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The influence of immature density and nutrient addition on adult *Culex quinquefasciatus* weight, emergence, and sex ratio in Atlanta, GA

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An abstract of
A thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

Environmental factors were manipulated to evaluate the effects on the development of mosquito, *Culex quinquefasciatus*. A study was designed to examine how time of year, food, and larval density influenced development as assessed through emerged adult weight and sex ratio. Immature mosquito larva density was manipulated during two seasons to determine the resulting adult mosquito weight. Development of adults from oviposited egg rafts was only successful in one of the two experimental breeding seasons due to weather constraints. In the summer, the addition of dog food nutrients to larval water increased oviposition, and when density of egg rafts was controlled, resulted in greater numbers of emerged adults. Elevated concentration of phosphate introduced at the beginning but not the end of the development period increased the dry weight of emerged mosquitoes. Both dog food and ammonia increased the ratio of females to males; phosphate had the opposite influence on sex ratios. However, the emerged dry weight of adult mosquitoes was not different between the nutrient-enhanced and unaltered stream breeding waters. In containers with higher densities of emerged adults, the dry weight of emerged mosquitoes was reduced. There is a balance between oviposition levels and the ability of nutrients to support growth, and an interaction between egg raft density and differential survival of males and females that is affected by nutrient levels. *Culex quinquefasciatus* are vectors of West NileVirus (WNV), and thus understanding mosquito population dynamics can lead to a better understanding of vectorial potential in urban areas.

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Introduction

Atlanta, Georgia has a combined sewer overflow (CSO) system, in which stormwater and domestic sewage are mixed and treated together. During dry and moderately wet conditions, domestic wastewater and stormwater are mixed and piped together to a treatment facility. However, after heavy precipitation, large volumes of stormwater in combination with wastewater exceed the maximum capacity of the sewer systems and are expelled into nearby natural streams with only minimal treatment. The Atlanta area contains seven CSO facilities that store, minimally treat, and release large amounts of water when the water volume exceeds what the pipes to water treatment plants can accommodate. Many of the Atlanta CSO facilities are located in residential areas with high population densities. The overflow pulses carry high levels of nutrients, which provide ideal breeding conditions for certain mosquito species including *Culex quinquefasciatus* (CALHOUN et al., 2007). Elevated nutrients in the water have been found to increase the number of egg rafts oviposited by *Cx. quinquefasciatus* (BENTLEY and DAY, 1989). Oviposition is additionally enhanced in habitats containing water specifically from combined sewer overflows (CHAVES et al., 2009).

Culex quinquefasciatus is the most common vector in the southeastern United States for West Nile Virus (WNV), a mosquito-borne flavivirus that was first reported in the West Nile region of Africa (TURELL et al., 2001). In 1999, WNV was first detected in the U.S. in an outbreak in New York City. After its emergence, WNV spread all over the U.S. causing epidemics in several urban centers (TURELL, SARDELIS, DOHM and O'GUINN, 2001). Mosquitoes serve as vectors of the disease and include avian hosts in the primary transmission cycle. Female mosquitoes, the only sex capable of taking a blood meal, bite infected birds, contain the virus in their salivary gland, and then can transmit the virus to other birds or hosts. Birds are the main amplifying hosts; once infected, the virus replicates in their body and are capable of transmitting the virus to other mosquitoes that bite them. Mosquitoes may also infect larger mammals such as humans when taking blood meals, but these hosts are too large to be infectious to other biting mosquitoes.

The *Culex* mosquito goes through four distinct stages during its lifecycle: egg, larva, pupa, and adult. The immature stages are aquatic, while the adult mosquito is terrestrial. In the *Culex* species, eggs are laid on the surface of water in groups of hundreds or more called rafts. Most eggs hatch within 48 hours into larvae, which go through four larval instars. Larvae feed on micro-organisms and organic material in the water and development is dependent on temperature and the nutrient available. The fourth molt of the larva changes into a pupa; pupae do not feed, but rather this stage is a resting phase where the mosquito turns into an adult. Adults may again feed, this time on sugar from plant sources. Female mosquitoes must obtain a bloodmeal in order to produce viable eggs.

Previous research has found that the body size of a mosquito determines vectorial capacity through altering its susceptibility to infection (NASCI, 1986). In addition, once a mosquito is infected, body size affects its survivorship and potential to transmit the pathogen to other susceptible hosts. In a study of *Culex* conducted by Baqar et al. (1980), large females were not as likely as small females to be infected with WNV (BAQAR et al., 1980). It was proposed that small females ingest a proportionally larger infectious blood meal than larger mosquitoes, and therefore are exposed to a larger WNV load. Large-bodied mosquitoes contribute more to the maintenance and amplification of pathogens in mosquito-borne disease cycles than small-bodied individuals of the same species (BAQAR, HAYES and AHMED, 1980). Therefore, the distribution of body size in a mosquito population may be used as an indicator of vector potential. In this study, we aimed to understand the varying size in the *Cx. quinquefasciatus* mosquito to better understand its role as a vector.

The size attained by adult mosquitoes is attributed to life history traits including immature larval stages and the environmental events occurring within the larval habitat. Two possible environmental factors could limit growth: inadequate food resources, which may be due to competition, and temperature (CARPENTER, 1982; FISH, 1984). Studies that manipulated the *Culex* larval environment have shown that overcrowding and competition resulted in slower development rate, increased mortality, lighter dry weight, and lower fecundity (AGNEW et al., 2000; RAJAGOPALAN et al., 1976; REISKIND et al., 2004). Adult female size is also directly correlated with the size of the egg raft oviposited (BOCK and MILBY, 1981). Therefore, the size of the mosquito can influence the population growth rate as a whole. The starved dry weight of females, as proposed in this study, is sensitive to the effects of larval density because it is a fixed trait that pertains directly to the metabolic reserve acquired during larval growth (AGNEW et al., 2000). Lipid and glycogen reserves are higher in adults exhibiting larger dry weights (NAYAR and SAUERMAN, 1970).

Mosquito development is significantly enhanced in nutrient enriched waters (MOGI and OKAZAWA, 1990). Nutrient addition accelerates mosquito larval development as well as increase the survival of larvae, and the proportion of larvae that pupate (REISKIND, WALTON and WILSON, 2004). Specifically, phosphate concentration exerts a positive influence on late aquatic stages of mosquito larvae (SUNISH and REUBEN, 2001).

Various densities of mosquitoes in nutrient supplemented water and creek water were examined in this study. Other studies have shown no evidence for developmental delay of mosquito larvae due to overcrowding in natural conditions. Overcrowding effects can occur through food exploitation, chemical interference, and behavioral interference. Supplemental food does not raise survival and only accelerates development when the breeding water remains clear (MOGI and OKAZAWA, 1990).

In addition, temperature is a major factor affecting the size of adult mosquitoes (BOCK and MILBY, 1981). A California study of *Culex* found a negative correlation between the mean monthly wing length of resting male and non-bloodfed female mosquitoes and mean water temperature (BOCK and MILBY, 1981). Mosquito body size is typically found to be larger in cooler water and these larger mosquitoes fare better during stressful situations due to their ability to maintain increased nutrient reserves (DAY et al., 1990).

Mosquito breeding occurs throughout the year in Atlanta, Georgia, and thus through seasonal temperatures, resulting in a range of different mosquito sizes. A study done in India showed a clear tendency of seasonal change in survival rate of immature *Culex* larvae (RAJAGOPALAN et al., 1976). The carrying capacity in wells is reported to change seasonally; a very low carrying capacity in wells during a cold season (water temperature 18-24 C) resulted in heavy mortality at larval stages (RAJAGOPALAN, YASUNO and MENON, 1976). In another study, the mean size of the warmer air temperature July population of *Aedes vexans* was significantly smaller than the cooler temperature September population (FISH, 1984). The July population was also more variable in size compared to the September population (FISH, 1984). In addition, the length of time required for an *Aedes* mosquito to complete larval development is temperature dependent and development is more rapid at higher temperatures (NAYAR and SAUERMAN, 1968). The temperature in Atlanta, Georgia differs significantly between the summer and fall, and previous research indicated that the *Culex* mosquito development and transmission of disease will be different during these times.

In our study, we examined the effects of temperature, density, and nutrient addition on the total number, sex, and dry weight of emerged mosquitoes. We predicted that adult mosquito weight and immature larval density would be inversely related due to competition for space and nutrient resources. In addition, due to warmer weather in the summer, we predicted that mosquitoes would be smaller than those collected in the fall, and size will decrease with density. In containers where more egg rafts are initially oviposited, even after experimental redistribution of egg rafts, proportionally more mosquitoes will emerge due to female mosquito selection of maximum fitness environments.

Methods

Study site:

Tanyard Creek, located in a residential area of Northwest Atlanta, is a combined sewer overflow (CSO) stream in which the Tanyard Creek CSO wastewater facility often expels minimally treated water (Fig. 1). The site selected for the experiment was near Ardmore Park approximately 1260 m downstream from the Tanyard Creek CSO facility. The study was set on the banks of Tanyard Creek to ensure that experimental containers were not disrupted by creek flooding.

Procedure:

The study was conducted from July 10-29, 2009 to sample the summer breeding season and October 3-22, 2009 for the fall breeding season. The procedure in both parts of the study consisted of filling 28 19.92-liter dark blue Rubbermaid containers with 6 liters of Tanyard Creek water. Half of the containers were supplemented with 24g dog food (20% protein content) which is found to be an oviposition attractant (CHAVES, KEOGH, VAZQUEZ-PROKOPEC and

KITRON, 2009). Water supplemented with nutrients was integrated throughout the group of containers (Fig. 2).

In both experiments the 28 containers were arranged as seen in figure 2 and remained covered for 6 days with fine mesh to prevent mosquito oviposition. The 6-day period is required for the water to age so it may be an attractive breeding site for *Culex*. After the aging of the water, containers were opened for 1 day to allow for mosquito oviposition. After this time, the oviposited egg rafts were recorded and removed. Removing the oviposited eggs from the first day of the uncovered containers controls for several factors that can influence oviposition choice including chemical cues and the presence of microorganisms (BENTLEY and DAY, 1989). The containers were then left open for another 24 hours. The egg rafts were counted, collected, and redistributed in matched pairs. The number of egg rafts were collected, ordered ascendingly by the total number of egg rafts in each container, and then re-distributed across the containers randomly choosing one container with and without nutrient enrichment sequentially. The specific distribution that was used controlled for variability across the two types of habitat. During the growth period for the mosquitoes, the containers were covered by a clear plastic tarp to prevent the containers from overflowing with water during heavy rains.

After 11 days, adult mosquitoes were collected from each container using a mosquito aspirator (VAZQUEZ-PROKOPEC et al., 2009). Collected mosquitoes were placed in a -6°C refrigerator. Once killed, they were placed on petri dishes, sexed, and counted. Samples were stored in -6°C until all samples were collected. Specimens were placed in a 40°C incubator for 72 hours to dry. Once dried, they were identified and only the mosquitoes belonging to the *Culex* genus were weighed (Fig. 3, 4).

To assess the influence of seasonality on the density dependent development, the experiment was conducted during July and October in order to sample both the summer and fall breeding periods. The spring breeding period was not sampled because the study species *Culex quinquefasciatus* is not common during this time and is replaced by a related species, *Culex restuans* (Vazquez-Prokopec, unpublished results).

Water Samples

The water from each container was analyzed for ammonia and phosphate concentrations after the 6 days of aging and at the end of the adult mosquito collection. Water samples were measured quantitatively with CHEMetrics V-2000 multi-analyte photometer for ammonia (range .50- 7.00 ppm) and phosphate (range .30-8.00 ppm). Previous research indicated that concentrations of these nutrients were involved in oviposition preference and were examined in this study to determine influence on larval growth (BEEHLER and MULLA, 1995).

Statistical Analysis

An analysis of the co-variance (ANCOVA) was performed for the number of egg rafts oviposited as a function of nutrient addition and phosphate and ammonia concentrations before and after the experiment. The addition of dog food was a categorical variable, while all of the other variables were continuous; the combination of two types of variables calls for the use of an ANCOVA.

The effect of nutrient addition including phosphate and ammonia concentrations on the total number of emerged adult mosquitoes was studied using a binomial generalized linear model (BinGLM). The negative binomial test was used because the data were in counts and over-dispersed.

An Akaike information criterion (AIC) value computation was used to analyze the emerged adult mosquito weight as a function of nutrient addition, phosphate and ammonia concentrations before and after the experiment, natural egg raft oviposition, and experimental redistribution of egg rafts. The AIC selected for the variables which were of the highest importance to adult weight. A bootstrap analysis was performed to calculate the significance of the variables determined to be of highest importance. For this analysis we used a linear mixed effects model to account for the lack of independence associated with individuals emerging from the same container (VENABLES and RIPLEY, 2002).

Results

The oviposition portion of the October study was successful, but the egg rafts did not hatch, and thus there were no emerged adults. Egg rafts did not hatch at any density or in any container. The following results are from the July study.

Significantly more eggs rafts were oviposited on water in containers supplemented with dog food, relative to containers with only stream water ($P<.05$, Tbl. 1, Fig. 5). Experimental egg rafts were distributed successfully to allow for distribution across the two types of habitat (Fig. 1). Specifically, more egg rafts were oviposited in containers with higher initial phosphate concentrations in the breeding water ($P<.05$, Tbl. 1). The number of egg rafts oviposited were not significantly related to the concentrations of ammonia in the breeding water before and after the experiment (Tbl. 1). A negative binomial generalized linear model analysis revealed that the natural levels of oviposition in containers, both on the day of the experiment and the day preceding the experiment, did not significantly influence the total number of emerged adult mosquitoes from those containers (Tbl. 2).

Significantly more adult mosquitoes emerged from containers supplemented with dog food compared to those without nutrient addition (P<.05, Tbl. 2, Fig. 6). Figure 3 illustrates the resultant higher concentrations of ammonia and phosphate in the breeding water throughout the experiment in the containers supplemented with dog food (Fig. 7). Although phosphate and ammonia concentrations were not significantly related to the total number of emerged adult mosquitoes individually, their concentrations are both elevated in the dog food addition treatment and are thus significant (Tbl. 2).

A binomial generalized linear model was used to analyze the effects of nutrients and the distribution of egg rafts on the emerged mosquito sex ratio. The addition of nutrients to experimental containers decreased the proportion of emerged males by 99%. Pre-experiment phosphate concentration increased the proportion of males by 36% for each additional mg/L (P<.01, Tbl. 3). The phosphate concentration after the experiment increased the proportion of males by 4% for each additional mg/L (P<.01, Tbl. 3). The ammonia concentration before and after the experiment decreased the proportion of males by 10% for each additional mg/L (P<.01, Tbl. 3). The natural egg rafts oviposited the day before the experiment increased the proportion of males by 69% for each additional egg raft (P<.01, Tbl. 3). The natural egg rafts the day of the experiment increased the proportion of males by 7% for each additional egg raft (P<.01, Tbl. 3). The experimental redistribution of egg rafts decreased the proportion of males by 9% for each additional raft added to a container (P<.01, Tbl. 3).

Of the 1,638 adult *Cx. quinquefasciatus* mosquitoes collected in 21 containers, the average adult female dry weight was determined to be .882 mg (SE 3.5 mg), while males averaged .549 mg (SE .44 mg) (P<.01, Tbl.4, Fig. 8). The weight of adult mosquitoes increased by an average of .058 mg (SE .24 mg) with each additional mg of phosphate in the breeding water before the experiment started (P<.05, Tbl. 4, Fig. 9). The weight of adult mosquitoes decreased by an average of .0067 mg (SE .032 mg) with each additional adult mosquito that emerged from individual containers. The container and individual variabilities were .081 and .072 respectively, indicating more variability at the scale of the container (Tbl. 4). The average emerged adult dry weight was not significantly different in treatments with and without added nutrients (Fig. 8). There was more variability in the adult weights of emerged mosquitoes that developed in water supplemented with nutrients (Fig. 8).

Discussion

The observation that none of the October oviposited egg rafts resulted in visible larval instars indicates that the experimental densities and containers had no influence on mortality. Possible explanations include a frost in October, cooler than average temperatures, as well as heavy rains and flooding at the end of September. Cooler temperatures and frosts could have prevented the development of immature larvae from the egg rafts oviposited. In addition heavy rainfall could have washed out a majority of the immature larvae in Tanyard Creek prior to the study, thus decreasing the population of adults during the fall study. In hindsight, it would have been better to conduct the experiment earlier in the fall, but the constraints and effects of heavy rain and cooler temperatures cannot be controlled. As a result of the failure of the October sampling portion of the experiment, no seasonal comparisons can be made. However, the data collected from the July study are still relevant and the analysis follows below.

The addition of dog food to the oviposition traps increased the number of egg rafts oviposited by *Culex quinquefasciatus* in this experiment and confirmed previous results showing that nutrients augment raft densities (BENTLEY and DAY, 1989; CHAVES, KEOGH, VAZQUEZ-PROKOPEC and KITRON, 2009). Female mosquitoes preferentially oviposit in areas where the fitness of their offspring is optimized (MANGEL, 1987). This behavior may have an evolutionary basis due to the fact that larvae are more likely to develop faster in breeding habitats with elevated nutrients. It should be noted that oviposition is additionally enhanced in habitats containing water from combined sewer overflows (CSOs) (CHAVES, KEOGH, VAZQUEZ-PROKOPEC and KITRON, 2009). These data show evidence of the selection for polluted water by a vector of WNV. Thus, standing water with added nutrients are more likely to breed WNV positive vectors. Of these added nutrients, our study determined that higher phosphate concentration in the water was an oviposition attractant. Ammonia concentration was not a significant oviposition attractant alone, but concentrations were elevated in the containers with dog food, and thus combined with other nutrients, ammonia may be an attractant.

The addition of dog food nutrients to the breeding water was also found to increase the total number of emerged adult mosquitoes when immature density was controlled. Nutrient addition has been found to accelerate mosquito larval development and increase the survival of larvae and the proportion of larvae that pupate (REISKIND, WALTON and WILSON, 2004). Both ammonia and phosphate concentrations in the breeding water were elevated with the addition of dog food, and thus either or both may have contributed to the total number of emerged mosquitoes. Previous studies have found that phosphate concentration simulates pupation rate and total pupation of mosquito larvae (CARPENTER, 1982). The results of our study show that phosphate alone may not be responsible for the increase in emerged adults.

The natural numbers of oviposited egg rafts both on the day of the experiment and the day preceding the experiment did not significantly influence the total number of emerged adult mosquitoes from those containers. Thus, female mosquito oviposition preference did not impact the total number of emerged adults. This result is contrary to previous research stating that females can distinguish habitats that optimize the fitness of their offspring (MANGEL, 1987). Females may select for habitats in which their offspring may develop at a faster rate, but the proportion of emergence, or survival, may not be optimized.

The addition of nutrients to the breeding water greatly increased the percentage of females that emerged. This may be a response to a productive environment for the development of mosquitoes. The skewed sex ratio towards the production of females can exponentially increase the mosquito population numbers where the capacity of growth is not limited by nutrients. The sex ratio may also be due to differential male/female mortality rates (AGNEW, HAUSSY and MICHALAKIS, 2000). The increase in the proportion females, capable of taking bloodmeals and transmitting WNV, in nutrient rich waters may explain the high prevalence of the disease in polluted urban areas.

The initial phosphate concentration in the breeding water produced a male-biased sex ratio. Phosphate concentration has been shown to be an important factor in the development of mosquito larvae (CARPENTER, 1982; PIYARATNE et al., 2005; SUNISH and REUBEN, 2001), but its effects on sex ratio have not been thoroughly examined. Elevated phosphate concentration introduced at the end of the experiment had a lesser increase in the emerged proportion of males. Thus, elevated phosphate concentration is more important for regulating sex ratio at the beginning of development. Interestingly, increases in ammonia concentration increased the proportion of females that emerged. Females may be more tolerant of high ammonia concentrations than males. Ammonia has been found to be one of the primary toxic compounds limiting *Cx. quinquefasciatus* from developing in environments containing high concentrations of waste material (IKESHOJI, 1965). The concentration of ammonia before and after the larval development influenced the sex ratio in exactly the same percentage before and after. The ammonia concentrations changed over the course of the experiment but still produced the same sex ratio, showing that its toxic effects were consistent throughout the experiment.

The water where females oviposited egg rafts at the start of the experiment and the day before the experiment increased the proportion of males by 69% and 7% respectively. This indicates that females laid eggs in water that was conducive to the growth and development of male larvae. Previous research has found that females oviposit in areas in which the fitness of their offspring is maximized (MANGEL, 1987). Male larvae may be more efficient at utilizing the resources available or more competitive than females, and therefore emerge in greater proportions than females in 'maximized fitness' environments.

The dry weight of emerged females was significantly more than the male weight in all trials and is consistent with previous research (AGNEW, HAUSSY and MICHALAKIS, 2000; REISKIND, WALTON and WILSON, 2004). Sexual differences in weight are inherently fixed and not due to faster or slower development of either sex as affected by rearing environmental factors (NAYAR and SAUERMAN, 1970). Even when reared in isolation, adult females are considerably heavier than males (AGNEW, HAUSSY and MICHALAKIS, 2000).

The average emerged adult dry weight was not significantly different between treatments with and without added nutrients. More adults may have emerged from the nutrient-supplemented water, but they did not emerge with larger weights. This finding is consistent with a study done with *Cx. quinquefasciatus* in Puerto Rico where mosquitoes that developed in septic tanks and surface containers did not differ in size (MACKAY et al., 2009). This could be explained by competition or mechanical interference associated with more larvae that were able to survive in nutrient-rich waters (MOGI and OKAZAWA, 1990). The similar weights of adults in treatments with and without added nutrients show that nutrients do not affect adult size, but rather the total number that can be supported in a specific volume of water. There was more variability in the adult weights of emerged mosquitoes that developed in water supplemented with nutrients, which may be evidence of competition and the range of fitness of larvae (AGNEW, HAUSSY and MICHALAKIS, 2000; REISKIND, WALTON and WILSON, 2004).

Higher concentrations of phosphate in the breeding water served as a developmental stimulant in that it increased the emerged dry weight of adults. Previous studies have also found that larval population growth is stimulated by phosphate (CARPENTER, 1982; PIYARATNE et al., 2005). Specifically, phosphate exerts a positive influence on late aquatic stages of mosquito larvae (SUNISH and REUBEN, 2001). Larger adult females, although less likely to become infected, are more likely to transmit WNV to other hosts once infected (BAQAR, HAYES and AHMED, 1980). This study found that phosphate increases the proportion of females that emerge and they are larger at emergence. Therefore phosphate is a key nutrient to the development of mosquitoes capable of transmitting WNV. Minimizing phosphate concentrations in breeding environments may result in a lower prevalence of WNV.

Density was a significant factor limiting larval development because the average dry weight of adults decreased with each additional adult mosquito that emerged. This is not a surprising result because increased density leads to more competition and mortality among larvae (AGNEW, HAUSSY and MICHALAKIS, 2000). However, increased nutrient input has been found to decrease density-dependent reductions in growth (REISKIND, WALTON and WILSON, 2004). Therefore nutrient concentrations in breeding environments are paramount to the regulation of development of *Culex* mosquitoes.

Environmental factors such as phosphate concentration in the water can be reduced and thus decrease the size of adult mosquitoes, limiting their vectorial capacity of WNV. In addition avoiding nutrient addition to breeding waters can eliminate the possibility of increased proportions of emerged females, capable of taking bloodmeals and transmitting WNV. This study demonstrated the importance of recognizing specific breeding habitats for the *Culex* mosquito and offers methods for altering them so as to decrease their productivity, thus decreasing the prevalence of WNV.

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Table 1. Analysis of co-variance (ANCOVA) of number of egg rafts oviposited as a function of nutrient addition and phosphate and ammonia concentration before and after the experiment.

Factor	df	Sum Sq.	Mean Sq.	F value	P-value
Nutrient Addition	1	167.38	660.24	6.45	<0.02*
Phosphate before	1	152.64	645.50	5.88	<0.05*
Ammonia before	1	2.30	495.16	0.088	0.77
Phosphate after	1	0.081	492.94	0.0031	0.96
Ammonia after	1	54.25	547.11	2.09	0.16
Residuals	19	492.86	25.94		

*statistically significant (p<.05)

Table 2. Negative binomial generalized linear model analysis of deviance of the effects of nutrient addition, phosphate (before, after), ammonia (before, after), and natural egg rafts (day before, day of experiment) on the total number of adult mosquitoes. The experimental egg rafts were offset because more individuals were expected in locations with more egg rafts. LRT: Likelihood Ratio Test

Factor	df	Deviance	LRT	P-value
Nutrient Addition	1	33.40	5.19	<0.05*
Phosphate before	1	28.71	0.50	0.48
Ammonia before	1	28.96	0.75	0.39
Phosphate after	1	28.79	0.58	0.45
Ammonia after	1	28.70	0.49	0.49
Natural Egg Rafts (Day Before)	1	28.59	0.38	0.54
Natural Egg Rafts (Day of Exp.)	1	0.023	1.92	0.17

Table 3. Binomial generalized linear model analysis of deviance of the effects of nutrient addition, phosphate (before, after), ammonia (before, after), natural egg rafts (day before, day of experiment), and experimental egg rafts on the sex ratio of adult mosquitoes. The odds ratio is presented as the proportion of males that increase with the addition of each additional mg of nutrient or each additional egg raft added.

Factor	Odds Ratio	df	Deviance	LRT	P-value
Nutrient Addition	0.0024	1	73.8	37.11	P<.0001*
Phosphate before	1.36	1	79.3	42.57	P<.0001*
Ammonia before	0.90	1	46.7	10.02	P<.01*
Phosphate after	1.04	1	41.0	4.31	P<.05*
Ammonia after	0.90	1	65.7	29.0	P<.0001*
Natural Egg Rafts (Day Before)	1.69	1	83.2	46.49	P<.0001*
Natural Egg Rafts (Day of Exp.)	1.07	1	57.4	20.71	P<.0001*
Experimental Egg Rafts	0.91	1	79.5	42.78	P<.0001*
Residuals			36.7	115.92	

*statistically significant (p<.05)

Table 4. Parameter estimates for adult *Culex quinquefasciatus* adult weight from a hierarchical linear mixed effects model. Data consist of a total collection of 1,638 individuals from 21 containers.

Parameter	Estimate (mg)	Std. Error	P*
Female	.882	0.35	P<0.0001
Male	.549	0.04	P<0.0001
Phosphate After	0.0058	0.02	P<0.05
Total Num. of mosquitoes	-0.0007	0.003	P<0.05
Container Variability	0.081	-	
Individual Variability	0.072	-	

*Obtained by bootstrap

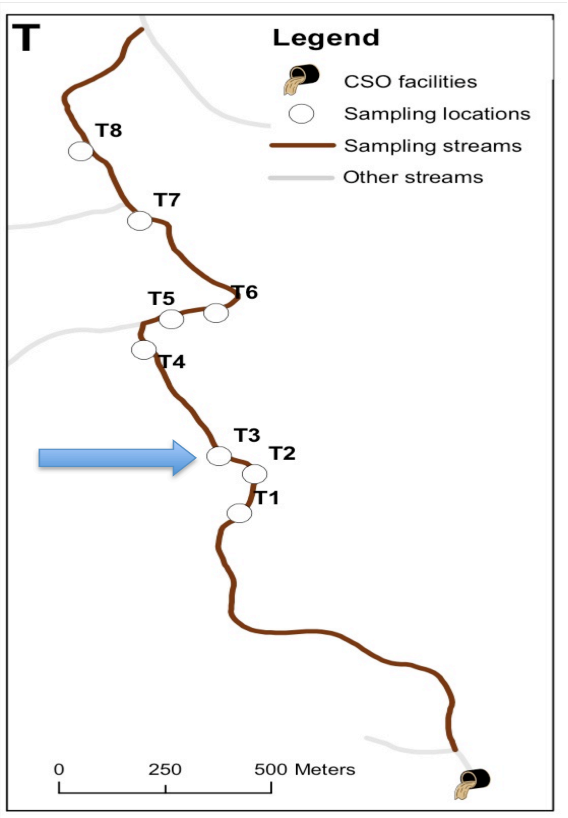

Figure 1. Map of study site at Tanyard Creek. Experimental containers were set on the bank of Tanyard Creek located at site T3 on the map, 1260m downstream of the Tanyard Creek CSO facility.

+	-	+	-	+	-	+
-	+	-	+	-	+	-
+	-	+	-	+	-	+
-	+	-	+	-	+	-

Figure 2. Experimental setup of 28 ovipostion/ breeding containers. Rectangles represent 19.92-liter dark blue Rubbermaid containers. (+) creek water plus nutrient addition (dog food), (-) creek water.

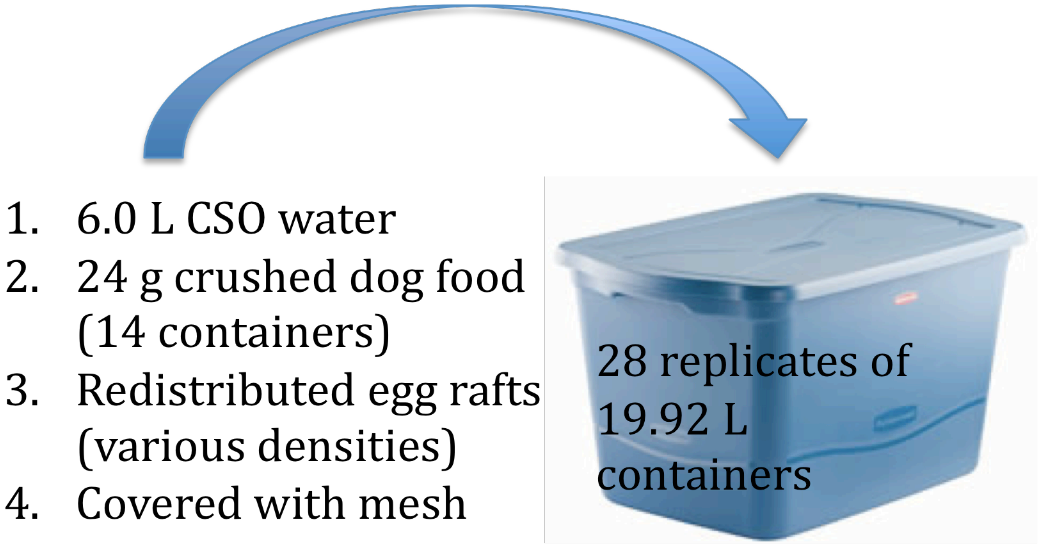

Figure 3. Contents of the mosquito breeding containers.

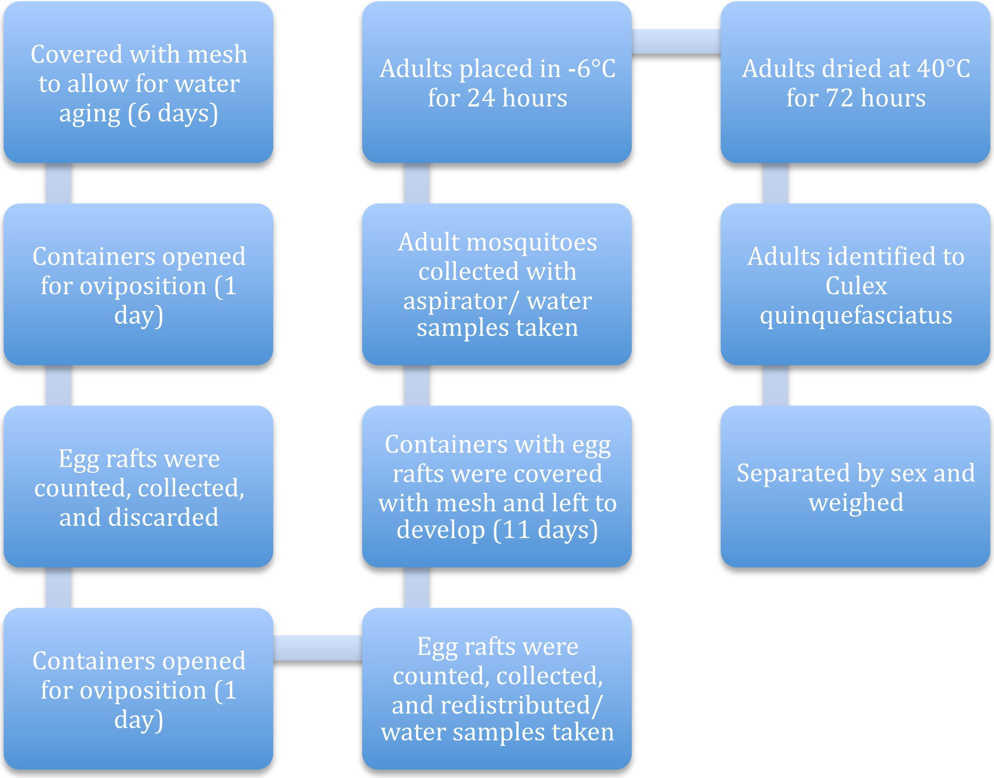

Figure 4. Flow diagram of the experimental procedure.

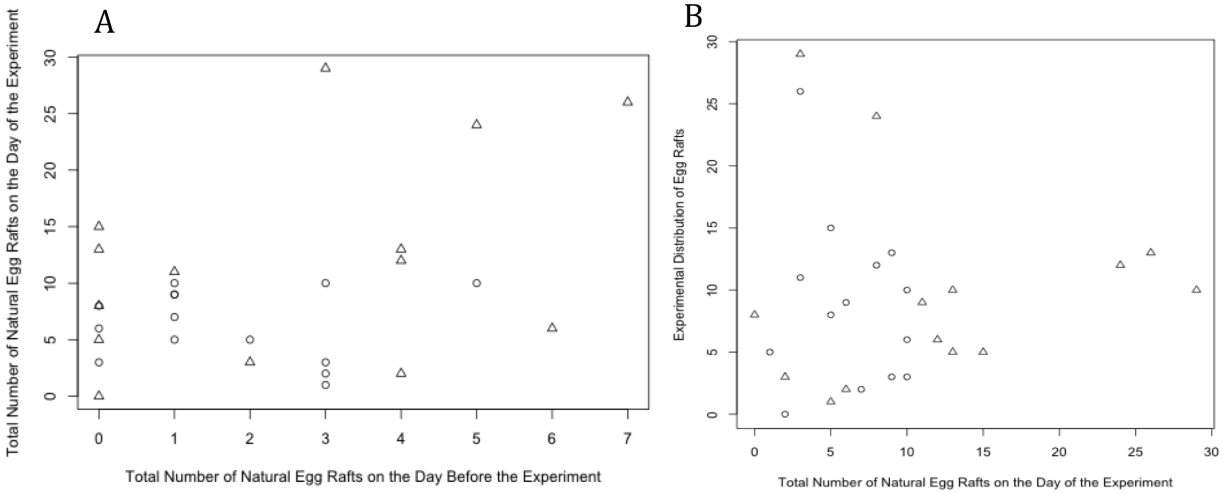

Fig. 5. Natural and experimental distribution of egg rafts. (A) The total number of natural egg rafts oviposited on the day of the experiment compared to the total number of natural egg rafts oviposited the day before the experiment. (B) The experimental redistribution of egg rafts compared to the number of egg rafts oviposited on the day of the experiment. Egg rafts oviposited in containers with creek water supplemented with nutrients are represented with triangles, while circles represent egg rafts oviposited in containers with only creek water.

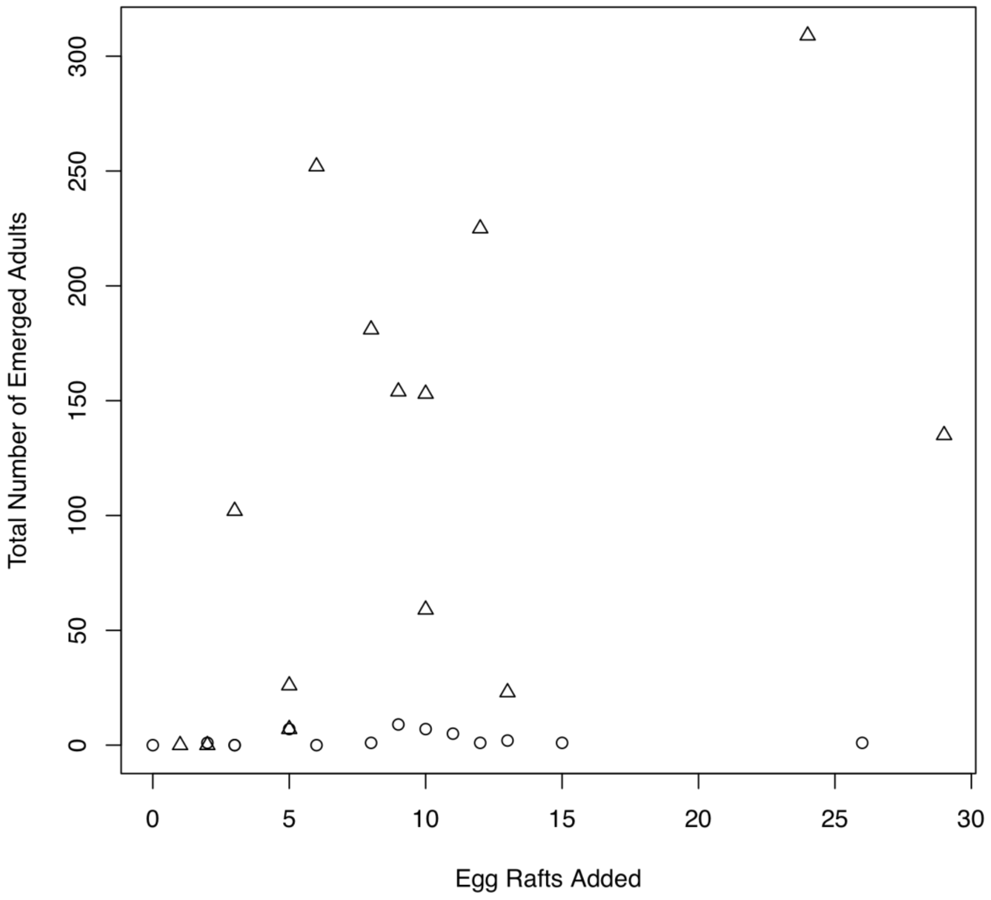

Fig. 6. The total number of emerged *Cx. quinquefasciatus* adults as a function of the number of egg rafts added to experimental containers. Adults from containers with creek water supplemented with nutrients are represented with triangles, while circles represent adults emerged from containers with only creek water.

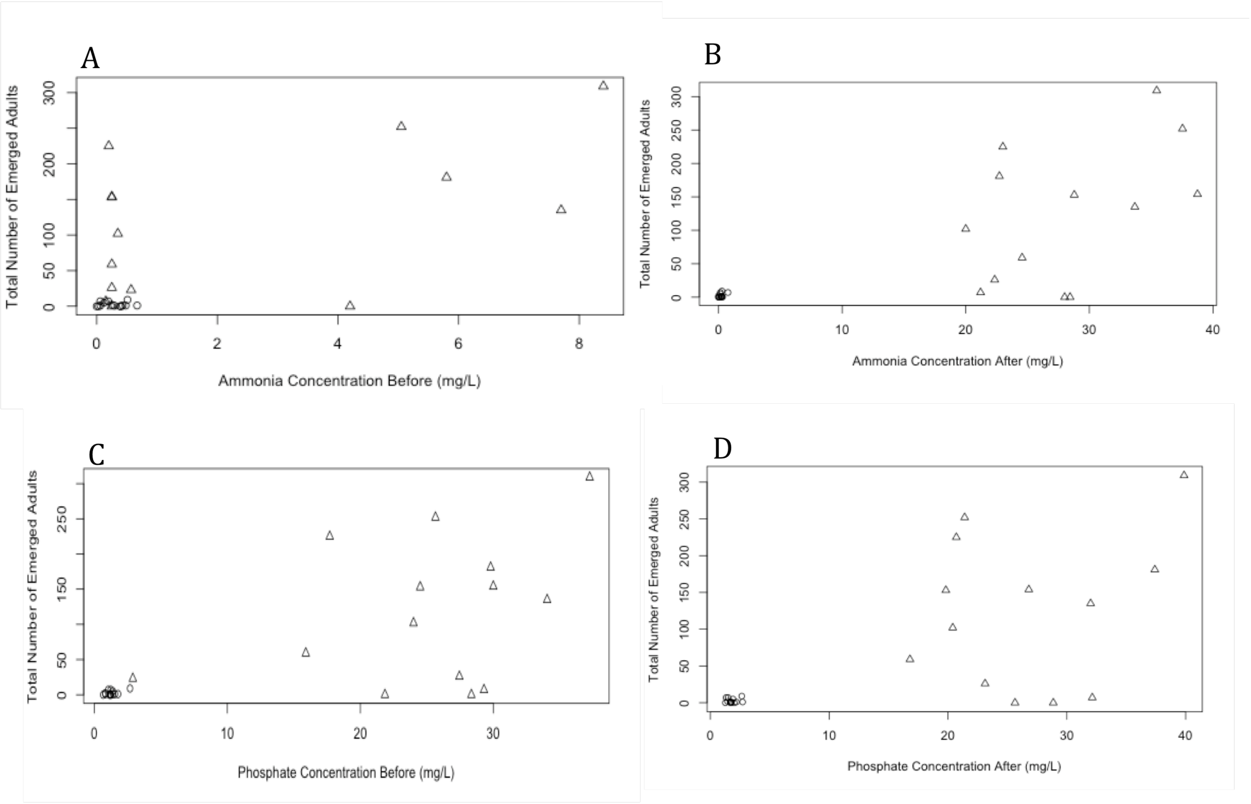

Fig. 7. The total number of emerged *Cx. quinquefasciatus* adults as a function of nutrient concentrations in the experimental rearing containers. (A) Total number of emerged adults compared to the ammonia concentration before the start of the experiment. (B) Total number of adults compared to the ammonia concentration after the experiment. (C) Total number of emerged adults compared to the phosphate concentration before the start of the experiment. (D) Total number of adults compared to the phosphate concentration after the experiment. Adults from containers with creek water supplemented with nutrients are represented with triangles, while circles represent adults emerged from containers with only creek water.

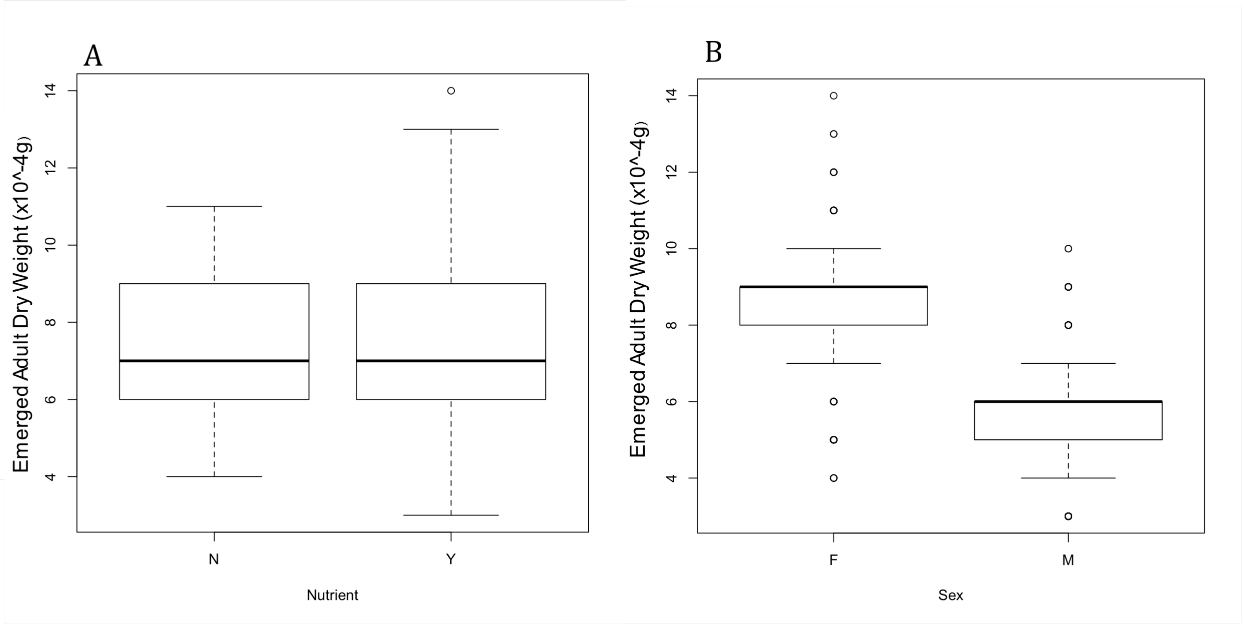

Fig. 8. Emerged adult *Cx. quinquefasciatus* dry weight (*10^{-4} g). (A) Boxplot of emerged adult *Cx. quinquefasciatus* dry weight as a function of nutrient addition to the stream water in each experimental container: N-no addition, Y-addition of dog food. (B) Boxplot of emerged adult *Cx. quinquefasciatus* dry weight separated by sex: F-female, M-male.

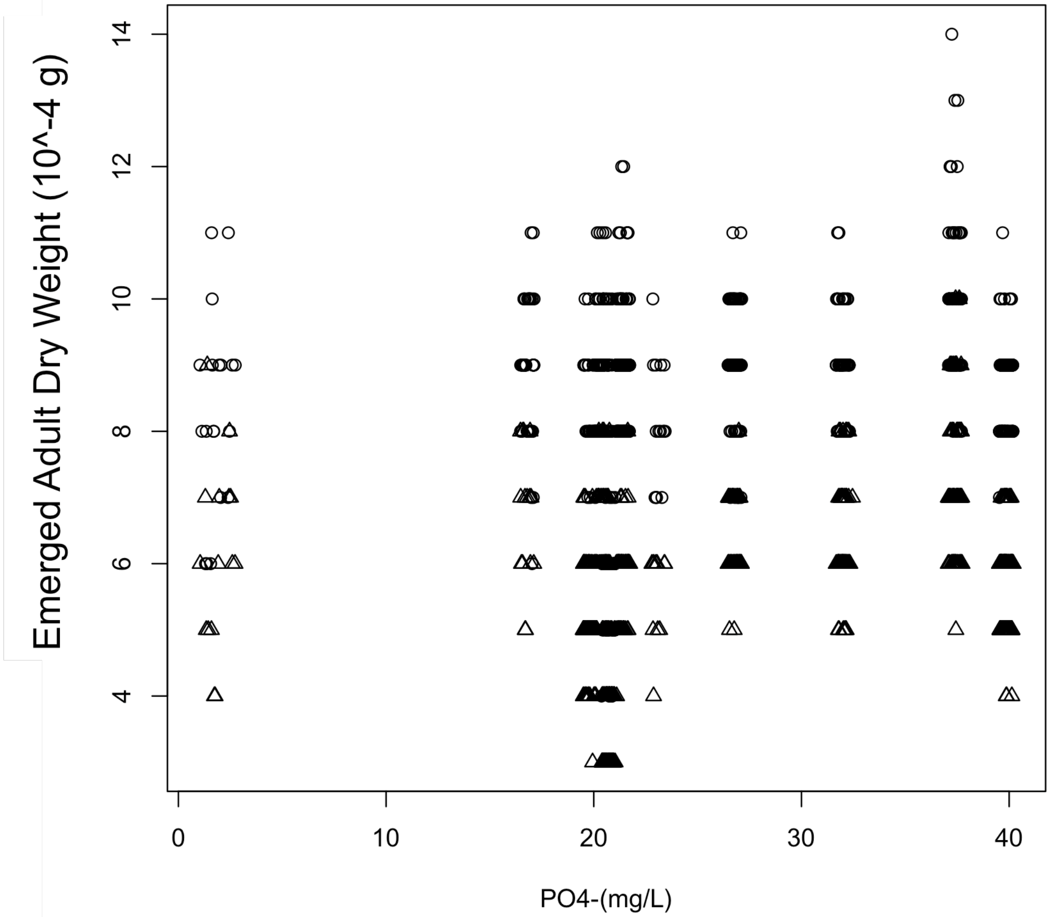

Fig. 9. Emerged adult *Cx. quinquefasciatus* dry weight (*10^{-4} g) as a function of phosphate concentration in the breeding containers before larval development. Adults from containers with creek water supplemented with nutrients are represented with triangles, while circle represent adults emerged from containers with only creek water.