Environmental Effects on Pupation of *Aedes aegypti* Larvae

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Abstract

Worldwide, the range of mosquito species is increasing at an alarming rate. One of the suspected causes for this is the changing climate, from a more temperate environment, to one that is increasingly hospitable for many arthropods. Because of this, many vector-borne diseases, such as Chikungunya, Dengue, and Yellow Fever are spreading into non-endemic areas. For this reason, it is vital to examine the effects environmental conditions have on the development of mosquito larvae, specifically the larvae of the Yellow Fever Mosquito *Aedes aegypti*. The effects of humidity, temperature, suspended nutrient availability, and nutrient density in the environment on *A. aegypti* larvae are explored, as these factors are subject to polarize as a result of human actions both globally via industry and locally via property embellishments. With this, the concerns around the effects of nutrient concentration and availability are explored, as are varying levels of artificial fertilizers and regular increases in turbidity. Additionally, the known effects of the urban environments and how these affect the larval development of the domestic forms of *A. aegypti* are also considered. It was found that turbidity was often associated with high levels of nutrient availability, which was positively correlated with pupation rates.

Keywords*:* Fecundity, Turbidity, Aedes aegypi

Introduction

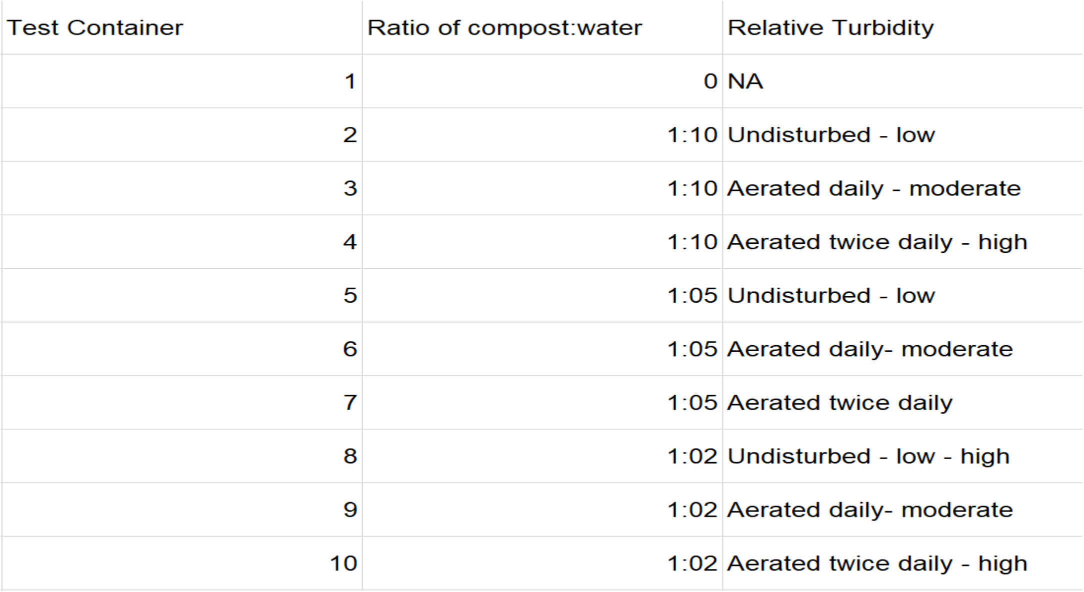
Mosquitos are holometabolous insects. Mosquitos spend the majority of their developmental in aquatic environments since eggs are laid on water, or areas that are to become flooded with water, so that when the larvae can hatch, they can develop through 4 instars. They begin life at only 1mm in length, and reach 8mm by the time the larvae pupate (Perez, 2012). The larval stage is the longest stage of development and is the only aquatic stage that feeds. The pupae have most adult features enclosed, then when the mosquito is ready, the fully formed adult mosquito will emerge and continue its life cycle. The conditions of larval development have a large impact on the size, fecundity, and health of adult mosquitos (Johnson, 2013). The time taken to complete this stage in development is highly indicative of the health of the mosquito as an inability to feed regularly as a larva will increase the needs of the adult upon eclosion. Each of these stages requires a set of conditions to continue development: temperature, humidity, nutritional availability, and space all effect the progression of mosquitos through their life cycle. These conditions are subject to change depending on the environment mosquito is living in. It is important to understand the natural weaknesses in mosquito reproduction in order to understand how human interaction either causes increases or decreases in mosquito population increases. For this reason, the focus of this paper is to observe and examine the effects of environmental conditions on the population dynamics of *Aedes aegypti* larvae. The conditions considered are humidity, temperature, suspended nutrient availability, and nutrient density in the environment. These factors are primary as they are subject to polarize as a result of human actions both globally via industry and locally via property embellishments.

Some actions of humans have historically caused mosquito control to be particularly difficult, and when these actions were remedied, control was possible. This is seen in the attempt to reduce the effect of Yellow Fever in Panama during the construction of the Panama Canal. French gardens were constructed with standing water basins as common decor. These gardens served as havens for mosquito reproduction. Walter Reed and William Gorgas worked together with scientific community and the US military to make all standing water either nonexistent or undesirable (Milian, 2012). For this reason, understanding the effects of human desires on the environment can be crucial in knowing how to best control a specific pest. Flower beds containing fertilizers create run off with high nutrient densities. On an industrial level the mass of nutrients from run off of agricultural lands has been known to cause eutrophication leading to hypoxic zones in major bodies of water due to the reaction of the ecosystems to excess nitrogen (Rabotyago, 2014). Following this same line of reasoning it is possible that collections of water enriched with nutrients from commercially available fertilizers can cause an increase in the proliferation of mosquito larvae. This is why nutrient concentration and availability provided by varying levels of fertilizer and regular increases in turbidity are of particular concern in the focus of this study.

Climate is another relevant topic in mosquito research. Disease vectors of tropical ailments such as Yellow Fever, Dengue, and Chikungunya all thrive under conditions of high humidity and high temperatures as are indicative of the tropical climate. With the changing weather patterns observed across the globe, more areas are increasingly hospitable to mosquito development, which bring with them, increasing availability of tropical disease pathogens (Thompson, 2010). Cities are known to geographers and scientists to be urban heat islands due to the entrapment of heat. Urban areas can be over 12 degrees Celsius hotter than nearby rural areas due to the reduction of air flow because of building and the absorption of heat via asphalt and concrete (EPA, 2018). These pockets of high temperatures could mean attractive habitats for mosquito populations thus increasing the interactions between humans and the pathogens associated with mosquitos. Areas near water sources, natural or manmade, are more humid due to the flow of air across bodies of water, contributing to the humidity of the environment. This is why temperature and humidity were monitored throughout this experiment and are included as major focuses in this study.

Materials and Methods

In order to simulate mosquito larval development in various differing environments, lab cultivated *Aedes aegypti* eggs were placed in quantities of approximately 100 eggs per plastic 5.7-liter plastic container (Sterilite). The containers made from plastic shoe boxes that had a mesh covered hole in the lid (roughly 30 cm x 15 cm) for air flow. The containers were then filled with water and the designated amount of substrate (Super Compost Organic Fertilizer. 2-2-2 Concentrated (EarthSoil Inc.)). The substrate type, incubation temperature, species of mosquito, food type, and water source were constant between all test containers. The first container had no substrate, with the exemption of a tsp of fish food (TetraVeggie Algae Wafers Balanced Diet for Algae Eaters, TETRA) every other day to provide nutrients. The second container had a 1 to 10 ratio of compost to water and remained undisturbed throughout the experiment, with the exclusion of extracting pupae through the use of an eye dropper and the addition of food. The third had a 1 to 10 ratio and the compost and water was stirred once daily. The remaining containers are as described in the table below.

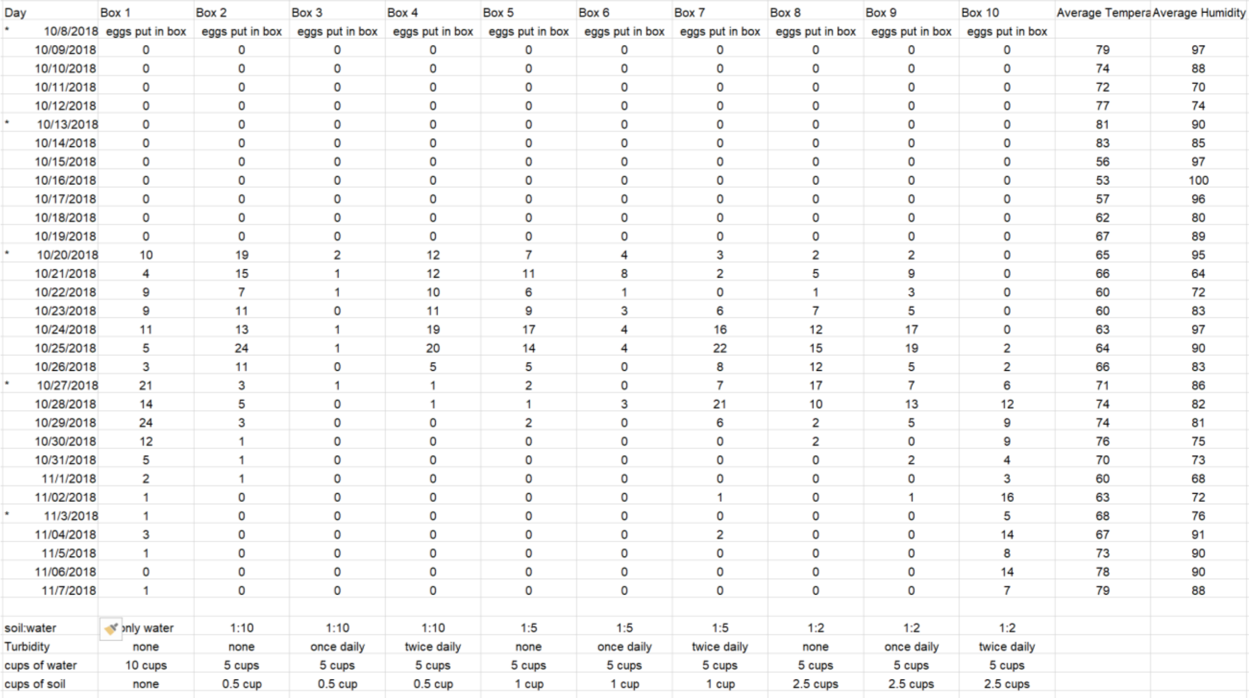


Test Container	Ratio of compost:water	Relative Turbidity
1	0	NA
2	1:10	Undisturbed - low
3	1:10	Aerated daily - moderate
4	1:10	Aerated twice daily - high
5	1:05	Undisturbed - low
6	1:05	Aerated daily- moderate
7	1:05	Aerated twice daily
8	1:02	Undisturbed - low - high
9	1:02	Aerated daily- moderate
10	1:02	Aerated twice daily - high

All of the above described systems were monitored for 30 days. The containers were not in a climate-controlled building so the data recorded included temperature, humidity, date of feeding, when pupae have developed and how many are present in each system on each day. Upon pupation the pupae were removed so to not affect the following days pupae count. The removed pupae were placed in a solution of bleach to kill the mosquito, ensuring that the lab bred insects were not introduced to the local environment. At the end of the trial all containers were terminated through the introduction of bleach to each system, killing all remaining eggs, larvae, and pupae

Results

The raw data obtained through the course of the study is indicated below. This data was obtained outdoors on the dates indicated in the left-hand column in College Station, Texas, USA. An asterisk in the date column indicates when all containers were fed. Climate information is listed at the far right, these values were obtained from Timeanddate.com and were verified via weather.gov. At the date of completion all larvae in each system had either pupated or died.



Day	Box 1	Box 2	Box 3	Box 4	Box 5	Box 6	Box 7	Box 8	Box 9	Box 10	Average Tempera	Average Humidity
* 10/8/2018	eggs put in box	eggs put in box	eggs put in box	eggs put in box	eggs put in box	eggs put in box	eggs put in box	eggs put in box	eggs put in box	eggs put in box		
10/09/2018	0	0	0	0	0	0	0	0	0	0	79	97
10/10/2018	0	0	0	0	0	0	0	0	0	0	74	88
10/11/2018	0	0	0	0	0	0	0	0	0	0	72	70
10/12/2018	0	0	0	0	0	0	0	0	0	0	77	74
* 10/13/2018	0	0	0	0	0	0	0	0	0	0	81	90
10/14/2018	0	0	0	0	0	0	0	0	0	0	83	85
10/15/2018	0	0	0	0	0	0	0	0	0	0	56	97
10/16/2018	0	0	0	0	0	0	0	0	0	0	53	100
10/17/2018	0	0	0	0	0	0	0	0	0	0	57	96
10/18/2018	0	0	0	0	0	0	0	0	0	0	62	80
10/19/2018	0	0	0	0	0	0	0	0	0	0	67	89
* 10/20/2018	10	19	2	12	7	4	3	2	2	0	65	95
10/21/2018	4	15	1	12	11	8	2	5	9	0	66	64
10/22/2018	9	7	1	10	6	1	0	1	3	0	60	72
10/23/2018	9	11	0	11	9	3	6	7	5	0	60	83
10/24/2018	11	13	1	19	17	4	16	12	17	0	63	97
10/25/2018	5	24	1	20	14	4	22	15	19	2	64	90
10/26/2018	3	11	0	5	5	0	8	12	5	2	66	83
* 10/27/2018	21	3	1	1	2	0	7	17	7	6	71	86
10/28/2018	14	5	0	1	1	3	21	10	13	12	74	82
10/29/2018	24	3	0	0	2	0	6	2	5	9	74	81
10/30/2018	12	1	0	0	0	0	0	2	0	9	76	75
10/31/2018	5	1	0	0	0	0	0	0	2	4	70	73
11/1/2018	2	1	0	0	0	0	0	0	0	3	60	68
11/02/2018	1	0	0	0	0	0	1	0	1	16	63	72
* 11/3/2018	1	0	0	0	0	0	0	0	0	5	68	76
11/04/2018	3	0	0	0	0	0	2	0	0	14	67	91
11/5/2018	1	0	0	0	0	0	0	0	0	8	73	90
11/06/2018	0	0	0	0	0	0	0	0	0	14	78	90
11/7/2018	1	0	0	0	0	0	0	0	0	7	79	88
soil:water	only water	1:10	1:10	1:10	1:5	1:5	1:5	1:2	1:2	1:2		
Turbidity	none	none	once daily	twice daily	none	once daily	twice daily	none	once daily	twice daily		
cups of water	10 cups	5 cups	5 cups	5 cups	5 cups	5 cups	5 cups	5 cups	5 cups	5 cups		
cups of soil	none	0.5 cup	0.5 cup	0.5 cup	1 cup	1 cup	1 cup	2.5 cups	2.5 cups	2.5 cups		

From this data many interesting trends were observed. The relationship between rate of pupation and humidity versus temperature was of major interest (Figure 1). Both factors are considered as highly relevant when discussing the development of mosquitos but the data indicated a higher sensitivity to humidity changes over temperature changes. This can be explained by the range in temperature fluctuations being within 30 degrees Fahrenheit. No correlation is evident between temperature and pupation but a clear correlation exists between humidity and pupation. Peaks in humidity precede peaks in pupation within 24-48 hours. Drops in humidity correlate with drops in pupation which is an observed trend in other experiments (Yamana, 2013). This trend is observed in each data set, however the vulnerability of each system to the effects of humidity varies from system to system.

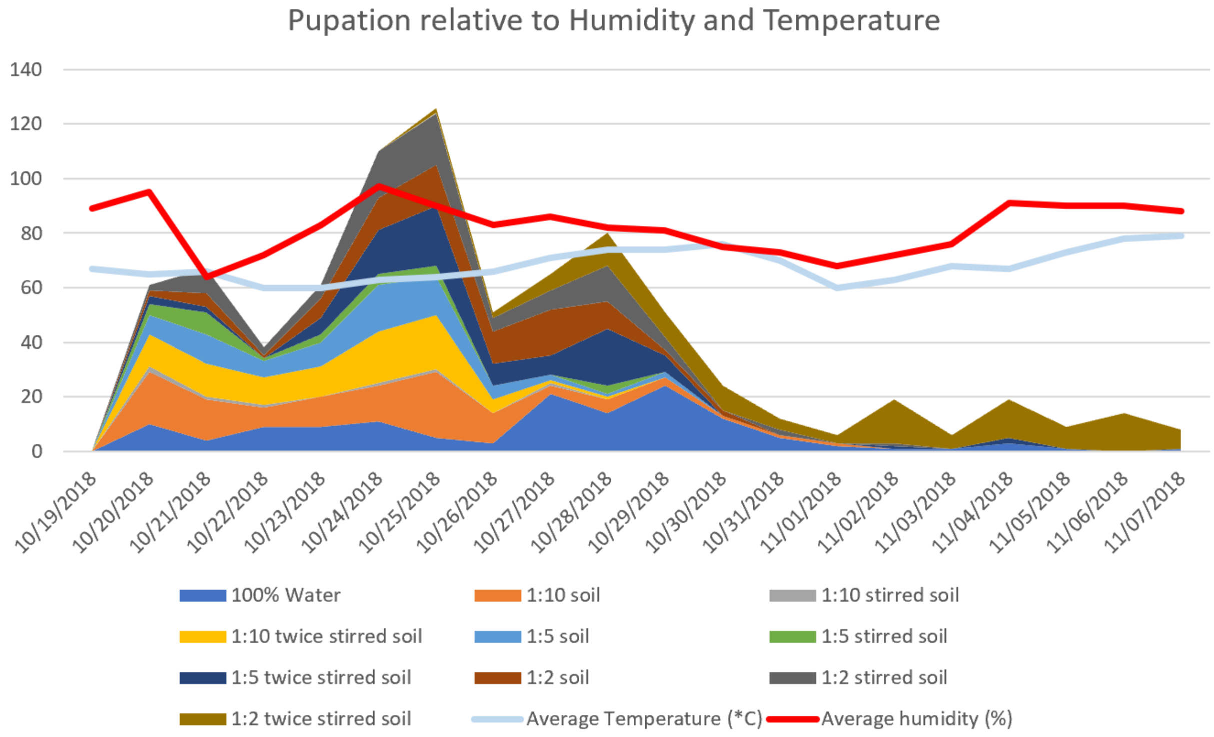


Figure 1. This stacked area graph illustrates pupae present in each system each day. The lines represent average temperature and humidity experienced at each respective day. Systems are described by their soil content and daily disturbances causing varying levels of suspended particulates.

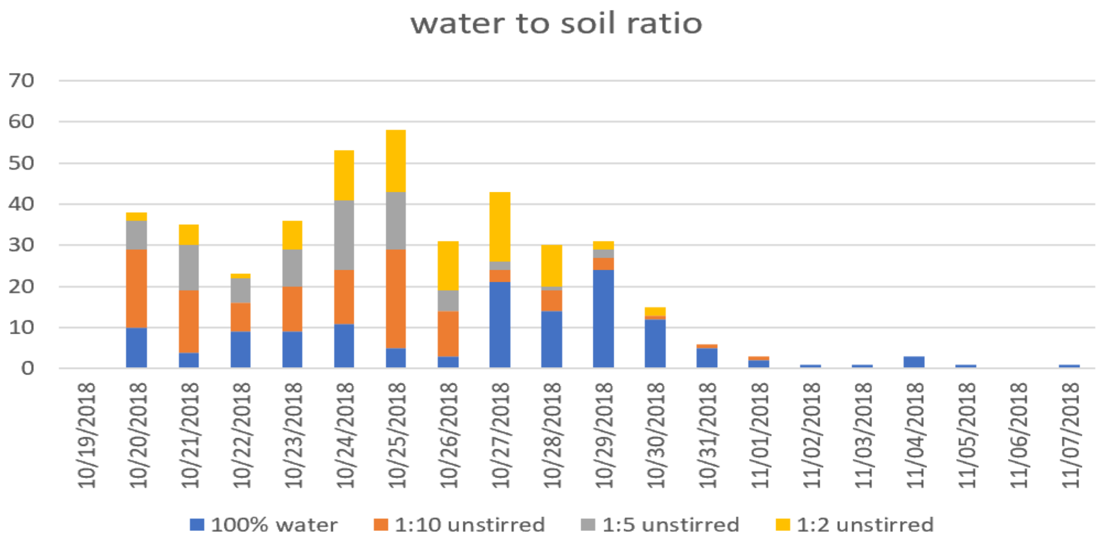


Figure 2. This stacked bar graph depicts the effects of the soil to water ratio on pupae present each day. Each system is described by their ratio of soil relative to water, in addition each system is unstirred meaning that each system is as close to neglected standing water as could be simulated in this experiment.

Based on the data in Figure 2, 100% water depends on nutritional supplementation of algae to determine the rate of pupation. The competitive atmosphere caused by limited nutrient availability causes a low rate of pupation which continues over a longer period of time than is observed in more nutrient dense systems. The positive correlation between nutrient density in aquatic environments with prolificacy of mosquitos is shown through examining areas of high disease burden by mosquito vectored diseases. Areas of India that are burdened with poor sanitation leading to high quantities of nutrients for mosquito development such as Odisha are also home to the highest distributions of malaria sufferers in India, claiming up to 40% of the nation's malaria burden (WHO, 2018). The connection between the disease burden and the sanitation are not coincidental. In containers with ample nutrition, greater quantities of larvae can feed and pupate sooner with decreases in pupation associated with poor environmental conditions only. All containers were present in the same environmental conditions thus it is reasonable to assume these conditions affect all containers in the same approximate way, as can be seen in Figure 1, where peaks and depressions in pupation of all containers coincide with decreased humidity (Duguma, 2014). The heights of each peak in the 100% water container are lower than in the nutrient dense counterparts, in addition more peaks and valleys exist in the 100% water container that are not explained by the weather and do correlate with algae feeding. This observation is depicted in Figure 3.

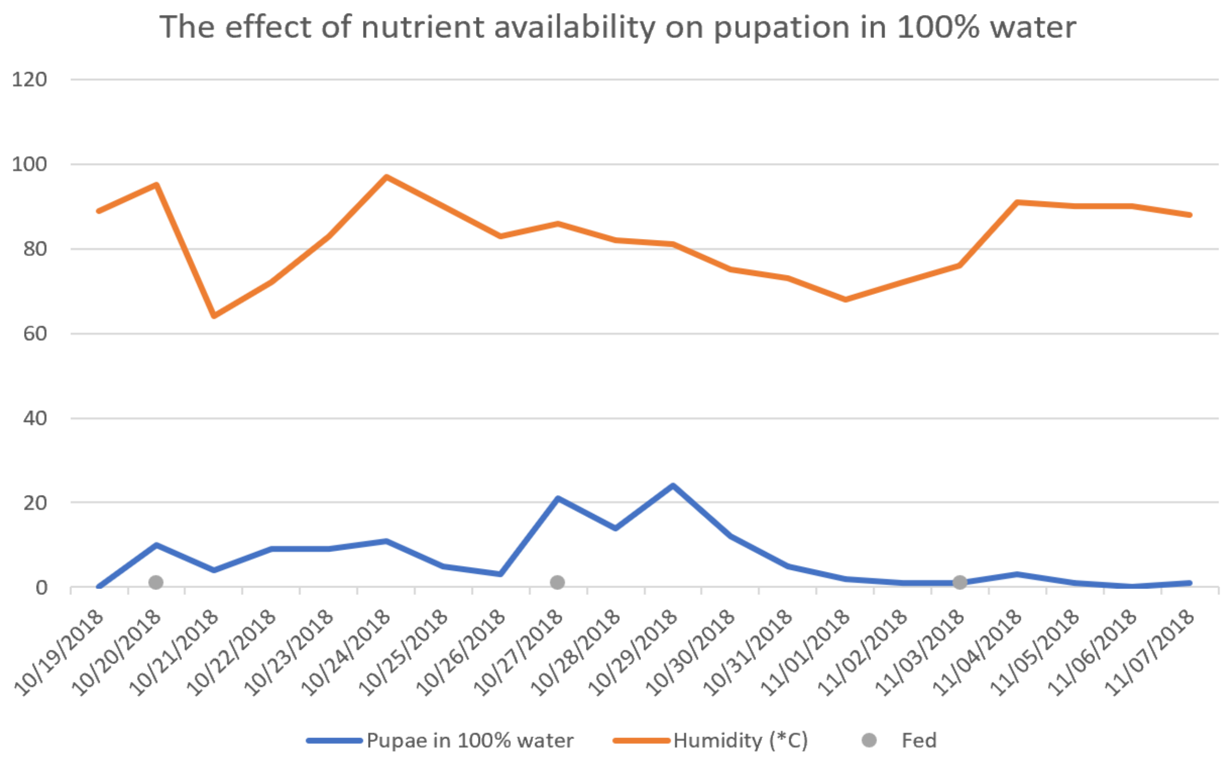


Figure 3. This composite graph is composed of two lines, one of which is indicating the number of pupae present in the pure water system on each day while the other line represents the average humidity each day. The dots at the bottom of the graph indicate when the system received algae supplementation.

Pupation follows the general trend described previously that is closely associated with humidity, but varies in aspects the other systems do not. Peaks at 10/27 and 10/29 are in periods of relatively stable average humidity and thus are not explained by weather. These peaks do occur immediately after food is reintroduced to the system. The increase in food availability appears to cause a major rise in pupation within the following 24 hours after feeding. Feeding occurred in the morning of the 27th. Pupation subsides after the initial peak while it is assumed that larvae previously unfit to successfully compete, gain the necessary nutrition to complete their larvae stage and pupate (Murrell, 2011). The second peak in pupation then occurs on the 29th or shortly after with the help of the remaining nutrients. By 11/2 nutrition had most likely run out, as indicate in the depressions in pupation rates the day before each feeding as seen on 10/19, 10/26, and 11/2. Immediately after feeding occurred on 11/3 the last few larvae present in the system pupate, leaving excess food in the system which ensured all viable larvae had pupate.

Both sets of data follow trends established by environmental factors, this explains the trimodal pattern in the 100% water system and the bimodal pattern in the 1:2 twice stirred system. The length of time taken for all larvae to pupate is largely different between the data sets and serves to suggest that higher nutrient concentrations create a more uniform population. This is indicated by the small window of time required for all of the larvae to pupate within the 1:2 system while the larvae in the 100% system pupate over a long period of time suggesting that the larvae present at any period existed at different instars, thus being able to pupate either immediately or after some time with the introduction of available nutrients causing them to develop further. When nutrient concentration is high, larvae do not have to compete for nutrition and thus their development is more constant throughout the population.

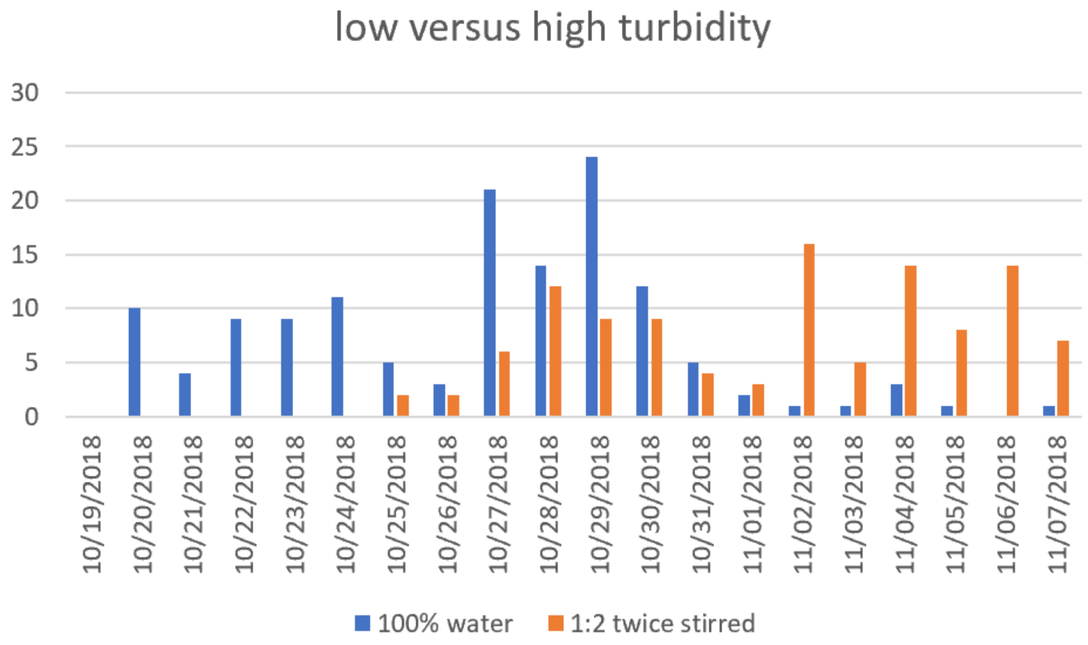


Figure 4. This bar graph is composed of the pupae present each day in the system with the least available nutrition, which was the 100% water system, as compared with the system where the most nutrients were readily available. This system was the 1:2 soil water ratio that was stirred twice daily.

Discussion

This study examines many factors and the interplay between these factors. As can be seen in natural environments, there is no perfect isolation of variable to draw a clear cause and effect but trends can be analyzed in the presence of other conditions. The first major consideration was nutrient concentration and turbidity. Through the examination of the data presented one can conclude that nutrient concentration and suspended availability is relevant but since the tolerance range is extremely high, this variable is lower in importance when it comes to mosquito control. The tolerance range is indicated as extremely high due to there being no discernible difference in pupation between any of the trial containers except for the systems with no substrate and the container with a 1:2 ratio that homogenized twice daily. The effects of nutrient levels being outside of the tolerance range is described in Figure 4. Being outside of the tolerance range indicates less predictability and population homogeneity, neither of which is particularly relevant to control efforts. For this reason, run off is determined to still have drastic effects on the environment but appears to not be a major factor in mosquito population dynamics. The other major concern of this study was on climate. Temperature was indicated to have a limited effect on the rate of pupation when oscillations are common and within a 15-degree variation from the environment that is considered hospitable. Slight temperature variations are not relevant in mosquito control but should continue to be a major area of concern as the mosquitos were able to pupate with little to no effect at varying temperatures. This ability is a factor in the massive area distribution in which *Aedes* mosquitos can exist. Humidity as a factor did play a major role on pupation rates but even at the lowest humidity observed, the effect on pupation was slight. At the highest humanities pupation increased drastically meaning a reduction in humidity is not effective enough as a control method but high humidity is a definite cause for concern.

An observational study of simulated environments with humidity, temperature, nutrient concentration, and nutrient availability as factors is useful in understanding the effects of the environment on mosquito larvae success in development. Population dynamics are important in understanding vulnerabilities and areas of concern. In systems with what is traditionally considered perfect conditions (Duguma, 2014), mosquito populations are highly efficient and uniform. This is the same concept observed in microbial cultivation. For microbes however this is also the time in which population are the most vulnerable to treatment (Pinfold, 2003). This very well may also be the case for mosquitos and should be researched further. Other continuations of research into this topic are in reference to more extreme environment. A study similar to this one based in a different climate zone could be helpful in drawing conclusions from the accumulation of these data sets. The continuation of this type of work is useful in understanding the effects of multiple variables, and how those variables interplay. Experimentation in isolation is extremely important in understanding cause and effect, but multivariable studies are applicable in understanding ecosystem dynamics as relevant to control.

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