0	14.0
2	69.0	72.0	69.0	17.0
3	81.5	82.0	77.0	16.0
4	66.0	83.0	70.0	18.0
5	81.0	80.0	68.0	18.0
6	71.0	76.0	57.0	14.0
7	69.0	75.0	60.0	13.0
8	71.0	79.0	74.0	14.0
9	69.0	86.0	70.0	18.0
10	74.5	71.0	61.0	16.0
11	59.5	85.0	64.0	18.5
12	85.0	91.0	80.0	18.0
13	79.0	82.0	73.0	17.5
14	74.0	80.0	59.0	16.0
15	75.0	82.0	60.0	20.0
16	72.0	89.0	76.0	20.0
17	81.0	83.0	70.0	21.0
18	85.0	82.0	73.5	15.0
19	67.0	80.0	71.5	15.0
20	85.0	82.0	51.0	21.0
21	70.0	79.0	69.0	19.0
22	79.0	82.0	73.0	18.0
23	75.0	91.0	75.0	17.0
24	81.0	84.0	61.0	19.0
25	75.0	89.0	74.0	19.5
26	71.0	83.0	75.0	17.0

27	59.0	85.0	75.0	16.0
28	81.0	82.5	69.0	18.0
29	74.0	77.0	70.0	15.0
30	73.5	73.0	63.0	18.0
31	75.0	84.0	73.5	17.0
32	84.0	87.5	75.0	20.0
33	81.0	78.0	69.0	19.0
34	64.0	86.0	69.0	15.0
35	67.0	78.5	65.0	16.0
36	69.0	70.0	59.0	15.0
37	72.0	92.0	72.0	19.0
38	67.0	72.0	47.0	18.0
39	77.0	82.0	79.5	17.0
40	84.0	88.0	70.0	15.0
41	64.0	87.0	79.0	17.0
42	80.0	74.0	74.0	15.0
43	86.0	86.0	78.0	16.0
44	71.5	87.0	72.5	18.0
45	80.0	90.0	69.0	15.0
46	74.0	67.0	59.0	17.0
47	75.5	86.0	79.0	19.0
48	74.0	70.0	67.0	17.0
49	81.0	80.0	62.0	19.0
50	74.0	80.0	78.0	15.0
51	70.0	71.0	72.0	16.0
52	72.0	79.5	69.0	17.0
53	70.0	79.0	73.0	17.0
54	79.0	75.0	70.0	17.0
55	73.0	71.0	70.0	16.0
56	57.0	92.0	67.0	15.0
57	70.5	85.0	70.0	16.5
58	83.0	85.0	75.0	18.0
59	70.0	82.0	74.0	19.0
60	79.0	82.0	78.0	17.0
61	81.0	77.0	70.0	18.0
62	68.0	76.0	65.0	16.0
63	72.0	71.0	72.0	17.0
64	60.0	86.0	67.0	18.0
65	74.0	82.0	74.0	18.0
66	68.0	92.0	76.0	16.0
67	66.0	84.0	75.0	16.0
68	59.0	86.0	65.0	13.0
69	71.0	91.0	75.0	18.0

70	65.0	77.0	75.0	14.0
71	70.0	86.0	64.0	16.0
72	61.0	82.0	75.0	15.5
73	82.0	77.0	71.0	18.5
74	75.0	86.0	67.0	20.0
75	66.0	86.0	77.0	15.0
76	69.5	85.0	78.0	16.0
77	67.0	90.0	75.0	20.0
78	66.5	77.0	72.0	15.0
79	66.0	86.0	70.0	19.0
80	83.0	86.0	73.0	15.5
81	70.5	75.0	69.0	19.5
82	68.5	76.0	64.5	15.0
83	79.5	91.0	65.0	20.0
84	78.0	76.0	71.5	18.5
85	84.0	75.0	77.0	16.0
86	83.5	79.0	69.0	17.5
87	69.0	73.0	71.0	15.0
88	65.0	81.0	72.5	17.0
89	69.5	80.0	65.0	19.0
90	72.5	76.0	69.0	21.0
91	72.5	73.5	66.0	19.0
92	80.0	79.0	70.0	18.0
93	76.0	87.0	76.0	20.0
94	60.5	86.0	65.0	18.0
95	75.0	87.0	72.0	15.0
96	80.0	76.0	70.0	16.0
97	71.0	71.0	61.0	16.0
98	86.0	75.0	60.0	18.0
99	73.0	84.0	81.0	17.0
100	87.0	79.0	68.0	15.0
AVG	73.5	81.1	69.9	17.1
SD	7.1	6.0	6.2	1.9
SE	0.7	0.6	0.6	0.2

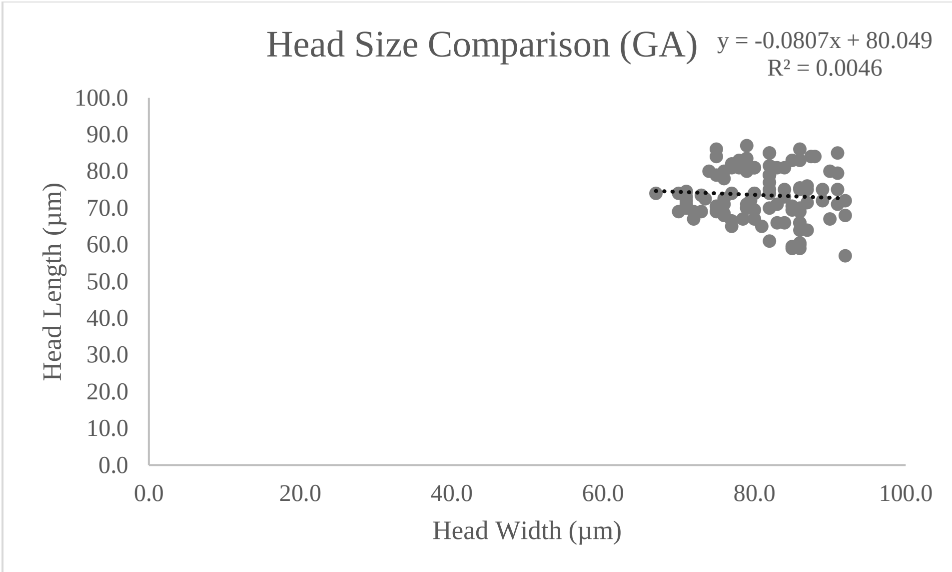

Figure 4. Comparison of the head widths (µm) and head lengths (µm) of the mosquitoes collected from the GA location in 2017.

Table 8. Calculated areas for the one-hundred mosquitoes collected from the Tattnall County, GA location in 2017.

Tattnall County, GA (2017)			
	Head Area (μm^2)	Siphon Area (μm^2)	Total Area (μm^2)
1	5084.7	682.5	5767.2
2	3901.9	879.8	4781.6
3	5248.8	924.0	6172.8
4	4302.4	945.0	5247.4
5	5089.4	918.0	6007.4
6	4238.0	598.5	4836.5
7	4064.4	585.0	4649.4
8	4405.3	777.0	5182.3
9	4660.5	945.0	5605.5
10	4154.4	732.0	4886.4
11	3972.1	888.0	4860.1
12	6075.0	1080.0	7155.0
13	5087.8	958.1	6045.9
14	4649.6	708.0	5357.6
15	4830.2	900.0	5730.2

16	5032.8	1140.0	6172.8
17	5280.2	1102.5	6382.7
18	5474.2	826.9	6301.1
19	4209.7	804.4	5014.1
20	5474.2	803.3	6277.5
21	4343.2	983.3	5326.5
22	5087.8	985.5	6073.3
23	5360.3	956.3	6316.6
24	5343.8	869.3	6213.1
25	5242.5	1082.3	6324.8
26	4628.3	956.3	5584.6
27	3938.8	900.0	4838.8
28	5248.4	931.5	6179.9
29	4475.2	787.5	5262.7
30	4214.1	850.5	5064.6
31	4948.0	937.1	5885.1
32	5772.7	1125.0	6897.7
33	4962.1	983.3	5945.4
34	4322.8	776.3	5099.1
35	4130.8	780.0	4910.8
36	3793.5	663.8	4457.2
37	5202.5	1026.0	6228.5
38	3788.8	634.5	4423.3
39	4959.0	1013.6	5972.6
40	5805.7	787.5	6593.2
41	4373.1	1007.3	5380.3
42	4649.6	832.5	5482.1
43	5808.8	936.0	6744.8
44	4885.6	978.8	5864.3
45	5654.9	776.3	6431.1
46	3894.0	752.3	4646.3
47	5099.6	1125.8	6225.3
48	4068.4	854.3	4922.6
49	5089.4	883.5	5972.9
50	4649.6	877.5	5527.1
51	3903.4	864.0	4767.4
52	4495.6	879.8	5375.4
53	4343.2	930.8	5274.0
54	4653.5	892.5	5546.0
55	4070.7	840.0	4910.7
56	4118.6	753.8	4872.4
57	4706.5	866.3	5572.7
58	5541.0	1012.5	6553.5

59	4508.2	1054.5	5562.7
60	5087.8	994.5	6082.3
61	4898.5	945.0	5843.5
62	4058.9	780.0	4838.9
63	4015.0	918.0	4933.0
64	4052.7	904.5	4957.2
65	4765.8	999.0	5764.8
66	4913.4	912.0	5825.4
67	4354.2	900.0	5254.2
68	3985.1	633.8	4618.9
69	5074.5	1012.5	6087.0
70	3930.9	787.5	4718.4
71	4728.1	768.0	5496.1
72	3928.6	871.9	4800.4
73	4959.0	985.1	5944.1
74	5065.8	1005.0	6070.8
75	4457.9	866.3	5324.2
76	4639.7	936.0	5575.7
77	4735.9	1125.0	5860.9
78	4021.6	810.0	4831.6
79	4457.9	997.5	5455.4
80	5606.2	848.6	6454.8
81	4152.8	1009.1	5161.9
82	4088.8	725.6	4814.4
83	5682.0	975.0	6657.0
84	4655.8	992.1	5647.9
85	4948.0	924.0	5872.0
86	5180.9	905.6	6086.5
87	3956.0	798.8	4754.8
88	4135.1	924.4	5059.5
89	4366.8	926.3	5293.1
90	4327.5	1086.8	5414.3
91	4185.2	940.5	5125.7
92	4963.7	945.0	5908.7
93	5193.0	1140.0	6333.0
94	4086.4	877.5	4963.9
95	5124.7	810.0	5934.7
96	4775.2	840.0	5615.2
97	3959.2	732.0	4691.2
98	5065.8	810.0	5875.8
99	4816.1	1032.8	5848.8
100	5398.0	765.0	6163.0
AVG	4681.2	895.1	5576.2

SD	557.7	122.1	619.3
SE	55.8	12.2	61.9

Table 9. Measurements of one-hundred mosquitoes collected from the Apalachicola, FL location in 2017.

Apalachicola, FL (2017)				
	Head Length (µm)	Head Width (µm)	Siphon Length (µm)	Siphon Width (µm)
1	54.0	83.0	77.0	19.0
2	64.0	86.0	57.0	17.5
3	89.0	79.0	75.0	16.0
4	58.0	77.0	66.5	14.0
5	72.0	75.0	75.0	16.0
6	86.0	71.0	84.0	17.0
7	60.0	76.0	68.0	19.0
8	50.0	78.0	67.0	18.0
9	62.0	50.0	62.0	15.0
10	65.0	84.0	78.0	16.5
11	63.0	88.0	62.0	13.0
12	57.0	78.0	59.0	17.0
13	78.0	74.0	63.5	16.0
14	74.0	72.0	79.0	16.0
15	80.0	85.0	62.0	20.5
16	69.0	74.0	60.0	13.0
17	74.0	73.0	81.0	17.0
18	82.0	87.0	72.0	18.0
19	81.0	71.0	81.0	17.0
20	86.0	78.0	67.0	19.0
21	71.0	62.0	64.0	19.0
22	65.0	77.5	74.0	16.0
23	70.0	83.5	63.0	16.0
24	66.5	84.0	70.0	18.0
25	63.0	83.0	55.0	16.0
26	63.0	93.0	80.0	19.0
27	91.0	81.0	76.0	18.0
28	66.0	85.0	76.0	17.5
29	75.0	74.0	83.0	13.0

30	75.0	82.0	76.0	14.0
31	75.0	81.0	80.0	17.0
32	60.0	81.0	77.0	16.5
33	88.5	73.0	79.0	18.0
34	63.0	84.0	73.0	17.5
35	77.5	80.0	69.0	17.0
36	78.0	75.5	64.0	16.0
37	77.0	71.0	72.0	19.0
38	86.0	84.0	66.0	19.0
39	77.0	74.0	80.0	17.0
40	73.0	74.0	83.0	18.0
41	58.0	82.0	84.0	15.0
42	79.0	74.0	77.0	16.0
43	72.0	78.0	77.0	18.0
44	69.0	65.0	73.0	15.5
45	80.0	64.0	80.0	14.0
46	84.0	79.0	75.0	19.0
47	71.0	83.0	70.0	16.0
48	76.0	79.5	80.5	16.0
49	60.0	74.0	84.5	18.0
50	73.5	75.0	76.0	16.0
51	75.0	82.5	77.0	17.0
52	61.0	82.0	75.5	155.0
53	72.0	77.0	75.5	15.0
54	72.0	84.0	77.0	18.0
55	70.5	73.0	85.5	18.0
56	55.0	72.0	73.5	15.5
57	85.0	85.0	83.0	21.0
58	62.0	80.0	82.0	19.0
59	77.0	75.5	77.0	17.0
60	70.0	59.0	71.0	18.0
61	76.0	73.5	72.0	16.5
62	85.5	84.5	74.0	18.0
63	76.0	79.0	71.0	18.0
64	80.0	77.0	79.5	18.0
65	83.0	75.0	72.0	15.5
66	61.5	83.0	78.0	15.0
67	65.0	72.0	77.0	17.0
68	76.0	78.0	78.0	16.0
69	72.0	82.0	73.0	17.5
70	86.0	76.0	75.0	17.0

71	73.0	71.0	72.0	19.0
72	56.0	88.5	70.0	15.0
73	75.0	80.0	74.0	16.0
74	73.0	88.0	79.5	18.0
75	74.5	78.0	81.0	18.0
76	70.0	82.0	81.5	17.0
77	80.0	79.0	69.0	19.0
78	75.0	69.0	54.0	18.0
79	80.0	73.0	78.0	15.0
80	79.0	77.0	76.0	17.0
81	73.0	79.0	75.5	17.0
82	68.0	76.0	77.0	14.0
83	80.0	76.0	66.0	19.0
84	71.5	88.0	85.0	14.0
85	77.0	76.0	75.0	16.0
86	72.0	75.0	72.0	15.0
87	75.0	79.0	71.0	16.0
88	79.0	78.0	73.0	16.0
89	72.0	80.0	73.0	16.0
90	79.0	82.0	70.0	16.5
91	63.0	75.0	74.0	13.0
92	74.0	76.0	79.0	16.0
93	67.0	82.0	85.0	15.0
94	80.0	83.0	89.0	20.0
95	61.0	91.0	82.0	14.0
96	84.0	77.0	71.0	19.0
97	79.0	75.0	71.0	19.0
98	81.0	78.0	61.0	13.0
99	77.0	77.0	76.0	15.5
100	77.0	80.0	79.0	18.0
AVG	72.7	77.9	74.0	18.2
SD	8.7	6.5	7.1	13.9
SE	0.9	0.6	0.7	1.4

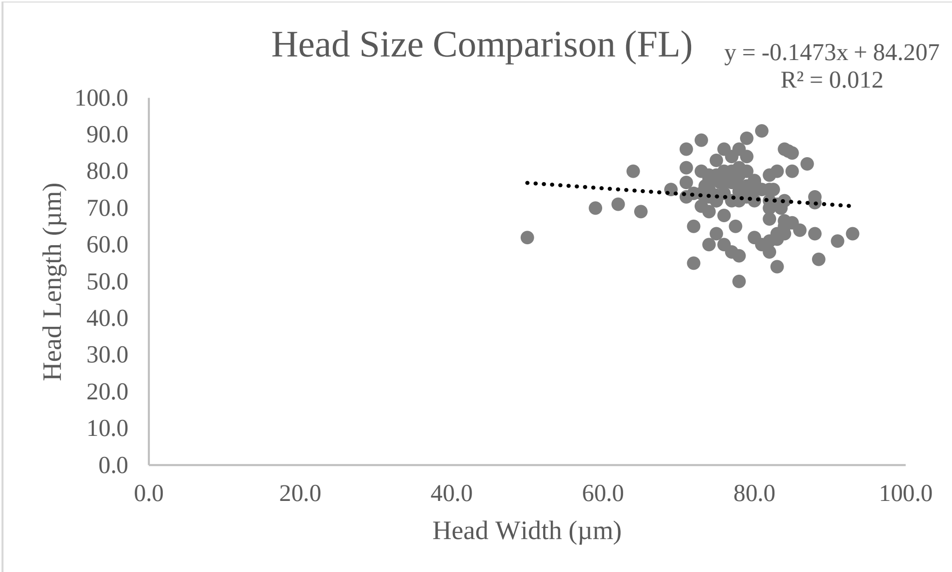

Figure 7. Comparison of the head widths and head lengths of the mosquitoes collected from FL in 2017.

Table 10. Calculated areas for the one-hundred mosquitoes collected from the Apalachicola, FL location in 2017.

	Apalachicola, FL (2017)		
	Head Area (μm^2)	Siphon Area (μm^2)	Total Area (μm^2)
1	3520.2	1097.3	4617.4
2	4322.8	748.1	5071.0
3	5522.1	900.0	6422.1
4	3507.6	698.3	4205.8
5	4241.1	900.0	5141.1
6	4795.6	1071.0	5866.6
7	3581.4	969.0	4550.4
8	3063.1	904.5	3967.6
9	2434.7	697.5	3132.2
10	4288.3	965.3	5253.5
11	4354.2	604.5	4958.7
12	3491.9	752.3	4244.1
13	4533.3	762.0	5295.3
14	4184.6	948.0	5132.6
15	5340.7	953.3	6294.0

16	4010.2	585.0	4595.2
17	4242.7	1032.8	5275.5
18	5603.0	972.0	6575.0
19	4516.8	1032.8	5549.6
20	5268.4	954.8	6223.2
21	3457.3	912.0	4369.3
22	3956.4	888.0	4844.4
23	4590.6	756.0	5346.6
24	4387.2	945.0	5332.2
25	4106.8	660.0	4766.8
26	4601.6	1140.0	5741.6
27	5789.2	1026.0	6815.2
28	4406.1	997.5	5403.6
29	4359.0	809.3	5168.2
30	4830.2	798.0	5628.2
31	4771.3	1020.0	5791.3
32	3817.0	952.9	4769.9
33	5074.1	1066.5	6140.6
34	4156.3	958.1	5114.4
35	4869.5	879.8	5749.2
36	4625.2	768.0	5393.2
37	4293.8	1026.0	5319.8
38	5673.7	940.5	6614.2
39	4475.2	1020.0	5495.2
40	4242.7	1120.5	5363.2
41	3735.4	945.0	4680.4
42	4591.4	924.0	5515.4
43	4410.8	1039.5	5450.3
44	3522.5	848.6	4371.1
45	4021.2	840.0	4861.2
46	5211.9	1068.8	6280.6
47	4628.3	840.0	5468.3
48	4745.4	966.0	5711.4
49	3487.2	1140.8	4627.9
50	4329.5	912.0	5241.5
51	4859.6	981.8	5841.4
52	3928.6	8776.9	12705.4
53	4354.2	849.4	5203.6
54	4750.1	1039.5	5789.6
55	4042.0	1154.3	5196.3
56	3110.2	854.4	3964.6

57	5674.5	1307.3	6981.7
58	3895.6	1168.5	5064.1
59	4565.9	981.8	5547.7
60	3243.7	958.5	4202.2
61	4387.2	891.0	5278.2
62	5674.3	999.0	6673.3
63	4715.5	958.5	5674.0
64	4838.0	1073.3	5911.3
65	4889.1	837.0	5726.1
66	4009.1	877.5	4886.6
67	3675.7	981.8	4657.4
68	4655.8	936.0	5591.8
69	4637.0	958.1	5595.1
70	5133.4	956.3	6089.6
71	4070.7	1026.0	5096.7
72	3892.4	787.5	4679.9
73	4712.4	888.0	5600.4
74	5045.4	1073.3	6118.6
75	4563.9	1093.5	5657.4
76	4508.2	1039.1	5547.3
77	4963.7	983.3	5947.0
78	4064.4	729.0	4793.4
79	4586.7	877.5	5464.2
80	4777.6	969.0	5746.6
81	4529.4	962.6	5492.0
82	4058.9	808.5	4867.4
83	4775.2	940.5	5715.7
84	4941.7	892.5	5834.2
85	4596.1	900.0	5496.1
86	4241.1	810.0	5051.1
87	4653.5	852.0	5505.5
88	4839.6	876.0	5715.6
89	4523.9	876.0	5399.9
90	5087.8	866.3	5954.1
91	3711.0	721.5	4432.5
92	4417.1	948.0	5365.1
93	4315.0	956.3	5271.2
94	5215.0	1335.0	6550.0
95	4359.7	861.0	5220.7
96	5080.0	1011.8	6091.7
97	4653.5	1011.8	5665.2

98	4962.1	594.8	5556.9
99	4656.6	883.5	5540.1
100	4838.0	1066.5	5904.5
AVG	4446.7	1009.4	5456.1
SD	610.4	796.0	985.4
SE	61.0	79.6	98.5

Table 11. Measurements of ten mosquitoes collected from the Highlands, NC location in 2012.

	Higlands, NC (2012)			
	Head Length (µm)	Head Width (µm)	Siphon Length (µm)	Siphon Width (µm)
1	80.0	77.0	83.0	16.0
2	62.5	83.0	90.0	16.0
3	72.0	81.0	80.0	14.0
4	77.0	83.0	83.5	16.0
5	69.0	86.0	82.0	19.0
6	71.0	81.0	86.0	14.0
7	83.5	82.0	82.0	19.0
8	77.0	83.0	84.0	17.0
9	69.0	77.0	82.0	16.0
10	84.0	84.0	81.0	18.0
Avg	74.5	81.7	83.4	16.5
SD	7.0	2.9	2.9	1.8
SE	2.2	0.9	0.9	0.6

Table 12. Calculated areas for mosquitoes collected from Highlands, NC in 2012.

Highland, NC (2012)			
	Head Area (µm2)	Siphon Area (µm7)	Total Area (µm2)
1	9676.1	996.0	10672.1
2	8148.5	1080.0	9228.5
3	9160.9	840.0	10000.9
4	10039.0	1002.0	11041.0
5	9321.1	1168.5	10489.6
6	9033.6	903.0	9936.6
7	10755.2	1168.5	11923.7
8	10039.0	1071.0	11110.0
9	8345.6	984.0	9329.6
10	11083.5	1093.5	12177.0
Avg	9560.3	1030.7	10590.9
SD	953.5	106.9	1000.2
SE	301.5	33.8	316.3

Table 13. Measurements of ten mosquitoes collected from the Tattnall County, GA location in 2012.

Tattnall County, GA (2012)				
	Head Length (µm)	Head Width (µm)	Siphon Length (µm)	Siphon Width (µm)
1	72.0	83.0	74.0	15.0
2	67.0	87.0	72.0	15.0
3	65.0	82.0	70.0	17.0
4	69.0	78.0	67.5	16.0
5	74.0	84.5	70.0	20.0
6	53.0	83.5	78.0	15.0
7	75.0	79.0	70.0	16.0
8	75.0	86.0	78.0	20.0
9	75.0	87.0	78.0	16.0
10	74.0	84.0	78.0	17.0
Avg	69.9	83.4	73.6	16.7
SD	7.0	3.1	4.2	1.9
SE	2.2	1.0	1.3	0.6

Table 14. Calculated areas for the ten mosquitoes collected from the Tattnall County, GA location in 2012.

Tattnall County, GA (2012)			
	Head Area (μm2)	Siphon Area (μm2)	Total Area (μm2)
1	9387.1	832.5	10219.6
2	9156.2	810.0	9966.2
3	8372.3	892.5	9264.8
4	8454.0	810.0	9264.0
5	9822.2	1050.0	10872.2
6	6951.6	877.5	7829.1
7	9307.0	840.0	10147.0
8	10131.6	1170.0	11301.6
9	10249.4	936.0	11185.4
10	9764.1	994.5	10758.6
Avg	9159.5	921.3	10080.8
SD	1000.2	118.3	1066.5
SE	316.3	37.4	337.2

Table 15. Measurements of mosquitoes collected from Apalachicola, FL in 2012.

Apalachicola, FL (2012)				
	Head Length (μm)	Head Width (μm)	Siphon Length (μm)	Siphon Width (μm)
1	56.0	80.0	83.0	16.0
2	69.0	83.0	77.0	16.0
3	56.0	81.0	75.0	17.0
4	60.5	76.5	70.5	16.0
5	74.0	73.0	75.0	14.0
6	75.0	84.0	84.0	17.0
7	65.0	82.0	75.0	17.0
8	63.0	81.0	87.0	16.0
9	69.5	74.0	68.5	15.0
10	66.0	80.0	80.0	15.0
Avg	65.4	79.5	77.5	15.9
SD	6.7	3.7	5.9	1.0
SE	2.1	1.2	1.9	0.3

Table 16. Calculated areas for the ten mosquitoes collected from the Apalachicola, FL location in 2012.

Apalachicola, FL (2012)			
	Head Area (μm2)	Siphon Area (μm2)	Total Area (μm2)
1	7037.2	996.0	8033.2
2	8995.9	924.0	9919.9
3	7125.1	956.3	8081.4
4	7270.0	846.0	8116.0
5	8485.4	787.5	9272.9
6	9896.0	1071.0	10967.0
7	8372.3	956.3	9328.6
8	8015.8	1044.0	9059.8
9	8078.6	770.6	8849.2
10	8293.8	900.0	9193.8
Avg	8157.0	925.2	9082.2
SD	884.0	101.1	910.1
SE	279.5	32.0	287.8

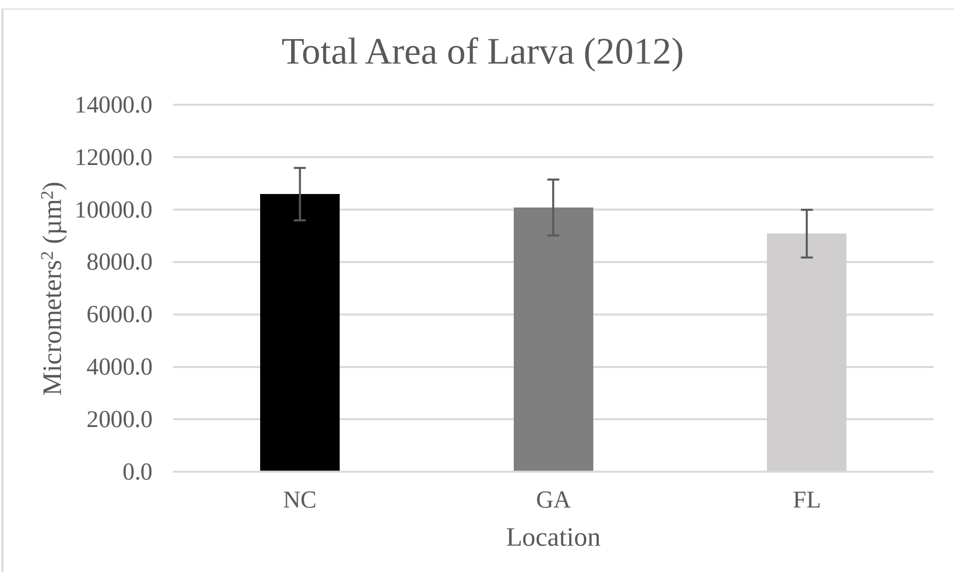

Figure 8. Average total area comparison of the mosquitoes from each location from 2012.

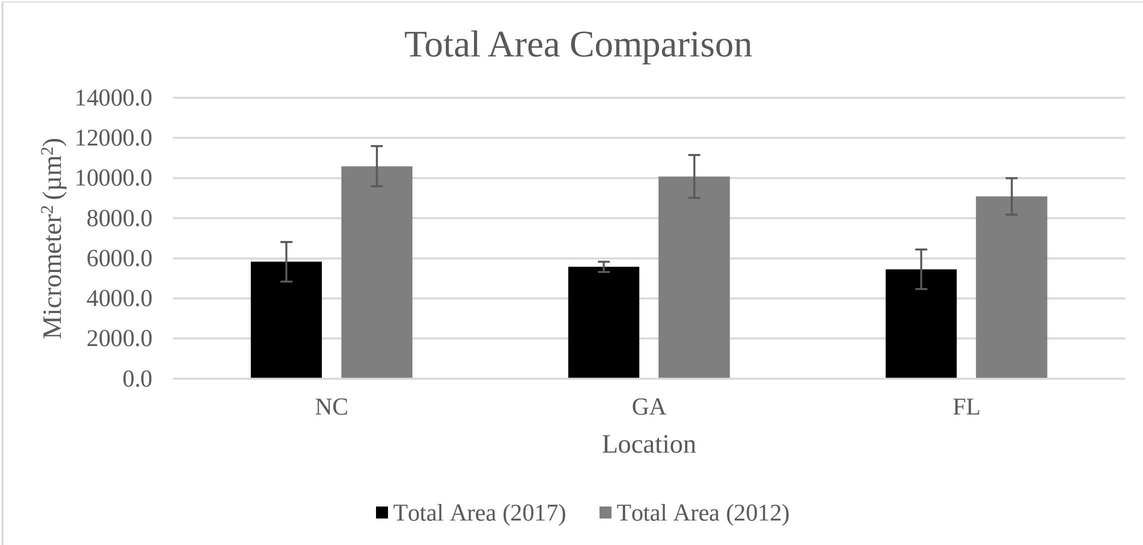

Figure 9. Total area comparison, in micrometers^2 (µm^2), of mosquitoes from the NC, GA, and FL locations from 2017 and 2012.

Table 17. Values for the measurements of the mosquitoes from the 1940s.

1940s Mosquitoes				
	Head Length (µm)	Head Width (µm)	Siphon Length (µm)	Siphon Width (µm)
Kingston, RI (04/05/1949)	56.0	63.0	52.5	14.0
Kingston, RI (04/15/1949)	61.0	56.0	65.0	13.0
Columbia, SC (07/13/1946)	79.0	76.0	75.0	19.5
Boca Raton, FL (09/06/1947)	72.0	85.0	109.0	16.0
Boca Raton, FL (09/06/1947)	79.0	89.0	113.0	17.0
Boca Raton, FL (09/06/1949)	79.5	88.0	113.0	16.0
Boca Raton, FL (09/06/1949)	80.0	95.0	95.0	22.5
Boca Raton, FL (09/06/1949)	76.0	86.0	96.5	20.0
Boca Raton, FL (09/06/1949)	92.0	80.0	96.0	21.0

Table 18. Calculations for the areas of the mosquitoes from the 1940s.

1940s Mosquitoes			
	Head Area (µm2)	Siphon Area (µm2)	Total Area (µm2)
Kingston, RI (04/05/1949)	2770.9	551.3	3322.1
Kingston, RI (04/15/1949)	2682.9	633.8	3316.7
Columbia, SC (07/13/1946)	4715.5	1096.9	5812.4
Boca Raton, FL (09/06/1947)	4806.6	1308.0	6114.6
Boca Raton, FL (09/06/1947)	5522.1	1440.8	6962.9
Boca Raton, FL (09/06/1949)	5494.6	1356.0	6850.6
Boca Raton, FL (09/06/1949)	5969.0	1603.1	7572.1
Boca Raton, FL (09/06/1949)	5133.4	1447.5	6580.9
Boca Raton, FL (09/06/1949)	5780.5	1512.0	7292.5