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HOW SEWAGE POLLUTION AFFECTS DISTRIBUTION AND LIFE HISTORY TRAITS OF THE SOUTHERN HOUSE MOSQUITO, CULEX QUINQUEFASCIATUS

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by

Catherine Dean Bermond

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ABSTRACT

Eutrophication from wastewater can cause fluxes of nutrients like and carbon and nitrogen in urban waters. One source of nitrogen in freshwater is from untreated sewage discharging from septic tanks. This causes a variety of environmental issues including harmful algal blooms, harming natural predators, and increased production of mosquitoes. *Culex quinquefasciatus* is the vector of West Nile Virus in St. Tammany Parish, Louisiana. St. Tammany Parish has over 600 miles of septic ditches, which receive effluent directly from aerated treatment units. *Culex quinquefasciatus* oviposit in polluted waters, and larvae perform well in water with high levels of decomposing organic matter. The goal of my thesis was to investigate the effects of septic ditches on the stoichiometry of *Cx. quinquefasciatus*. I collected mosquitoes and larvae from septic and rainwater ditches in the parish and used that water to rear *Cx. quinquefasciatus* larvae to see how water type affects their life history strategies. I found that species composition and water quality differed significantly between ditch type, and nutrient composition differed significantly between *Ae. albopictus* and *Cx. quinquefasciatus* within ditches. My data show that *Cx. quinquefasciatus* are larger with higher survival, develop faster, and different nutrient signatures when reared in septic and rainwater when compared to RO water. This study shows the effect of sewage on *Cx. quinquefasciatus*, and more research needs to be conducted to know how different sewerage strategies can influence mosquito production. This is important for mosquito abatement districts and need for sewage reform in St. Tammany Parish, Louisiana.

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CHAPTER I - INTRODUCTION

1.1 Nitrogen

Eutrophication of urban waters is major environmental issue due to increased fluxes of nutrients, including carbon (C), nitrogen (N), and phosphorous (P) that are associated with increased urbanization (Grimm et al. 2008). Inputs of nitrogen into freshwater waterbodies can be from a variety of sources including fertilizer, atmospheric disposition, and wastewater discharge (Lusk 2017). Besides these, septic tank systems have been shown to be a source of nitrogen pollution in the environment (Elliott et al. 2007; Hossain et al. 2010; Kaushal et al. 2011; Law et al. 2004; Oakley et al. 2010). Investigating the transportation and fate of nitrogen in septic systems is of importance due to the capacity of nitrogen to affect the quality of ground and surface water (Beal 2005, Withers 2011). Valeila and Costa (1988) found that in Buttermilk Bay, MA, 40% of nitrogen and phosphorous entering the watershed originated from septic systems, and in 1997 they found that in Waquoit Bay, MA, 48% of the N loading was from septic systems. A study in the Florida Keys by Lapointe et al. (1990) found that due to septic systems, the concentration of dissolved inorganic nitrogen in the ground water could be enriched up to 400-fold.

Nitrogen exists in various forms in the environment due to its oxidation states. These forms consist of ammonia (NH_4), nitrate (NO_3), nitrite (NO_2), nitric oxide (NO), nitrous oxide (N_2O), and nitrogen gas (N_2) (Lusk 2017). The oxidation states of these forms are predictable and depend on many factors. The main form of nitrogen that is of concern to public and environmental health is NO3. Consumption of water that is containimated with NO_3 can cause health problems in infants such as methemoglobinemia (i.e., blue baby syndrome), and in response the U.S. Environmental Protection Agency (EPA) (1999) and the World Health Organization (WHO) (2006) set a NO_3 limit of 10mg/L in drinking water. Nitrogen poses a threat to ecosystem health as N is the limiting nutrient in many bodies of freshwater and has been connected to harmful algal blooms, hypoxia, fish kills, and loss of biodiversity. In Florida, Hazen and Sawyer (2009) found that as little as 1 mg/L of N can cause algal growth in a natural spring.

1.2 Nitrogen in wastewater

Urine and fecal matter are the main source of nitrogen found in wastewater/sewage, with urine accounting for 91% of N (Henze et al. 2002). Other sources of nitrogen found in wastewater include, but are not limited to, food from kitchen sinks and dishwashers. In 1992, the U.S. EPA estimated that one person produces up to 11.2 g of total N/day, with 78% (8.7g) from toilets, 17% (1.7 g) from baths, sinks, and appliances, and 5% (0.6 g) from kitchen sinks. The most common forms of nitrogen found in domestic wastewater are organic nitrogen (40%), ammonia (60%) and less than 1% of both nitrate and nitrite (von Sperling 2007, Harden et al. 2008). The amount of nitrogen concentration in wastewater is dependent on the amount of nitrogen loading and the amount of water that is mixed with the nitrogen. Lowe (2009) found that total nitrogen concentrations in raw wastewater typically ranges from 20 to 85 mg/L, but higher nitrogen values have been reported in areas with lower water use.

1.3 Septic tanks

Septic tanks are an alternative option for wastewater treatment if a centralized sewer system is not available. Septic tanks store wastewater and operate as a "bioreactor" and provides a location for microorganisms to break down organic matter and separate it into solids, liquids, or gasses from the original sewage (EPA 2022) Systems in the soil discharge the liquid waste, or effluent, through a series of pipes that are intended to release the effluent into the soil. Although an underground absorption field is the ideal method for secondary sewage treatment for effluent to discharge, soil conditions in some regions can create an inadequate absorption field since the effluent cannot seep through the soil. If an area cannot support an underground absorption field, another method using filter beds or Aerobic Treatment Units (ATUs) is used (Lusk 2017). A filter bed is a collection of pipes laid on top of a thick bed of gravel or sand with an effulent collection pipe located underneath the gravel bed. Effluent drains from the septic tank through the top pipes, is filtered through the gravel, collected in the bottom pipe, and then discharged into the environment (EPA 2022). Unfortunately, these filter bed systems frequently fail to properly filter effluent properly before discharging it into the environment (e.g., a ditch, stream, or bayou), resulting in sewage pollution. (EPA 2022). Consequently, and appropriately, filter beds are no longer authorized for installation in some areas, including the area of study of this proposal, St. Tammany Parish, Louisiana, U.S.A. (St. Tammany Parish Gov.).

In St. Tammany Parish, ATUs are an alternate secondary treatment approach. These units are designed to treat water as effectively as municipal treatment plants, but on a smaller scale. An ATU feeds oxygen into the treatment tank after the sewage has separated into layers. As a result, anaerobic microorganisms thrive in this oxygen rich environment, further reducing organic waste into carbon dioxide and water. Aerobic bacteria break down waste materials faster than anaerobic bacteria, therefore treated water from ATUs is cleaner than treated water from other systems if properly managed (Lusk 2017). In addition. ATUs also take up less space, process waste more efficiently, and reduce the level of odor associated with a septic tank. There are also disadvantages to these systems. In general, ATUs are expensive due to having more parts than conventional systems as they need electrical components (e.g., pumps and compressors) to be able to pump oxygen into the tank. They also require more costly maintenance than standard septic systems, including frequently checking the electrical components and aerator. If any of these components fail, it can cause polluted water to drain into the environment. Some septic systems are engineered to remove specific containments, for example, nitrogen. There are two components to septic systems that are designed for N reduction: (1) ATUs that cause the conversion of NH_4 to NO_3 in the wastewater and (2) an anaerobic treatment containing a reactive media to convert NO_3 to N_2 gas (e.g., wood chips for heterotrophic denitrification and sulfur (S) for autotrophic denitrification) (Xuan 2012, De and Toor 2016). Although these mechanisms exist, in 2008 the Water Environment Research Foundation (WERF) found that less than 5% of N is removed from the wastewater in a septic tank.

An improperly managed septic system can cause a variety of environmental issues due to it causing untreated wastewater to be released where it should not be. Problems with components of a septic tank can cause it to overload with waste liquids and solids, causing overflow into the home or contamination of nearby waterbodies. The discharge of untreated sewage into local waterways poses several environmental and public health risks (Tibbetts 2005). Septic tanks can be a source of nitrates and other harmful chemicals (Liu et al. 2005). Fecal contamination of a public water supply by a nearby septic system has been linked to increased incidents of viral (Borchardt 2003) and bacterial (Özkan et al. 2007) diseases and at least one outbreak of gastroenteritis (Weissman et al. 1976). Untreated wastewater can cause elevated levels of N and P to be released into the environment. Excess levels of N and P in waterbodies can cause an over-production of algae and microorganisms, which can cause a variety of problems including reducing the aesthetic value of the water, increasing the occurrence of harmful algal blooms, harming naturally occurring aquatic predators, and loss of aquatic biodiversity.

1.4 Mosquitoes

Mosquitoes are insects within the Order Diptera and the Family Culicidae with 3,715 described species (Mosquito Taxonomic Inventory, 2023). Approximately 2.5% to 9.3% of all mosquito species have been found to transmit pathogens (Yee et al. 2022) that are of importance for human health (Clements 2000) with those species most concentrated in the genera *Aedes*, *Culex,* and *Anopheles*. Mosquitoes go through complete metamorphosis, with an egg, larval, pupal, and adult phase. The egg, larval, and pupal phase are aquatic, and a terrestrial adult emerges from the pupa (Clements 2000).

Mosquitoes use a variety of natural and artificial habitats for larval development. These can include habitats such as containers (e.g., cemetery vases, abandoned tires, and tree holes) or open-water systems (e.g., ditches and ponds) (Laird 1988). Depending on the species, gravid adult females will lay eggs either on top of the water or around the water line on the edge of the container. Artificial containers are used as larval rearing sites by many species of medically important mosquito species (Vezzani 2007). In container systems, most essential nutrients and energy come from detrital pathways. In these systems, primary production is essentially absent, and depends on allochthonous detrital inputs from the surrounding terrestrial matrix (Vezzani 2007, Yee and Juliano 2006).

1.5 *Culex* mosquitoes

Multiple species of mosquitoes in the genus *Culex* are part of the *Culex pipiens* species complex. A species complex is generally defined as a group of closely related species that are often difficult to separate morphologically (Collins and Paskewitz 1996). The *Culex pipiens* complex includes multiple taxonomic entities including: *Cx. pipiens pipiens*, *Cx. pipiens quinquefasciatus* Say, and *Cx. pipiens* form *molestus* Forskal and various other hybrids. Species within the *Cx. pipiens* complex have been found in human settlements worldwide (Calhoun 2007). *Culex quinquefasciatus*, the southern house mosquito, tends to prefer more subtropical climates as found in the southern U.S., and shares many biological traits and behaviors with *Cx. pipiens*, the northern house mosquito, which prefers cooler climates as in the northern U.S. Mosquitoes within the *Cx. pipiens* complex have been commonly observed to enter human houses, which gave rise to the common name "house mosquito" for the species *Cx. quinquefasciatus* and *Cx. pipiens*. The primary method to distinguishing between species in this complex is the by the shape of the male genitalia (Barr 1957; Dobrotworsky 1967) or the larval siphonal index (Becker et al. 2010), the ratio of length to width of the respiratory siphon, but this does not allow for identification of female adults, which are the primary focus of mosquito control surveillance efforts. Where their ranges overlap, *Cx. pipiens* and *Cx. quinquefasciatus* can extensively hybridize shown by genetic and microsatellite analysis. *Culex quinquefasciatus* is a peridomestic mosquito that has a worldwide distribution in tropical and subtropical areas including the Americas, Africa, Asia, and Australia (Fonseca et al. 2004). This species is an urban species (Subra 1981, Harbison et al. 2009) and is known as the main vector of multiple pathogens that affect humans, and both domestic and wild animals. *Culex quinquefasciatus* is considered the vector of West Nile Virus (WNV) (Sardelis et al. 2001, Goddard et al. 2002, Molaei et al. 2007) and St. Louis encephalitis virus (SLEV) (Hardy et al. 1984, Savage et al. 1993) in the southern U.S.

Adults of the species *Culex quinquefasciatus* are characterized as a medium-sized golden-brown mosquito with a white banded abdomen (Fig. 1). Females of this species are anautogenous, meaning they require blood meals for development of eggs and gravid females will lay a single floating egg raft (~155 eggs) on the surface of a suitable body of water (Roberts and Kokkinn, 2010). Gravid females often oviposit in highly organic waters, and larval habitats for *Cx. quinquefasciatus* include artificial containers (e.g., abandoned tires), man-made impoundments (e.g., ditches, ground pools) or in natural areas (e.g., rock pools, tree holes, stream margins). Eggs usually hatch within 48 hrs of oviposition, and larval develop through four instars over the course of 5-10 days. Larvae are filter feeders on various types of detritus and microorganisms from the water column and surface (Merritt et al., 1992). Larvae do well in aquatic habitats that are highly eutrophic with high concentrations of decomposing organic matter, including water associated with septic tanks, sewage drains, and cesspools (Subra 1981, Harbison et al. 2009, Reisen 2012, Yee and Skiff 2015). After the larval stage of development, there is a pupal stage that lasts ~48 hrs, and then male adults usually emerge first, followed by females. *Culex quinquefasciatus* adults are considered peridomestic and feed on blood from a variety of vertebrates including humans, dogs, and birds (Reisen, 2012). Birds make up a large portion of blood diet of *Cx. quinquefasicatus*, therefore adult females encounter blood infected with avian viruses such as West Nile Virus (Fonseca et al. 2004).

Figure 1.1 *Photo of adult female Culex quinquefasciatus on a flower. Photo by D.A. Yee.*

CHAPTER II - EFFECTS OF NUTRIENT LOADING FROM SEWAGE POLLUTION ON CULEX QUINQUEFASICATUS

2.1 Introduction

An ecological and public health hazard associated with septic tanks and discharge of untreated sewage into waterways is the increased production of medically important mosquito species that are considered vectors of human pathogens. Multiple species of mosquitoes, including *Ae. aegypti* and *Cx. quinquefasciatus*, have been found emerging from septic tanks (Irving-Bell, 1987) and septic tanks can serve as refugia for *Cx. quinquefasciatus* (Kay et al., 2000). There are multiple factors that contribute to the success of *Cx. quinquefasciatus* in polluted waters, such as faster larval development (Curtis and Feachem 1981) and in conjuncture with reduced larval predation and interspecific competition (Agnew et al. 2000, Beketov and Leiss 2007, Mogi and Sota 1996, and Yee and Skiff 2015) and a tendency for females to prefer to oviposit in organically enriched waters (Chaves et al. 2009, Murrell et al., 2011, and Nguyen et al., 2014). In Puerto Rico, Mackay et al., (2009) investigated the dynamics of the mosquito species *Ae. aegypti* and *Cx. quinquefasciatus* in septic tanks. Their data showed that septic tanks produce large numbers of *Ae. aegypti* and *Cx. quinquefasciatus* throughout the year and the production numbers of *Ae. aegypti* were 3 to 9 times larger and produced adult size were significantly larger than in surface containers. Following Mackay in Puerto Rico, Burke et al. (2010) investigated the relationship of structural variables of septic tanks and the chemical properties of water and immature mosquito abundance. They found immatures of the species *Cx. quinquefasciatus* in 74% of septic tanks and larval presence was negatively associated with TDS, while larval abundance was positively associated with cracked septic tank walls.

Medically important mosquitoes have also been associated with combined sewage overflow (CSO) systems. These systems collect industrial wastewater, domestic sewage, and stormwater in the same pipe and direct it to wastewater treatment facilities (Bernheardt et al., 2008). Research has shown a strong association between WNV infection in mosquitoes, corvids, and humans and proximity to CSO-affected urban streams (Vazquez-Prokopec et al., 2010). In urban creeks that receive combined stormwater and wastewater effluent in Atlanta, GA, Calhoun (2007) found that *Cx. quinquefasciatus* was the dominant mosquito species, mosquito densities were higher in areas of stagnant water, and mosquito numbers were largely associated when the urban creek flooded from the input of effluent. Also in Atlanta, GA, Chaves et al. (2009), studied the relationship of CSOs and *Cx. quinquefasciatus* oviposition preference. Their data show that CSO water, especially when enriched, was a more attractive oviposition site. Additionally, research suggests that the potential effect of sewage polluted streams could extend further than the drainage path because larval productivity levels of *Cx. quinquefasciatus* is not significantly affected by the distance from the source overflow discharge (Calhoun 2007). In CSO basins, larval abundance of *Cx. pipiens* was significantly higher compared to stormwater-only basins (Marini et al., 2020).

St. Tammany Parish (STP) is located in the New Orleans-Metairie metropolitan area. Rapid population growth following Hurricane Katrina in 2005 led to decentralized sewerage practices in areas of STP. Surface water quality has declined in STP due to the installation of thousands of individual aerated treatment units which are usually operated and maintained by the homeowner. Many of these units are poorly maintained and in inadequate position due to homeowners being unaware or unable to afford the maintenance needed for ATUs. An improperly managed septic system can cause untreated sewage water to discharge out of the tank and contaminate nearby water bodies, specifically roadside ditches in STP. There are over 600 mi (965 km) of septic ditches in STP, which have been shown to be a major production site for medical and nuisance mosquito species such as *Cx. quinquefasicatus*.

2.2 Goal of chapter

My goal for this chapter is to examine how nutrient signatures differ among mosquitoes and water collected from septic ditches and rainwater ditches in St. Tammany Parish. I selected septic ditches that had a drainage pipe from the tank into the ditch, and rainwater ditches in areas where there are larger sewage treatment plants. At each ditch I collected mosquito larvae, ditch water onto filters, and adult mosquitoes via CDC no-light traps. I analyzed each component for its carbon and nitrogen nutrient signatures.

2.3 Study Area

St. Tammany Parish (STP) is located in the state of Louisiana in the southern United States. St. Tammany Parish is included in the New Orleans-Metairie metropolitan area, includes cities such as Slidell, Covington, and Mandeville, and has an estimated population of 233,740 (2020 US Census). The parish covers approximately 2,910 km^2 of which 2,190 km^2 is land and 720 km^2 is water.

2.4 Research Questions and Hypotheses

This chapter will answer and test the following research questions and hypotheses:

I. What are the effects of effluent flowing from septic tanks into ditches in St. Tammany Parish?

   a. How do the nutrient signatures (C, N) differ in adult mosquitoes trapped near septic and rainwater ditches?

      i. H_A: Nutrient signatures of trapped adults will be significantly different between septic and rainwater ditches.

      ii. H_o: Nutrient signatures of trapped adults will not be significantly different between septic and rainwater ditches.

   b. Is the species composition of larval and adult mosquito species collected from septic and rainwater ditches different?

      i. H_A: Species composition of larval and adult mosquitoes will vary between septic and rainwater ditches.

      ii. H_o: Species composition of larval and adult mosquitoes will not vary between septic and rainwater ditches.

   c. Are there differences in water quality parameters between septic ditches and rainwater ditches?

      i. H_A: Water quality parameters will be significantly different between septic and rainwater ditches.

      ii. H_o: Water quality parameters will not be significantly different between septic and rainwater ditches.

I predict that nutrient signatures and water quality parameters will vary between septic ditches and rainwater ditches, owing to differences in inputs of N and P into these systems. Moreover, I predict that septic ditches will have higher nutrient signatures in the larvae, water filters, and adults based on increased nutrient influxes into septic ditches. I also predict that adult and larval species composition will vary between septic and rainwater ditches. Specifically, I predict that I will mostly find the species *Cx. quinquefasciatus* in septic ditches due to their ability to tolerate highly organic waters. I predict that in rainwater ditches, that I will find species that tend to use flood waters for larval production (e.g., *Aedes vexans*, *Culex salinarius*).

2.5 Materials and Methods

2.5.1 Methods

I sampled ditches within St. Tammany Parish from May to September of 2021 that were each designated in areas that have sewerage strategies of either municipal sewage or unsewered/septic sewage. I randomized my sampling area and sewage strategy type to avoid spatial or temporal bias while sampling. For septic ditches (Fig. 2.1), I tried to select ditches that were positive for mosquito larvae and have a pipe that directly drained sewage effluent from individual houses into the ditch. For ditches in municipal sewage areas, I attempted to select for rainwater ditches (Fig. 2.2) that were positive for mosquito larvae. If I could not find ditches with larvae in them, I selected ditches that look like they would be a suitable habitat for larvae based off absence of predators (e.g., *Gambusia* sp. [mosquito fish], frogs, turtles), shade to prevent desiccation, and presence of detritus. At each ditch, I sampled at the 'center' of the ditch and then at 1 m away from the center on either side. In ditches that have mosquito larvae in them, the 'center' was my first sighting of larvae in the ditch. In ditches with no larvae, the center is an area I chose with at least 2 m of water on either side. For each sampling area I collected mosquito larvae, water quality parameters, organic material on filters, and trapped adult mosquitoes. I used a water sampling dipper to collect one sample of mosquito larvae from each sampling location in the ditch (center, and 1 m away on both sides) and placed samples into a 500 mL container. I used a YSI ProDSS Multiparameter Digital Water Quality Meter to measure the following parameters in the water: temperature (C˚), dissolved oxygen (DO), total dissolved solids (TDS), nitrates (NO_3), conductivity (µS/cm), salinity (psu) and pH. I collected adult mosquitoes by placing two CDC miniature no light traps that are baited with dry ice (CO_2 lure) at the 1 m and 3 m mark in the ditch. Traps were hung directly above the ditch on shepard's hooks (Fig. 2.1). Traps and adults were collected within 24 hrs of trap placement. Mosquito larvae and adults were brought back to the Yee Mosquito Lab at The University of Southern Mississippi (USM) to be processed. Larvae and adults were identified to species using Darsie and Ward (2005). Trapped adults were oven dried for ≥ 48 h and then weighed to the nearest 0.001 mg using a XP2U ultra microbalance (Mettler Toledo, Ohio). Trapped adults were analyzed for carbon and nitrogen values by me using an ECS 4010 Elemental Combustion System in the lab of Dr. Kevin Kuehn at USM.

Figure 2.1 *Photo of a septic ditch in St. Tammany Parish, LA with CDC no light traps set as described in methods.*

Figure 2.2 *Rainwater ditch found in St. Tammany Parish, LA*

Figure 2.3 *Map of sampling locations in St. Tammany Parish, LA.*

Septic ditches are represented by blue circles and rainwater ditches are represented by black diamonds.

2.5.2 Statistical Analysis

Data were checked to confirm they meet the assumptions of parametric tests. A Multivariate Analysis of Variance (MANOVA) was used to test for effects of ditch type (2) and species on the nutrient signatures (C, N, C:N) of the collected adults. Standardized canonical coefficients (SCCs) were used to find the important variables accounting of observed multivariate effects (Scheiner 2001).

The raw data met the assumption of homogenic variances but not the assumption of normality. No transformation improved normality, and because MANOVA is robust against deviations of normality the raw data were used. The species used in the analysis were reduced to those that occurred in both septic and rainwater ditches.

2.6 Results

2.6.1 Species Composition

Mosquito species composition varied between both septic and rainwater ditches for adults and larval (Table 2.1). In total, 1034 mosquitoes were collected near septic ditches and 217 mosquitoes were collected near rainwater ditches. Fifteen species were identified from septic ditches with all species found as adults and four species (*Cx. nigripalpus, Cx. quinquefasciatus, Cx. salinarius, Ps. ferox)* also collected as larvae. Ten species were collected from rainwater ditches with all 10 found as adults and four species collected as larvae (*Cx. nigripalpus, Cx. quinquefasciatus, Cx. salinarius, Ps. ferox*). Species that were found in both septic and rainwater ditches include *Aedes albopictus, Anopheles quadrimaculatus, Culex erraticus, Cx. nigripalpus, Cx. quinquefasciatus, Cx. salinarius*, and *Psorophora ferox*.

Table 2.1 *Identified mosquito species collected from CDC no light traps or larval sampling in septic and rainwater ditches*

Septic ditch species (n = 15)	Rainwater ditch species (n = 10)
Aedes albopictus[A]	*Aedes albopictus*[A]
Aedes dupreei[A]	*Aedes vexans*[A]
Aedes triseriatus[A]	*Anopheles crucians*[A]
Aedes sollicitans[A]	*Anopheles quadrimaculatus*[A]
Anopheles quadrimaculatus[A]	*Culex erraticus*[A]
Coquillettidia perturbans[A]	*Culex nigripalpus*[AL]
Culex coronator[A]	*Culex quinquefasciatus*[AL]
Culex erraticus[A]	*Culex salinarius*[AL]
Culex nigripalpus[AL]	*Psorophora columbiae*[A]
Culex quinquefasciatus[AL]	*Psorophora ferox*[AL]
Culex restuans[A]	
Culex salinarius[AL]	
Psorophora columbiae[A]	
Psorophora ferox[AL]	
Psorophora howardii[A]	

Superscripts denote life stage the species was in when collected (A = adult, L = larvae)

2.6.2 Water quality parameters

The effect of ditch type on water quality parameters (e.g., conductivity, specific conductivity, salinity, total dissolved solids (TDS), temperature pH, and NO_3) was significant (Pillai's Trace = 0.548, $F_{6,16} = 3.24$, $p = 0.028$). Based on SCCs, the differences were mainly due to conductivity, salinity, and TDS. Septic ditches had significantly higher means than rainwater ditches for conductivity, specific conductivity, salinity, and TDS. Septic ditches also had significantly less NO_3 than rainwater ditches (Table 2.2).

2.6.3 Stoichiometry and mass of adults

The effect of ditch type on the nutrient signatures (C, N, and C:N) and mass of adults was not significant (Pillai's Trace = 0.039, $F_{4,159} = 1.63$, $p = 0.169$) but the species effect was significant (Pillai's Trace = 0.631, $F_{28,648} = 4.33$, $p < 0.001$) as was the interaction of ditch type and species (Pillai's Trace = 0.349, $F_{28,648} = 2.22$, $p < 0.001$). Based on SCCs, significant differences were mostly due to nitrogen (SCC = 0.943) instead of carbon (0.522), mass (0.503), or C:N (0.398). For differences in species between ditches, %C for *Ae. albopictus* was significantly higher in rainwater ditches (62.89 %) compared to septic ditches (41.57%). There were no significant effects of N or C:N across ditch types. For %C in species within rainwater ditches, *Ae. albopictus* was significantly different than *Ps. columbiae, An. quadricmaculatus*, *Cx. nigripalpus*, and *Cx. quinquefasciatus*, with *Ae. albopictus* (62.89%) having higher %C than *Cx. quinquefasciatus* (51.3%). In septic ditches, *Ae. albopictus* had lower %C (41.57%) than *Cx. quinquefasciatus* (52.95%). For %N of species within rainwater ditches, *Ae. albopictus* had higher values (10.89%) than *Ps. columbiae* (7.79%) and *Cx. salinarius*

(7.83%). There were no significant differences of %N among species within septic ditches or in C:N among species in both septic and rainwater ditches. For mass of trapped adults between ditches, there was no significant difference between ditch types, but there were multiple significant differences in mass between species within ditch type (Figure 2.4).

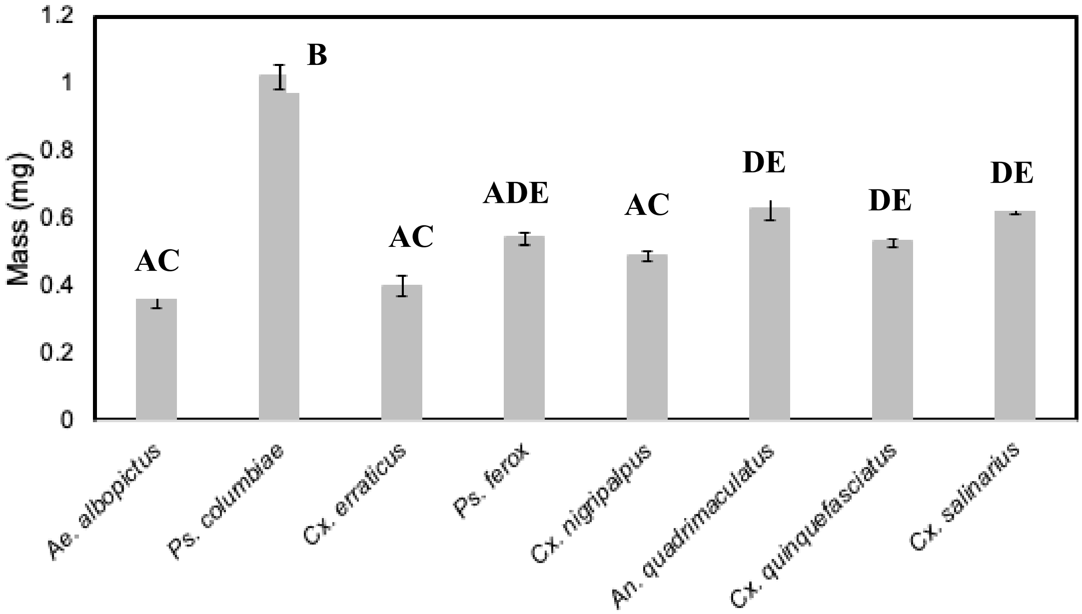

Figure 2.4 *Values of mass between species within ditches. Bars represent means ± SE. Means that share a letter are not significantly different than each other.*

Table 2.2 *Values of water quality parameters that were measured at each ditch using a YSI ProDSS Multiparameter Digital Water Quality Meter.*

Water quality parameter	Septic Ditch	Rainwater Ditch	*P*
Conductivity (µS/cm)	677.89	333.08	**0.02**
Specific Conductivity (µS/cm)	645.20	320.99	**0.03**
Salinity (psu)	0.31	0.15	**0.03**
Total Dissolved Solids (mg/L)	419.42	208.60	**0.03**
Temperature (°F)	81.83	81.63	0.94
pH	6.78	6.54	0.35
NO3 -N (mV)	148.09	202.70	**0.02**

Note: Values are averages. Significant *p* values in bo

2.7 Discussion

My data support my hypothesis that there would be differences in species composition of both adults and larvae between septic and rainwater ditches. In total, I collected 1034 adult mosquitoes near septic ditches and 217 mosquitoes near rainwater ditches. I collected 17 species overall between ditch type and adult and larval collection. I found 15 of those species in septic ditches and 10 in rainwater ditches with most species found as adults and four species found as larvae in both rainwater and septic ditches (*Cx. quinquefasciatus*, *Cx. nigripalpus*, *Cx. salinarius*, and *Ps. ferox*). In total, 47 species have been found in St. Tammany Parish and the most common species found are *Cx. quinquefasciatus, Cx. salinarius, Ae. albopictus, Ae. aegypti,* and *Ae. solicitans* (STMAD, 2023). I collected all of these species except *Ae. aegypti*, which at the time of collection was not prevalent in the parish. Even though there have been 47 species collected in STP, my collection was limited spatially to adults trapped above ditches and larval subsamples in rainwater and septic ditches. Thus, the way in which I collected the samples (i.e., right above the ditches) may be expected to primarily collect those species that use ditches as larvae rearing sites, and not container species (e.g., *Ae. aegypti*). My collection was also limited temporally as collections ranged from May 2021 to September 2021, so I may have missed species more commonly found in early spring or late fall. I also only used CO_2-baited CDC no light traps which are designed to attract host seeking mosquitoes, and the removal of the light is effective at trapping *Culex* females (McNamara et al., 2021).

In the U.S., water pollution from inputs of sewage from sewage facilities or septic tanks is considered a major source of pollution and a human health concern (EPA 2009).

I found significant differences for water quality parameters between septic and rainwater ditches. The parameters of conductivity, specific conductivity, salinity, and total dissolved solids were significantly higher in septic ditches than in rainwater ditches. Conductivity is a measurement of the ability of water to conduct an electrical current, and higher conductivity is a result of the ion concentration in the water that originates from dissolved solids and inorganic materials (Ribeiro de Sousa, 2014). A higher conductivity can imply that there are higher levels of total dissolved solids, which is consistent with my data. Research has shown that water with decaying animal detritus (i.e., dried crickets) has higher conductivity than water with decaying plant detritus (Yee et al., 2006). Increasing the amount of animal detritus has positive effects on life history traits including faster development times, larger adult mass, greater survivorship to adulthood, and high estimated population growth (Yee and Juliano 2006, Murrell and Juliano 2008, Winters and Yee 2012, Yee et al. 2015). Unexpectedly, nitrate (NO_3) levels were significantly lower in septic ditches compared to rainwater ditches. Mechanisms that may explain lower levels of nitrate include denitrification, where inorganic nitrate is reduced to nitrous oxide and nitrogen gas (Knowles, 1982) and dissimilatory nitrate reduction that reduces nitrate to ammonium (Burgin and Hamilton, 2007). Ditches that receive inputs from CSO systems have been found to have higher amounts of ammonium when compared to streams that do not receive inputs from CSO systems (Chavez et al. 2011). The most common forms of nitrogen found in domestic wastewater are organic nitrogen (40%), ammonia (60%) and less than 1% of both nitrate and nitrite (von Sperling 2007, Harden et al. 2008). Further research into the effects of sewage loading should investigate ammonium levels over nitrate levels. It is possible that there were errors in my nitrate measurements due to my improper calibration of the probe.

My research partially supports my hypothesis that there would be differences in the nutrient stoichiometry in adults between septic ditches and rainwater ditches. *Aedes albopictus* had higher %C values in rainwater ditches than in septic ditches. When comparing *Ae. albopictus* to *Cx quinquefasciatus* within ditch type, *Ae. albopictus* had higher %C values. However, in septic ditches *Ae. albopictus* had lower %C values than *Cx. quinquefasciatus*. Traps were hung above the ditch on shepards hooks with the intention of collecting adult mosquitoes emerging from the ditch. Species can differ in how far they travel, with *Ae. albopictus* having an average flight range of 100-200 m (Vavassori et al., 2019) compared to *Cx. quinquefasciatus* with ranges up to 3.2 km (CDC, 2022). Most of the traps were hung in ditches in residential areas that may contain a variety of habitats (e.g., artificial containers) for mosquitoes to develop. As no *Ae. albopictus* larvae were collected in my data, we can assume that they may have developed in a different nutrient environment, which could be why they have different nutrient composition than other species collected near ditches.

Aedes albopictus is an invasive species (Paupy et al., 2009) and has been found to be a superior competitor to *Ae. aegypti* (Juliano, 1998; Murrell and Juliano, 2008), *Culex pipiens* (Carrieri et al., 2003; Costanzo et al., 2005), *Ae. triseriatus* (Yee et al. 2007), and *Cx. quinquefasciatus* (Allgood and Yee, 2014). The competitive superiority of *Ae. albopictus* over other mosquito species seems to be condition specific. The competitive advantage of *Ae. albopictus* has been reduced in conditions that are dry (Costanzo et al., 2005), in the presence of predators (Griswold & Lounibos, 2005), and in labile resources

(Yee et al., 2007; Murrell & Juliano, 2008; Costanzo et al., 2011). In rainwater ditches, *Ae. albopictus* had higher %C values than *Cx. quinquefasciatus*. There are two possible explanations for this, either *Ae. albopictus* developed in waters different than *Cx. quinquefasciatus* resulting in different nutrient composition, or *Ae. albopictus* is a superior competitor to *Cx. quinquefasciatus* in rainwater ditches and is better able to assimilate carbon from the water than *Cx. quinquefasciatus*. In septic ditches, I found that *Ae. albopictus* had lower %C values than *Cx. quinquefasciatus*. There is the possibility that they developed in different habitats so they would have different nutrient compositions, but *Ae. albopictus* has been found to have a lower tolerance to polluted waters (Murrell & Juliano, 2008), while *Cx. quinquefasciatus* has a higher tolerance. *Culex quinquefasciatus* performs well under polluted environments when compares to *Ae. albopictus*, so there is a possibility that in polluted conditions *Ae. albopictus* forgoes carbon assimilation for survival.

Overall, I found support for my hypotheses that septic and rainwater ditches vary in their species composition, water quality parameters, and nutrient composition of the adults collected around the ditch. There were spatial and temporal limitations to my data collection, and additional research needs to be conducted to show how different ditch types can affect production and distribution of mosquitoes. Understanding the ecological dynamics of the WNV vector, *Culex quinquefasciatus*, is important for control practices within mosquito abatement districts where the species is prevalent. *Culex quinquefasciatus* has been shown, now and historically, to be productive in water with high levels of decomposing organic matter including septic tanks and ditches (Subra 1981, Harbison et al. 2009, Reisen 2012, Yee and Skiff 2015). Septic tanks can be sources of nitrogen pollution of urban waters. Understanding the fate and transport of nitrogen in this system could help create control efforts to help create models to predict larval mosquito presence. Additionally, the knowledge of about how different sewerage strategies can influence mosquito production is important when understanding this mosquito's nutritional needs and will be helpful in serving as evidence of the need for sewage reform in St. Tammany Parish. Although my data did not show differences in the nutrient content of Cx. quinquefasciatus between ditch types, these data only consider total nutrient content (e.g, %N is all forms of nitrogen). Data using a stable isotope approach, where a specific isotope of nitrogen is examined may be able to reveal such differences.

CHAPTER III - SEWAGE AND CULEX QUINQUEFASCIATUS LIFE HISTORY TRAITS

3.1 Introduction

Increased population growth and urbanization has caused aquatic ecosystems to change due to eutrophication of waters from increases fluxes of nutrients, primarily nitrogen (N) and phosphorous (P). Inputs of nitrogen can come from a variety of sources, including fertilizers and wastewater discharge from septic tanks. In St. Tammany Parish, the majority of septic systems are aerobic treatment units (ATUs). These units are a good alternative method for wastewater treatment if either centralized sewage systems are not available or if the soil in the area cannot support drain fields to filter septic wastewater effluent. These units feed oxygen into the sewage treatment tank after the sewage has been separated into layers. As a result, aerobic microorganisms thrive in this environment and can break down any remaining organic matter from the effluent before it is discharged from the tank. Advantages of using ATUs include efficient wastewater treatment (Lusk 2017), less space taken up by the septic system, and reduced odors associated with wastewater treatment. There are also disadvantages associated with ATUs. Aerobic treatment units are generally more expensive to purchase, install and maintain due to having more complex parts than conventional septic tanks since ATUs require electrical components to pump oxygen into the tank. If any of the components in an ATU fail, it can cause polluted wastewater to drain into surrounding waterbodies including ditches, streams, and ponds.

Discharge of untreated sewage can cause several environmental and public health problems. Improperly treated sewage can be a source of N and P into nearby waters. This can cause eutrophication of waters which can cause harmful algal blooms, harm to naturally occurring aquatic predators and aquatic biodiversity, and provide excess nutrients that benefit medically important or nuisance species of mosquitoes (e.g., *Cx. quinquefasciatus*).

Adults of the mosquito species *Cx. quinquefasciatus* have been known to prefer to oviposit in waters that have a high concentration of decomposing organic matter associated with septic tanks, septic ditches, and sewage drains (Subra 1981, Harbison et al. 2009, Reisen 2012, Yee et al. 2015). *Culex quinquefasciatus* adults have been found emerging (Irving-Bell, 1987) and occupying (Kay et al., 2000) septic tanks. In Puerto Rico, Mackay et al., (2009) investigated the dynamics of the mosquito species *Ae. aegypti* and *Cx. quinquefasciatus* in septic tanks. Their data showed that septic tanks produce large numbers of *Ae. aegypti* and *Cx. quinquefasciatus* throughout the year and the production numbers of *Ae. aegypti* were 3 to 9 times larger and produced adult size were significantly larger than in surface containers. Following Mackay in Puerto Rico, Burke et al., (2010) investigated the relationship of structural variables of septic tanks and the chemical properties of water and immature mosquito abundance. They found immatures of *Cx. quinquefasciatus* in 74% of septic tanks and larval presence was negatively associated with TDS, whereas larval abundance was positively associated with cracked septic tank walls.

Nutrient pulses from septic systems into ditches cause a change in habitat quality and nutritional resources for native fauna. Most organisms are negatively affected by these pulses of N and P due to increasing the concentration of these nutrients in the water. This can render the habitat unsuitable for many organisms due to not being able to tolerate large changes in nutrients. However, some species of mosquito larvae are more likely to benefit from the changes in N concentration. Nitrogen is a limiting factor in many types of aquatic habitats, and increasing the N concentration can also increase the amount of basal resources available for mosquito larvae (Mogi and Okazawa, 1990). Understanding how nutrient pulses affect the life history traits (e.g., mass, development time, percent survival of certain mosquito species is important for understanding the population dynamics and transmission of vector-borne diseases.

3.2 Goal of Chapter

The goal of this chapter is to investigate the effect of different water sources on the life history traits of the southern house mosquito, *Culex quinquefasciatus*. I reared *Culex quinquefasciatus* larvae in four water treatment levels: 100% septic water, 50% septic water, 100% rainwater, and reverse osmosis [RO] water, with grass as a food source (0.01g/larvae) to investigate the effect of sewage on their life history traits (size, % survival, development time), population growth (estimate of the finite rate of increase, λ'), and nutrient signatures (C, N).

3.3 Research Questions and Hypotheses

I tested the following research questions and hypotheses:

I. How does rearing *Cx. quinquefasciatus* on different water treatment levels affect their life history traits?

   a. Specifically, what affect does water type (septic vs. rainwater) have on the size (mass in mg) of *Cx. quinquefasciatus* adults?

      i. H_A: Water type will show a significant difference in the size (mass in mg) of produced *Cx. quinquefasciatus* adults.

ii. H_o: Water type will show no significant difference on the size (mass, mg) of produced *Cx. quinquefasciatus* adults.

b. What affect does water type (septic vs. rainwater) have on *Cx. quinquefasciatus* survival (%) from egg to adulthood?

i. H_A: Water type will show a significant difference on the survival of *Cx. quinquefasciatus* from egg to adulthood.

ii. H_o: Water type will show no significant difference on the survival of *Cx. quinquefasciatus* from egg to adulthood.

c. What affect does water type (septic vs. rainwater) have on *Cx. quinquefasciatus* development time from egg to adulthood?

i. H_A: Water type will show a significant difference on the development time of *Cx. quinquefasciatus* from egg to adulthood.

ii. H_o: Water type will show no significant difference on the development time of *Cx. quinquefasciatus* from egg to adulthood.

d. What affect does water type (septic vs. rainwater) have on *Cx. quinquefasciatus* population growth?

i. H_A: Water type will show no significant difference on the population growth of *Cx. quinquefasciatus*.

ii. H_o: Water type will show a significant difference on the population growth of *Cx. quinquefasciatus*.

e. Are there differences in nutrient (C, N) signatures of adults produced from septic water verses rainwater?

i. H_A: There will be a significant difference in nutrient signatures (C, N) of *Cx. quinquefasciatus* adults produced.

ii. H_o: There will be no significant difference in nutrient signatures of *Cx. quinquefasciatus* adults produced.

3.4 Materials and Methods

3.4.1 Methods

Egg rafts were collected by placing black bins filled with oviposition water near ditches that are known to have *Cx. quinquefasciatus* larvae. Oviposition water was made by mixing 1 tbsp of fish meal (Earth Safe Organics) mixed with 3.8 L of water. Bins were left out over-night and rafts were collected the next day.

Water types consisted of water collected from septic and rainwater ditches in STP and RO water. I collected septic water from three ditches in areas of the parish with existing septic ditches. Water was filtered through a sieve (106 µm) into a 5-gal bucket and then sealed. with the same process was used to collect water from three rainwater ditches. Egg rafts and sealed buckets of ditch water were brought back to The University of Southern Mississippi in Hattiesburg, MS to conduct the experiment.

Four water levels (100% septic water, 50% diluted septic water, 100% rainwater, and RO water) were crossed with two densities of larvae (i.e., 10, 20), with combination replicated six replicates resulting in a total of 48 microcosms and 720 larvae. Two densities of larvae were chosen because density-dependent competition has been shown to influence *Culex* larval mosquito performance (Agnew et al., 2000, Alto et al., 2012).

Each microcosm additionally received grass as a detritus source. I chose grass because grass has been shown to be a suitable detrital source for *Culex* larvae (Costanzo

2014) and is commonly found in rainwater and septic ditches (personal observation). Grass was freshly cut and collected from St. Tammany Parish, LA, and dried in an oven at 50 °C for > 48 h. I allocated 0.01 g of grass detritus per larvae, and this number was based off previous research (Costanzo 2014, Murrell and Juliano 2011, and Yee et al. 2007) and preliminary runs of this study. To isolate the effect of density and not resources microcosms with 10 larvae had 0.1 g of dried grass and microcosms with 20 larvae had 0.2 g of dried grass.

Two days prior to larval introduction, 400 mL tri-pour beakers were filled with 239 mL of water. I added the grass detritus, and each treatment received 1 mL of inoculum collected from a rainwater ditch to allow for microorganism growth needed for larval development. Microcosms were kept in an environmental chamber (Percival Scientific, Inc., Perry, IA, USA) at 28 ºC on a 12h:12h light:dark cycle to duplicate average mid-summer conditions found in south Louisiana. Because I used field collected egg rafts, and there is no way to identify 1st instars of *Culex* larvae to species, I allocated a single egg raft to each replicate and identified the larvae when they reached the 3rd/4th instar stage; replicates with non-target species were discarded. Egg rafts were placed in RO water in an environmental chamber for 24 h to hatch, and 1st instar larvae were transferred to the microcosms. Larvae were allowed to develop to pupae. Water levels in the microcosms were checked daily and refilled to 400 mL using the respective water type. I rotated the microcosms within the incubator to homogenize intra-incubator variation as well as check for pupae. Pupae were removed daily and placed individually into 0.25 mm dram shell vials until the pupae eclosed into an adult. As adults emerged, development time and sex were recorded. I dried the adults in an oven at 50°C for > 48 h.

Size was determined by measuring the dried weight of the adults to the nearest 0.0001 mg using a XP2U ultra-microbalance (Mettler-Toledo Inc., Columbus, OH, USA). Three females were selected from each replicate to be analyzed for carbon and nitrogen stoichiometry using methods from Chapter 1.

3.4.2 Population Growth Estimate

Population growth is estimated by calculating an estimate of the finite rate of increase (λ') (lambda prime) [λ = exp(r)], where r is the per captia rate of population change (dN/N dt). Lambda prime values are used to estimate the effect of treatments on population performance for mosquito species in the genera *Aedes* (e.g., Juliano 1998, Lounibos et al., 2002, Yee et al., 2007) and *Culex* (Costanzo et al. 2011). The estimated finite rate of increase is calculated using the following formula:

$$\lambda' = \exp(r') = \exp \frac{\ln [(1/N_o) \sum_x A_x f(w_x)]}{D + [\sum_x x A_x f(w_x) / \sum_x A_x f(w_x)]}$$

where r' is an estimate of r derived by Livdahl and Sugihara (1984), No represents the initial number of females in a cohort (assumed to be 50%), A_x represents the number of females eclosing on a particular day (x), *D* represents the amount of time (days) that it takes for a newly eclosed female to mate, obtain a blood meal, and oviposit (assumed to be 10 d for *Culex* (Vinogradova, 2000), *f(wx)* represents a function relating mosquito fecundity to adult female size (*f(wx)* based on regressions in the literature. For C*x. quinquefasciatus*, regressions relating female mass to fecundity are not available, so instead a function relating wing length (l) to fecundity [f(l) = -123.88 + 90.311] (McCann et al., 2009) was modified using regressions using wing length and female mass. When solved for wing length, the regression for wild *Cx. quinquefasciatus* is l = [(w + 0.162)/0.021]1/3 (Allgood and Yee, 2014). This wing length regression is substituted into the fecundity function to give the function f(w) = -123.88 + 90.31*[(w + 0.162/0.021]1/3 relating mass to fecundity for wild *Cx. quinquefasciatus*. Values of λ′ > 1 indicates the population size is increasing, whereas a λ′ value of < 1 indicates the population size is decreasing, and λ′ equal to one indicates a stable population.

3.4.3 Statistical Analyses

All raw data were checked to ensure it meets the assumptions of parametric tests. A multivariate analysis of variance (MANOVA) was used to test for effects of water (4), food (2), and density (2) as well as their interaction on the dependent variables (i.e., male and female development time, mass, and survivorship to adulthood). Standardized canonical coefficients (SCCs) were used to find the important variables accounting of observed multivariate effects (Scheiner 2001).

Raw values of λ′ did not meet the assumptions of normality or homogeneous variances for parametric tests. Because no simple transformation of λ′ values met the appropriate assumptions, a randomization ANOVA (Manly 1991, Cassell 2002) was used to test the effects of water type (4) and larval density (2) on λ′ values. This test allows comparison of the original data arrangement to the random rearrangement of data (Cassell 2002) by using randomized subsamples (n = 2000) while preserving order of the independent variables (water type and density). Pairwise differences among means were found by using Tukey’s mean separation.

A Multivariate Analysis of Variance was used to test the effects of 3 independent variables (water type, sex, larval density) on mosquito adult stoichiometry (C, N, and C:N). When checking for assumptions of parametric tests, the raw stoichiometric data met the assumption that the variances are equal but did not meet the assumption of normality. When transforming the data (both sqrt and log), the assumption of normality was not met. Because the variances were equal in the raw data, we decided to use it since a Multivariate Analysis of Variance (MANOVA) is relatively robust to deviations of normality. Standardized canonical coefficients (SCCs) were used to find the important variables accounting of observed multivariate effects (Scheiner 2001).

3.5 Results

3.5.1 Life history traits

There was a significant effect (Pillai's Trace: 1.019, $F_{12,90}$= 2.86, $p < 0.001$) of water type on life history traits (survival, development time, and mass). However, there was no significant effect of larval density (Pillai's Trace= 0.274, $F_{4,28}$= 1.916, $p = 0.136$) or the interaction of water type and density on life history traits (Pillai's Trace = 0.298, $F_{12,90}$= 0.827, $p = 0.136$).

For effects of water type on mass (mg) from eclosed adult *Cx. quinquefasciatus*, there was a significant difference in male and female mass. For males, RO water produced smaller adults than rainwater, full septic, and diluted septic (Fig 3.1). There was also a significant difference in mass between males produced from full septic and rainwater. For females, the control water type produced smaller adults than the rainwater, full septic, and diluted septic (Fig 3.1).

For effects of water type on percent survival from egg to adult, there was a significant difference between RO water and both full septic and the diluted septic, with survival being higher in both diluted and full septic water. (Fig. 3.2) compared to RO water with rainwater as intermediate.

For female development time, there were differences across water types, with female development time higher in RO water compared to full septic, diluted septic, and rainwater (Fig. 3.3).

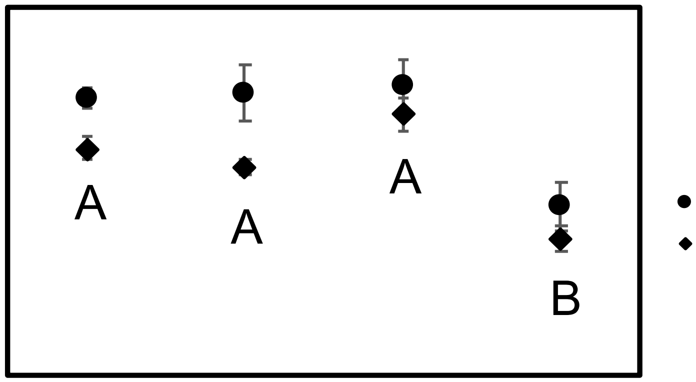

Figure 3.1 Mass (mg) (mean ± SE from 6 replicates) of adult male and female *Cx. quinquefasciatus* across different water types. Means which share a letter are not significantly different.

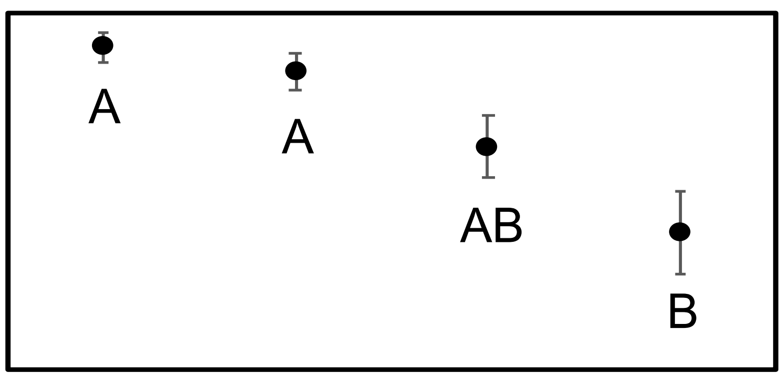

Figure 3.2 Percent (mean ± SE from 6 replicates) of *Cx. quinquefasciatus* larvae that survived to adulthood across different water types. Means which share a letter are not significantly different.

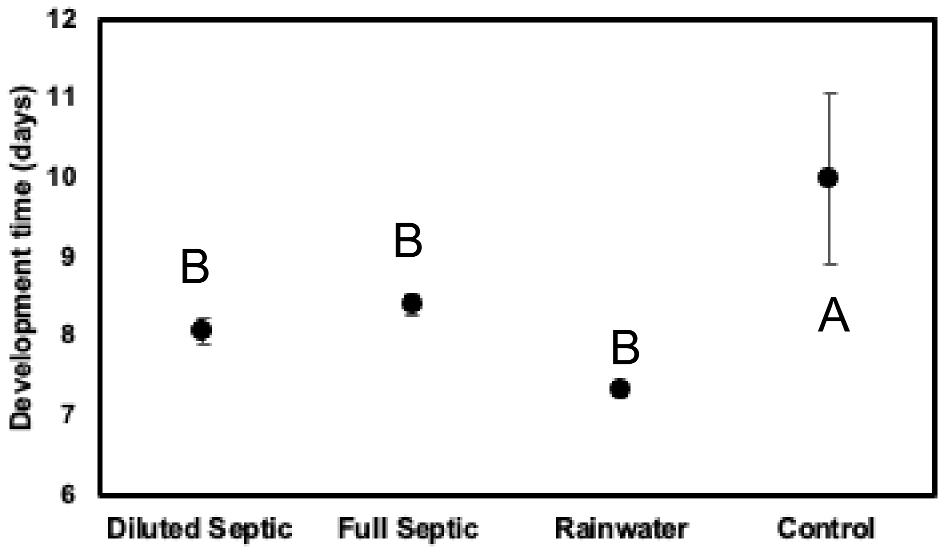

Figure 3.3 Development time (days) (mean ± SE from 6 replicates) from egg to adult for female *Cx. quinquefasciatus* across different water types. Means which share a letter are not significantly different.

The randomization ANOVA on λ′ showed a significant effect of water type ($p < 0.0001$) but no significant effect of larval density ($p = 0.676$) or the interaction of water type and density ($p = 0.802$). Results of mean separation showed the control water type was significantly different than both the diluted septic and rainwater ditch water and trending towards significance for the full septic water type (Fig 3.4).

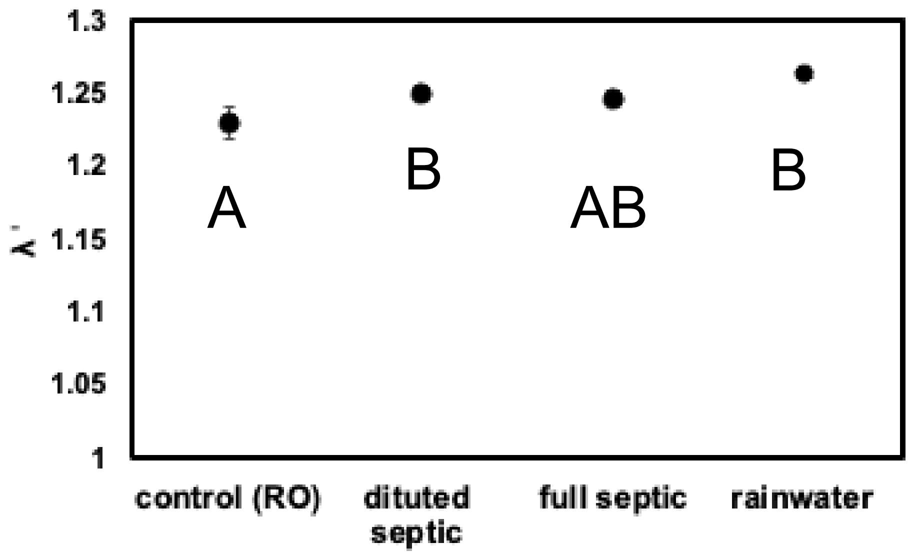

Figure 3.4 Values of the population growth estimate (λ′) (mean ± SE from 6 replicates) for *Cx. quinquefasciatus* females across different water types. Means which share a letter are not significantly different.

3.5.2 Stoichiometry

For the MANOVA on adult C, N, and C:N, there was a significant effect of water type (Pillai's Trace= 0.314, $F_{9,195}$= 2.53, p = 0.009), sex (Pillai's Trace=0.194, $F_{3,63}$ = 5.06, p = 0.003), and larval density (Pillai's Trace= 0.129, $F_{3,63}$ = 3.11, p = 0.032) on adult mosquito stoichiometry. There are significant effects of the interaction of water type and sex (Pillai's Trace= 0.267, $F_{9,195}$= 2.21, p = 0.029) and the interaction of density and sex (Pillai's Trace= 0.136, $F_{3,63}$ = 3.31, p = 0.026). There were non-significant effects of the interaction of water type and density (Pillai's Trace= 0.245, $F_{9,195}$= 1.93, p = 0.050) and the interaction of water type, density, and sex (Pillai's Trace= 0.212, $F_{9,195}$= 1.65, p = 0.105).

For the interaction of water type and sex on C, N, and C:N stoichiometry within water level significant differences were due mostly to C:N and not for C or N (Table 3.1). There was a significant difference between males and females produced from rainwater, with males having higher C:N values than females (Fig. 3.5). There was also a significant difference in C:N values between males and females produced from full septic, with males having higher C:N values compared to females (Fig. 3.5). There was no significant difference of C:N between males and females produced from RO water or diluted water. There was also a significant effect of the interaction of density and sex, with males in the higher density having a higher C:N (3.96 ± 0.02) than females in the higher density (3.83 ± 0.02), females in low density (3.82 ± 0.02), and males in low density (3.85 ± 0.02).

Table 3.1 *Values of %C, %N, and C:N (mean ± SE for 6 replicates) by weight of produced male and female Cx. quinquefasciatus across different water treatment levels.*

	C		**N**		**C:N**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
RO Water	44.93 ± 1.10	43.14 ± 1.10	11.87 ± 0.30	11.17 ± 0.30	3.79 ± 0.04	3.86 ± 0.04
Rainwater	43.97 ± 0.82	44.74 ± 0.82	10.94 ± 0.22	11.58 ± 0.22	4.03 ± 0.03	3.87 ± 0.03
Diluted Septic	43.36 ± 0.78	42.18 ± 0.78	11.13 ± 0.21	11.14 ± 0.21	3.89 ± 0.03	3.79 ± 0.03
Full Septic	44.34 ± 0.78	43.61 ± 0.82	11.33 ± 0.21	11.54 ± 0.22	3.92 ± 0.03	3.78 ± 0.03

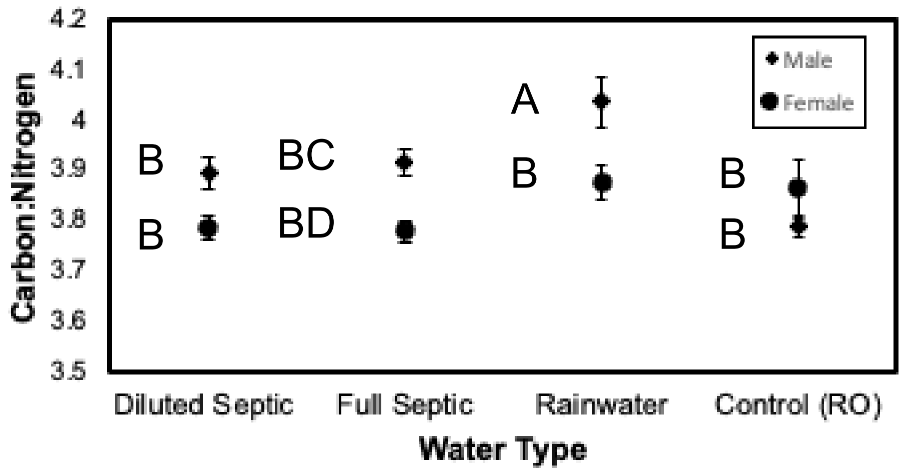

Figure 3.5 *Values of C:N ratio of produced male and female adults across different water types.*

Note: Values are means ± SE of 6 replicates. Values that share a letter are not significantly different at $p < 0.05$.

3.6 Discussion

My hypothesis that rearing *Cx. quinquefasciatus* larvae in difference water types would show a significant difference in their life history traits was supported. Specifically adult male and female mass was highest in all water types compared to control (Fig 3.1). Understanding how body size can influence other life-history traits of *Cx. quinquefasciatus* is of importance to public health because research suggests that body size can affect mosquito vector competence for pathogenic infections for certain species-virus combinations. Paulson and Hawley (1991) found that *Aedes triseriatus* larvae that were nutritionally deprived developed into small adults that were more susceptible to LaCross (LAC) virus infection and were able to transmit the virus more frequently than larger adults from well-fed larvae. The same pattern has also been shown in *Culex tritaeniorhynchus* and Japanese encephalitis virus (Takahashi 1976), WNV (Bagar et al, 1980), LaCrosse encephalitis (LAC) virus (Grimstead and Walker 1991), and with *Aedes aegypti* and Zika virus (Paige et al. 2019). However, conflicting research shows that larger females are more susceptible to infection than smaller females in *Aedes aegypti* and Ross River virus (Nasci and Mitchell 1994). Mosquitos that were produced from all water types other than RO water produced larger adults, this is probably because both rainwater and septic ditches receive inputs of nutrients from allochthonous detritus, autochthonous detritus, and water run off which serve as the nutritional basis for microorganisms needed for mosquito growth.

Water type also had a significant effect on the percentage of adult *Cx. quinquefasicatus* that survived from each treatment. Adult survival was significantly higher in full and diluted septic (>80%) than RO water (40%) with rainwater as an intermediate (60%). Water bodies that contain inputs from sewage contaminated water can cause increased pulses of nutrients required for mosquito development and survival, specifically increases in reduced biological forms of nitrogen (e.g., NH_4^+) (Chavez et al. 2010). Nitrogen is typically a limiting resource for the bacteria that form the base of the mosquito food chain (Fish and Carpenter 1982), so increasing the amount of nitrogen available likely increased the basal resources needed for mosquito survival from egg to adult. Previous research found that the addition of nutrients to the water that mosquito larvae develop in increases the survival of larvae and accelerates larval development (Reiskind et al. 2004) whereas other research suggest that survival response is dependent on the type and amount of nutrients that are added (Noori 2015). Even though rainwater ditches do not receive inputs of sewage, they still receive inputs from surrounding detritus and sediments in the ditch itself, which would help increase mosquito survival in comparison to the RO water.

In addition to mass and survival, the data support my hypothesis of a significant difference in the amount of time it takes for *Cx. quinquefasciatus* eggs to hatch and develop into adults across treatment levels. Development time was significantly higher (i.e., took longer) in the control water compared to the full septic, diluted septic, and rainwater water treatments (Fig. 3.3). Previous research shows that the addition of nutrients to water can increase larval survival and accelerate larval development time (Reiskind et al. 2004). Additionally, *Cx. quinquefasciatus* larvae that were reared in water collected from a combined sewage overflow stream developed faster and had larger adult body sizes than their tap water control (Chaves et al. 2009). Septic and rainwater ditches have inputs of nutrients from the surrounding environment, whether from sewage or detritus, and these nutrients help increase the rate at which mosquito larvae will develop.

I also found differences in population growth across water types, with the data supporting my hypothesis that different water types will show a difference in the estimated population growth of *Cx. quinquefasciatus* (Fig. 3.4). In the control water type, the λ'values showed that those adults will have a lower population growth than the adults in the diluted septic and rainwater water treatment. There was also a marginally non-significant difference in the λ' values between the control treatment and the full septic treatment. This suggests that population levels of *Cx. quinquefasctiaus* production could be higher in areas that have ditches, including both rainwater ditches and septic ditches, however in all cases we would expect populations to be increasing.

The data support my hypothesis that difference water types will produce adults with different carbon and nitrogen nutrient signatures (Table 3.2). There are differences in the C:N ratio between males and females in the full septic and rainwater ditch water type, with males having a higher C:N ratio than females (Fig. 3.5). When looking at the nitrogen values between sexes, the values were lower in males than females. There is not a lot of research investigating sex – based stoichiometric differences in adult mosquitoes, but my results are consistent with Yee and coauthors (2015), which showed that males have higher C:N values (i.e., lower N) across differing detrital types. When comparing stoichiometric values for N and phosphorous (P) between sex in the species *Anopheles arabiensis*, Hood-Nowotny (2012) found that females had double the requirements for P than males and males and females had differences in their fatty acid composition. These differences could be related to sex requirements for reproduction. Other research has shown differences in some life history traits between males and females of the same species. In many arthropods, males are proandric, with adult males developing and emerging faster than adult females. (Kleckner 1995). Because of this, we can predict that males in females would vary in their nutrient assimilation from microorganisms in detritus. Because males develop faster and are smaller in mass than females, they may reach their nutrient threshold for nitrogen quicker than females and forgo nutrient assimilation for faster development times. Females may also have higher nutrient thresholds than males as they select for maximum size and development time and need to allocate resources for reproduction (Hood-Nowotny et al. 2012) Similarly, my data also show a significant effect of density and sex on C:N ratios. Males in the higher density treatments had a higher C:N ratio than males in the low-density treatment and females in both the low density and high-density treatments. Having a higher C:N means that the N values are lower in males in the high density treatments. In high density larval environments, males may have had to compete for the limited amount of nitrogen available.

This study shows the effects of water type in ditches on the life history traits, population growth, and carbon and nitrogen nutrient signatures of *Cx. quinquefasciatus*. St. Tammany parish has over 600 miles of septic ditches due to many houses using individual aerated treatment units as their method of wastewater treatment. If these units are damaged or improperly maintained, they can cause direct point pollution of untreated sewage into the ditch. Untreated sewage has high levels of N and P, and inputs of these nutrients into surrounding fresh water can have harmful environmental effects including harmful algal blooms, harming naturally occurring aquatic predators, loss of aquatic diversity, and increased production of mosquitoes. Female *Cx. quinquefasciatus* are known for ovipositing in and being tolerant of highly organic waters, and research has shown that mosquito larvae develop faster (Curtis and Feachem, 1981) and have larger body size (Mackay, 2009) when in polluted waters compared to non-polluted. My data shows that when in polluted waters (whether full or diluted septic, or rainwater) larvae developed faster, had higher survival, larger mass, and higher estimated population growth in comparison to the mosquitoes from RO water.

Culex quinquefasciatus is the main vector of multiple medically important pathogens, such as WNV. Calhoun and coauthors (2007) found that *Cx. quinquefasciatus* is the dominant species in CSO streams, and there has been an association between WNV presence and proximity to streams polluted with CSO (Vazquez-Prokopec et al. 2010). In the past decade, he majority of arbovirus activity in ATP has been due to WNV. Ditches in STP produce large numbers of *Cx. quinquefasciatus* during the summer months and understanding how polluted waters can affect their life history traits is of importance to mosquito abatement districts. Septic water produces larger females that develop faster and have a higher chance of survival and that can influence WNV presence in the parish. More research needs to be conducted to continue to investigate the effect of polluted waters on life history traits in *Cx. quinquefasciatus*, including how water with untreated sewage could affect its vector competence for medically important pathogens. These associations have been relatively unexplored but are very important in understanding how untreated sewage could affect human public health risk.

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