MOSQUITO SURVEY OF CAMP GRUBER TRAINING CENTER AND COMPARISON WITH OTHER OKLAHOMA MILITARY BASES

By

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Abstract: Abstract: Army National Guard training requires personnel to occasionally visit wild areas for one weekend a month and two weeks a year. During these times, the soldiers may be exposed to mosquitoes and be at risk of mosquito-borne pathogens. Our survey investigated mosquitoes inhabiting Camp Gruber Military Training Base, and in the adjacent town of Braggs, OK. Thirty traps (10 BG Sentinel Traps and 20 modified CDC light traps) using CO2 as attractant were emplaced every other weekend for 48 hours (Friday morning and-Sunday morning) for 23 collection periods between mid-April and late October 2018. A total of 10,405 mosquitoes from 7 genera and 26 species were collected, representing about 40% of the 64 species known from Oklahoma. The majority (98.4%) of mosquitoes were collected from CDC traps, which mainly collected Culex spp. (71.8% of total). Of the BG trap-collected mosquitoes, 40.2% were Aedes spp. The most abundant species Culex, of which some can transmit West Nile Virus. A single adult *Aedes aegypti* was captured on the base in August, and was genetically similar to a single larva captured in May. Compared with other military bases in Oklahoma, Camp Gruber had the only *Ae. aegypti*, and the most *Ae. vexans*, *Cx. erraticus*, and *An. quadrimaculatus* among bases. Tinker Air Force Base and Fort Sill Army Base had the most *Ae. albopictus* collected. The capture of *Ae. aegypti* along with other important disease vector species highlights the need for regular season-long monitoring to protect troops during training. Information generated from this study was used to provide mosquito warnings for troops training on base, and as well as a mosquito management plan for Camp Gruber.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Mosquito Systematics and Identification

Mosquito classification at the species level is subject to change often as more accurate genetic identification techniques are developed (Bui and Darsie, 2008). Mosquitoes can be differentiated from other dipteran groups by the possession of scales on the wing veins and a long proboscis that is adapted for piercing and sucking. Scales are also found on the thorax and are one of the principle structures used for identification. Unlike the setae that are also on the body of adult mosquitoes, scales are flat and widen from the base to the end, have longitudinal ridges and ae easily dislodged. These scales have three basic forms, flat and broad, curved and narrow, and upright with an apical fork. Scales located on wing fringes are classified as fusiform. These scales are a variety of shades of black, brown and gold, different yellows, or white and silvery and the color along with the pattern that is formed allow female mosquitoes to be identified to species (Ward 2005). However, identification of male mosquitoes by scale patterns and damaged specimens that have lost scales are more difficult to determine morphologically and often require molecular analysis for confirmation.

Mosquito Development

Mosquitoes develop through a holometabolous life cycle, with four stages: egg, larvae, pupa, and adult (Ward 2005). Mosquitoes spend their larval stages in an aquatic environment such as lakes, ponds, flood plains, puddles, and natural containers such as tree holes, as well as manmade structures that hold water. Some groups, including floodwater mosquitoes such as *Ae. vexans*, require a specific series of events to initiate hatching of eggs. Drying, aeration, submergence, and deoxygenation of the water are all required in that order to stimulate the eggs to hatch (Horsfall, 1956).

Mosquitoes have upper and lower temperature thresholds for ideal physical development, with fastest development occurring near the upper limit (Eisen et al. 2014). The range of suitable developmental temperatures can be wider for male mosquitoes (Loetti et al. 2011). The best development temperature can decrease slightly for some species as the mosquito progresses through each larval instar and becomes a pupa (Huffaker 1944) Some evidence also suggests that higher temperatures create higher competition for resources among mosquito larvae (Amarasekare and Coutinho, 2014), which can affect adult size and fecundity (Peters and Barbosa, 1977). Density-dependent competition is prominent for mosquito larvae. Higher densities of larvae decreases survival when nutrients are limited (Alto et al. 2012). Competition for nutrients affects vector competency of some mosquitoes. For example, low nutrient availability increases *Ae. albopictus*' competency to transmit dengue, but decreases *Ae. aegypti's* competency for the same disease (Alto et al. 2012).

Mosquito Transmitted Diseases

Insect transmitted diseases take a large toll on the world's human population, infecting approximately one billion people and causes millions of deaths every year Mosquitoes contributed to 17% of all human diseases in 2016 (Wang et al. 2016). However, it is important to note that only certain species of mosquitoes can transmit specific diseases (Dodson et al. 2018).

Even without a disease threat, mosquitoes can render outdoor spaces unusable when they occur in high numbers. A mosquito bite can cause itching, irritation, allergic reaction, general pain, and in some cases lead to secondary infection. Mosquito feeding activity also negatively impacts livestock by decreasing weight gain and milk production in dairy cows (Islam et al. 2017).

Human activities often enhance mosquito problems. Climate change and rising global temperatures will impact the future of mosquito transmitted diseases (Reiter 2001; Eisen and Moore 2013; Morin and Comrie 2013; Eisen et al. 2014; Equihua et al. 2017). This will inevitably lead to new disease pressure for more temperate regions when historically most of the world's insect transmitted diseases were confined to tropical regions (Ajelli 2017).

As global temperatures rise, areas usually geographically north of regions with endemic mosquito disease could seasonally or permanently experience an infiltration of mosquitoes and their associated pathogens. This pattern has been observed with other arthropod vectors such as *Ixodes spp.* ticks moving north into Canada (Eisen and Moore 2013). As a result of mosquito distribution changes, more areas will likely experience the establishment of newly arrived diseases, placing more of the world's population in danger (Eisen and Moore 2013; Equihua et al. 2017). For example, northern Italy has recently experienced outbreaks of mosquito borne diseases, including Chikungunya in 2007, when historically this location has not had any mosquito transmitted diseases (Ajelli 2017).

In addition to latitudinal expansion of mosquitoes, climate change also allows movement upward in elevation. Some cities, such as Xalapa, Mexico, that are at higher elevations are under new threat from dengue as their locations become able to support mosquitoes (Equihua et al. 2017). Additionally, higher temperatures can cause earlier emergence of mosquitoes and longer seasonal activity (Morin and Comrie 2013; Eisen et al. 2014). Warmer average temperatures may also allow more mosquito eggs to survive the winter, contributing to higher populations in the spring.

As human populations increase in underdeveloped countries, these governments are often unable to meet resource demands including access to clean running water (Eisen et al. 2014). This increases the need for homemade water storage, creating more mosquito habitat in close proximity to human dwellings. Housing in these countries often consists of open-air dwellings, allowing mosquitoes to enter, thereby compounding exposure to disease (Reiter 2001; Gubler 2011; Eisen and Moore 2013; Eisen et al. 2014). Socioeconomic factors are often linked with increases in mosquito transmitted disease cases. Unfortunately, many of the world's developing nations, which have the largest population growths, are also located in tropical areas with endemic mosquito-transmitted diseases (Reiter 2001; Eisen and Moore 2013; Eisen et al. 2014). Immigration can further exacerbate the spread of disease. For example, southern California, which has immigration from countries in Central and South America, has experienced cases of mosquito-borne disease that have been transported over the border by travelers (Porse et al. 2015).

In addition to natural range expansions in response to climate change, travel and transport across vast distances over the sea and through the air can also establish species in new areas (Ajelli 2017). Even the most remote islands in the South Pacific have established mosquitoes as a result of travel and trade (Nunn et al. 2011). Tourists who become infected can also bring back mosquito-transmitted diseases such as Zika, which has been reported in the United States and Brazil (Wang et al. 2016; Mainali et al. 2017).

Arthropod Vectors of Disease and Their Effects on Militaries

Throughout history, vector-borne diseases have hampered the ability of armies to wage war. However, after WW II, because of the discovery of penicillin, infectious disease were no longer the main cause of mortality for soldiers (Pages et al. 2010). Today, military personnel still face the threat of vector-borne disease during training, time at duty stations, and deployments (Iwakami and Reeves 2011). More recent conflicts involving Western armies traveling overseas has increased the risks to soldiers as well as for the human populations in their country of origin when they return home (Pages et al. 2010; Reeves and Bettano 2014). Some diseases, like bubonic plague, have become less important because of antibiotics and vector control. Others, like malaria, still hamper operations in much of the tropics (Pages et al. 2010). Because of this dynamic between the soldier and transmitted diseases, it is important to revise and implement new control strategies to protect military personnel abroad and at home.

As control strategies are implemented, insects and the disease organisms that they may carry adapt. The Vietnam War highlighted resistance to the contemporary treatments for the malarial parasite, which subsequently caused unsustainable losses. Poor compliance with prophylaxis, lack of repellent use, the improper use of chemical-infused uniforms, and the lack of the ability to perform environmental control resulted in outbreaks of malaria among many western troops, including those from America (Pages et al. 2010).

Mosquitoes, ticks, chiggers, sand flies, and biting midges all pose a threat to military personnel (Kitchen et al 2009; Lawrence and Coleman 2009; Pearce and Grove 1987). Biting and stinging can be an irritation even without disease risk. In addition to transmitting disease, arthropods can cause dermatitis, allergic reactions, and sleep loss (Kitchen et al. 2009).

Lyme disease caused by the bacteria *Borrelia burgdorferi* is the most common vector-borne disease in both U.S civilians and military personnel (Rossi et al. 2015). It is transmitted by the tick *Ixodes pacificus* in the western U.S. and *Ixodes scapularis* east of the Rocky Mountains. Military service and training exercises exposes troops to more ticks and mosquitoes than the average civilian (Rossi et al. 2015). A study from Australia found that some soldiers carried up to 100 tick nymphs after field exercises (Pearce and Grove 1987). States such as Arkansas and New Jersey have experienced notable tick disease outbreaks (Yevich et al. 1995).

Fort Chaffee, Arkansas, provides the species name for the bacteria, *Ehrlichia chaffeensis* that was described following a mysterious outbreak among National Guard soldiers (McCall et al. 2001). Fort Campbell, Kentucky, has also been monitored. There, the American dog tick, *Amblyomma americium*, transmits both *Borrelia burgdorferi* and *E. chaffeensis* within base borders (Murphree et al. 2009). Tick-borne disease can also be spread through troop movements. For example, U. S. Air Force personnel were bitten by ticks in Arkansas and then experienced symptoms that were diagnosed upon returning to Arizona (Warner et al. 1996). In locations were tick-borne diseases are endemic, the use of permethrin-treated uniforms has reduced tick bite likelihood (Yevich et al. 1995), but not all troops have access to these uniforms.

Another disease that impacts U.S. military personnel, particularly in southern states bordering Mexico, is Chagas disease (Harris et al. 2017). Chagas is caused by the protozoan *Trypanosoma cruzi* and is transmitted by triatomine bugs via excrement that is rubbed into feeding sites (Harris et al. 2017). Triatomine bugs can be abundant on military bases, but to date only a couple of species have been found positive for Chagas disease. It is suggested that military dogs, which can serve as blood meals and hosts, play a role in perpetuating Chagas on certain military bases (McPhatter et al. 2012).

Mosquitoes are not the only dipteran threat on military personnel. Conflicts in the deserts of the Middle East have highlighted problems caused by sand flies. Phlebotomine sand flies fed on soldiers at Tallil Air Force Base, Iraq, transmitting Leishmania, a parasitic protozoan (Coleman et al. 2006). However, only 12

Leishmania tropica cases were recorded during the Gulf War (Hyams et al. 1995). Arthropod transmitted disease was far less of an issue in Iraq than during WW II because of better medical care, preventative medicines, and a drier, more inhospitable environment (Hyams et al. 1995). To control Leishmania, the military applied insecticides targeting sand flies, used residual insecticides on tents and buildings, and destroyed breeding habitats (Coleman et al. 2006).

Because of concern that disease-carrying mosquitoes were being brought back from deployments, U.S. Army surveys of mosquito populations have been conducted on active duty bases since the early years of WW II (Rapp 1959; Foley et al. 2011). These surveys are often used to characterize an entire states' mosquito community (Sames et al. 2007). Mosquito survey data from 1947 to 2009 showed that *Ae. vexans*, *Ps. columbiae*, *Cx. pipiens*, and *Cx. tarsalis* were in the top 10 species collected on military bases and other Department of Defense (DoD) installations (Rapp 1959; Foley et al. 2011). The practice of characterizing an entire state's mosquitoes based on a limited survey could lead to improper control implementations as the phenology and prevalence of arthropodtransmitted disease can vary by regions and within a state (Stromdahl et al. 2014).

Although mosquitoes do not transmit as many diseases as ticks do within in the United States, they are the most important vector of human disease globally. In tropical regions, including southeast Asia, a number of mosquito-borne diseases have affected troops and continue to do so today. For example, during the Korean Conflict there were 300 Japanese Encephalitis Virus (JEV) cases and 30 deaths among United Nations (UN) forces (Kim et al. 2015). Today, mosquitoes are reported as possible vectors for *Rickettsia felis* in Korea (Terenius et al. 2017). In addition, the differences between North and South Korea and the presence of an unoccupied demilitarized zone have allowed continued presence of mosquito-borne diseases that affect troops. For example, between the years of 2001-2011 40% of malaria cases in South Korea, were among military personnel (Yoo et al. 2013).

Wetlands and rice farms maintain large mosquito populations in South Korea. The mountains and somewhat isolated valleys create the potential for focused malaria transmission. One study found that *An. sinensis* was the most common mosquito (70.4%) in the Republic of Korea (ROK) (Kim et al. 2007). Soldiers returning from ROK deployments could bring back *Plasmodium vivax* to U.S. mainland military installations, where relapses could occur. Examples of malarial relapse occurred after the Vietnam

War resulting in malaria (*Plasmodium vivax*) cases in the U.S., increasing civilian cases between 1957 and 1973. Malaria cases among military personnel also increased from 1966 to 1972 (Gibson et al. 1974; Chang et al. 2016) despite the use of chemoprophylactics (hydroxychloroquine; primaquine) among troops (Chang et al. 2016; Coleman et al. 2002).

It is not just military installations within tropical regions that face mosquito-borne disease threats. During Artic summers, swarms of mosquitoes can affect military operations. Thule Air Force Base, located within the arctic circle on an island near Greenland experiences yearly mosquito swarms. Although there was no mosquito-borne disease reported from the base as late as 2013, arboviruses are present in the Artic, Alaska, and Canada (Reeves et al. 2013).

Because of the movement of troops and equipment even massive bodies of water such as the Pacific Ocean are not barriers to mosquitoes. The island of Guam has cases of malaria, dengue fever, Chikungunya, dengue hemorrhagic fever, Japanese Encephalitis, Murry Valley Encephalitis, yellow fever, and filariasis. Higher numbers of military and civilian personnel on the island increase the risk if disease transmission. As the risk of vector-borne disease increases, so does the threat of transporting them from the island (Rueda et al. 2011).

In addition to soldiers being at risk, military working dogs (MWD) are also at risk of contracting vector-borne diseases. These dogs are used to find explosives, drugs, provide security for patrols, participate in search and rescue operations, and provide improved guard duty missions (Bell et al. 2012). The presence of MWDs can increase exposure to arthropod-transmitted disease for the handlers and associated personnel such as Army veterinary personnel (Reeves et al. 2015; Burke et al. 2012). The Vietnam War highlighted the importance of tick-borne diseases to MWDs and their handlers (Bell et al. 2012). The incidence of diseases have been suggested to be increasing in canines that can serve as hosts for some diseases that also affect humans (Alho et al. 2016). Stray dogs around military bases might pose risk to MWDs or police dogs using the base (Bell et al. 2012). MWDs that return from service could also allow new introductions of diseases to military instillations (Alho et al. 2016).

Because of the risks of vector and disease introductions associated with the movement of troops and equipment, the DoD has implemented a number of policies and procedures. Materials and supplies along with the vehicles are required to be inspected at each port or boarder the shipment crosses. However, inspection and cleaning can take long periods, even for small groups of soldiers, and if a foreign material is discovered shipments can be delayed. For example, a flight of C-17 transport aircraft was quarantined and fumigated because of snails (Cofrancesco et al. 2007).

The more time in the field, the more time invasive species have to colonize or stow away in military equipment. Cofrancesco et al. (2007) reports that cleaning programs excel at sanitizing equipment for return to the United States, but notes that some equipment is not always cleaned properly. Certain locations, such as Fort

Campbell, Kentucky, which is home to the 101st Airborne Division and 160th Special Operations Aviation Regiment, are likely at more risk of transporting organism on military vehicles. These special units often get sent to tropical locations in Africa and Latin America, and Fort Campbell houses over 300 military aircraft for troop transport purposes. Upon return, these aircraft provide many opportunities for invasive mosquitoes and other vectors to enter the base (Moore, 1999).

Mosquito Monitoring

Adult mosquitoes are monitored using a number of different methods. These methods have been developed to target specific genera based on their behaviors or responses to different host cues. The BG-Sentinel: Biogents' Professional Mosquito Trap (BG Trap) is a modified laundry hamper designed to capture medically important mosquitoes. The BG Trap consist of the hamper, which is supported by a wire skeleton, giving it an upright, cylindrical shape, with a perforated lid on top that contains a small black tube. At the bottom of this tube is a fan that disperses scent and as a capture device by pulling mosquitoes into the tube. Attracted mosquitoes are not sucked into the fan because of a mesh net that is attached to the bottom of the tube just above the fan. The air current is strong enough to capture mosquitoes on the net but prevents them from being damaged (Rithcie, et al. 2014). Typically, BG Traps are baited with human scented lure, CO_2, or a combination of both (de Valdez 2017). The traps require external power, either a 12V battery or wall outlet (Montgomery et al. 2017) and are favored for the capture of *Aedes spp.* (Ritchie et al. 2014).

A second type of trap, the Modified Center for Disease Control CO_2 light traps (CDC Trap), normally operate using a small light to attract mosquitoes. Similar to the BG Trap, CDC Traps have a battery-powered fan that is located at the top of the trap. The CDC trap captures most mosquito types that are attracted to CO_2 for feeding, and has the advantage of being small, portable, and relatively inexpensive.

Other traps such as the Mosquito Magnet can effectively trap and kill tens of thousands of mosquitoes each night by attracting them with large volumes of CO_2 (Kim et al. 2014). When placed at the corners of an area of approximately 4,050 m^2, a similar trap, the Blue Rhino, greatly reduced biting pressure from mosquitoes (Revay et al. 2013). These types of mosquito traps could be placed at the edges of troop assembly and resting areas for protection during field training exercises. The same study found that the Thermacell Mosquito Repeller device reduced biting pressure by almost 77% in a one square kilometer area, and more than 96% at a distances 4 meters or less (Revay et al. 2013).

Mosquito Management

Before implementing control measures, it is necessary to know if they are needed. Sampling through trapping and comparing the numbers of mosquitos captured to trap indices allows for Integrated Pest Management of mosquitoes. These techniques can be used to decide whether less environmentally friendly and more expensive methods of control, such as pesticide application, should be employed (Kim et al. 2007; Kim et al. 2009; Kim et al. 2009a).

Physical, chemical, and cultural control measures, and more recently, genetic modification have been employed in in an attempt to manage mosquito populations (Fikrig et al. 2017). Chemicals, insecticides, and habitat modification have been the main control methods. However, extensive use of chemical pesticides has led to the development of resistance in the target population. Additionally, the use of broad- spectrum chemicals such as DDT can impact non-target organisms as well as the environment as a whole (Davey and Meisch 1977; Islam et al. 2017).

The use of chemicals on DoD installations must be in compliance with federal regulations from the Environmental Protection Agency (EPA) along with other agencies including the Fish and Wildlife Service (USFWS). Because of the Endangered Species Act military bases such as Camp Gruber that harbor threatened or endangered species, may be limited in the types and frequency of pesticide applications. The federally designated endangered American Burying Beetle (*Nicrophorus americanus*) is present-on this installation (Butler et al. 2012; Jurzenski et al. 2014), limiting control options for adult mosquitoes (Kitchen et al. 2009). Biological pesticides based on naturally occurring compounds that are specific, such as Spinosid made from a soil fungus, *Saccharopolyspora spinose,* could be used in some instances (Pridgeon et al. 2008).

The gram-negative bacteria *Wolbachia* causes many different negative effects on mosquitoes and has been suggested as a biological control method (Suh et al. 2017; Telschow et al. 2017). Results of infection with this bacterium include feminization, parthenogenesis, killing of males, and cytoplasmic incompatibility (Suh et al. 2017). Cytoplasmic incompatibility occurs when infected males cannot fertilize the eggs of noninfected females. Some trains of *Wolbachia* from the fruit fly *Drosophila melanogaster* have been artificially injected into *Ae. aegypti*. This subsequently reduced the life span of these mosquitoes. Perhaps most encouraging of all is the ability of *Wolbachia* to lower the competence of mosquitoes to transmit important human diseases, including Zika, dengue, and Chikungunya (Suh et al. 2017).

Adult mosquitoes require a sugar-based energy source for flight and maintenance. For males sugars like nectar are their source to replenish energy. Because of this requirement, sucrose-based traps to control mosquito populations have been suggested (Fikrig et al. 2017). Unfortunately, artificial, floral-based attractants were not particularly attractive, even when paired with a gravid trap consisting of water infused with decaying vegetation. However 'Attractive Toxic Sugar Baits' that use natural, floral compounds from fruits and flowers combined with sugar, which encourages feeding have shown promise (Fikrig et al. 2017). Toxic Sugar Baits were injected into sponges and attracted males and both gravid and nulliparous females to feed, leading to their deaths (Fikrig et al. 2017).

An alternative source of control for male mosquitoes is to use sounds mimicking the same frequencies created by flying female mosquitoes. Fikrig et al. (2017) showed that sound-based traps could work for attracting males. It is important to note that many of these frequencies are species-specific, so control may only occur for one species of male.

Another proposed control method for mosquitoes is male-sterilization. This technique was successful in controlling screwworm in North and Central America. This technique has been used against mosquitoes but is dependent on how well the sterilized males compete with wild males (Vanickova et al. 2017). Unlike screwworm females that only mate once, female mosquitoes mate many times. Thus, higher numbers of sterile males must be released over longer periods to achieve control.

Often, the best way to protect an individual from mosquitoes is by giving them the means to protect themselves. Topically-applied repellants have long been used to provide personal protection from mosquitoes, and can be used by both military and civilian personal needing protection from mosquito-borne diseases (Islam et al. 2017).

Insect repellants work through interference of the olfactory and gustatory receptors of questing individuals. These odorants have several effects against mosquitoes: repelling, immobilizing, masking human scent, and reducing the number of human blood meals taken (Ray 2015). Unfortunately, these chemical compounds require high application rates on skin and can be expensive. They also contribute to dissolving of nylon and plastic, have unpleasant odors, and leave thick oily residues, which can limit their use (Ray, 2015).

Many repellents rely on the artificially created compound DEET, or N, NDiethyl-meta-toluamide (Revay et al. 2013). However, prior to the discovery of DEET, repellant compounds were derived from plants. Five essential oils from plants have been shown to repel mosquitoes and may have fewer undesirable effects. Results from one study indicated 37 plants from 14 families showed some mosquito repelling ability (Tisgratog et al. 2016). Costs, application rates, delivery methods, and duration of protection still require more research to determine if viable alternatives to DEET exist.

Another of the most common tools used against mosquitoes are bed nets, which can be treated with insecticide. Bed nets are relatively inexpensive, and can provide defense against species that transmit serious diseases. In the case of malaria, insecticidetreated bed nets have been an effective tool at preventing disease transmission, and have reduced childhood mortality by 20% and severe disease by 50% in some areas (Kitchen et al. 2009).

Unfortunately, the nets are often criticized for being too hot, having strong odors, or being against the personnel beliefs of those receiving them. These factors can lead to low usage numbers (Dumont and Thuilliez, 2016). The same qualities that make bed nets a viable counter-measure to mosquitoes in developing countries, makes them a good option for soldiers. Indeed, bed nets were necessary for troops fighting in the south Pacific during WW II (Kitchen et al. 2009). Improvement of bed nets has been in development, such as the SS-LP Bed Net, which resulted in a 97.9% protection rate when tested (Kitchen et al. 2009). In one study, deltamethrin-impregnated bed nets reduced malaria cases at a mock military location by 87% (Joshi et al. 2003).

Just like with civilians who receive bed nets, troops are also sometimes resistant to their use. Complaints about the fine mesh of bed nets restricting airflow and leading to overheating may result in non-compliance in hotter climates, echoing complaints given by civilians (Kitchen et al. 2009). Better educating people about the risks from insectvectored diseases is likely the key to better adoption (Dumont and Thuilliez 2016).

Another form of self-regulated protection against malaria is the use of chemical prophylactics. Normally these medications are taken orally to prevent contracting specific diseases. While these methods are effective in there intended use, compliance with courses is often not enforced (Kim et al. 2009). Additionally, prophylactics often take time to become effective, which is not a problem for soldiers going on planned, long-term deployments. However, the use of these methods for National Guard troops during weekend drills is not realistic. If troops are participating in an annual training event in an area where there is mosquito-disease risk and prophylactics are available for a particular disease, these could be a viable option.

Larval Management

Satellite imagery can also be used to estimate percent greenness, which can be correlated with both mosquito population increases and habitat for mosquito predators (Britch et al. 2008). Land usage is also an indicator of species presence and potential habit for mosquitoes along with potential for predator control of populations. The highest richness of mosquito predators was found in forest and grasslands, and lowest in human altered areas such as pastures (Foley et al. 2017). This relationship is reversed when mosquitoes are considered. Because urban habitats typically have smaller freshwater habitats than other human altered land areas, the potential for container breeding species to increase is greater. During hotter periods, aquatic habitats could become less hospitable and more fleeting, reducing the impact of mosquito predators (Hunt et al. 2017).

Because mosquitoes need a water source for their young to develop, the removal of habit can greatly decrease mosquito reproduction (Dumont and Thuilliez, 2016). Reducing the number of standing water locations that last 96+ hours reduces mosquito populations (Metzger et al. 2018). This is a particularly important strategy for combating *Aedes* species, including *Ae. aegypti* and *Ae. albopictus*, as they are container breeders (Dumont and Thuilliez 2016). In a suburban setting this can be as easy as cleaning gutters, removing containers that hold water after precipitation events, and changing the water in bird baths and pet water containers frequently (Fikrig et al. 2017). The same approach can be used by soldiers when they find themselves in the field near any easily removable standing water sources. On a broader scale, storm water best management practices are needed. These include the removal of debris from collapsed structures, creating drainage ports in large dumpsters, and filling in low areas that hold water (Metzger et al. 2018).

Mosquito larvae are also susceptible to predators including fish, other invertebrates such as dragonfly larvae, and even some genera of mosquitoes such as *Toxorhynchites*. Predation pressure is a known determinant for mosquito presence (Hunt et al. 2017) and fish and other species have been intentionally introduced to many areas. Predators affect mosquito populations by directly consuming larvae, and indirectly by causing defensive reactions from the prey. For example, mosquito larvae that were exposed to cues from back swimmers (Notonectidae) that had fed on mosquito larvae, showed decreased survival, delayed development, and smaller body size as adults (Beketov and Liess, 2007).

Similarly, *Ae. triseriatis* larvae fed less and rested more when pheromones of *Toxorhynchites* mosquito larvae, which are predatory, were present (Beketov and Liess 2007). Development rates and body sizes of females decreased more than males in response to predator chemical cues. Presumably, this is because females are larger as adults and have higher biological demands as adults (Beketov and Liess, 2007). Typically, species show less response to chemical cues from other species than from conspecifics. However, *Ae. triseriatus* larva showed the same response to cues from predators fed on conspecifics and predators that consumed *Ae. albopictus* (Beketov and Liess, 2007).

Some of the most effective predators of mosquitoes are small fish. The western mosquitofish (*Gambusia affinis*) can reduce larvae mosquito populations by 100% when stocked at 0.06 fish per 0.25 m^2. Another species, the green sunfish, *Lepomis cyanellus*, reduced populations by 99.3% when stocked at 0.03 per 0.25 m^2. While effective in controlling mosquito numbers in small areas, between 2,000 and 2,800 fish per acre are required to provide 90% reduction in larger habitats. When comparing these two fish, sunfish were more effective early in the season, but the higher reproductive capacity of the live-bearing mosquito fish allows it be more effective through the mid-to-late season. On average, mosquitofish require about 24 days to complete a reproductive cycle. These fish are considered extremely hardy, being able to exist in very small amounts of water (Davey and Meisch, 1977). Unfortunately, the combination of surviving harsh conditions, including low oxygen and warm water, being generalist predators, and not requiring specific substrate for spawning make these fish an invasive species.

While fish can provide mosquito control in larger, interconnected waterways, imposing control on standing water mosquito species, they have no ability to control treehole and container breeding species. Fortunately, a specialist predator exists that preys on mosquitoes in these habitats. This predator is another mosquito, *Toxorhynchites rutilus*, and they do not feed on vertebrate blood as adults (Williams et al. 1961; Steffan and Evenhuis, 1981). They are commonly called the “tree hole predatory mosquito” because their large larvae attack and consume juveniles of other mosquito species. In addition to consuming other larvae, their fourth instars are known to kill prey without consuming it, adding to their control ability (Steffan and Evenhuis, 1981).

Naturally, *Toxorhynchites* mosquitos use tree holes for oviposition, but they will use containers as well (Williams et al. 1961; Trimble and Smith 1978). These mosquitoes are considered effective predators of *Ae. aegypti* and *Ae. albopictus* in other parts of the world and have been documented over their four larval stages to kill an average of 110.5 larvae, with 80.5 of those being consumed (Trimble and Smith, 1978). They will also feed on insects at the surface, perhaps even adult mosquitoes. *Toxorhynchites* are ambush predators that do not pursue prey and are able to withstand starvation for 10 weeks or more (Steffan and Evenhuis, 1981).

Toxorhynchites females are unusual in oviposition behavior and can accurately
"shoot" their eggs through the air up to 18 cm to reach breeding pools (Williams et al. 1961; Steffan and Evenhuis 1981). Females prefer to oviposit in well-shaded, wooded habitats, and studies have shown that these mosquitos can lay 58.5 eggs per female on average (Steffan and Evenhuis, 1981). Unfortunately, larvae often engage in cannibalism (Trimble and Smith, 1978; Steffan and Evenhuis, 1981).

In addition to relying on predators for larval control, a natural insecticide that is specific to Diptera and is made from *Bacillus thuringiensis israelensis* (Bti) is available. This bacteria is a commonly found in commercial "mosquito dunks" that are circular, porous rings placed in the water, and are ingested during feeding by the mosquito larvae. After the dunk is consumed by the larvae, the Bti interacts with gut chemistry of the larvae and forms crystals that perforate their gut lining (Fikrig et al. 2017).

Treatment with BT dunks have some limits including that they often float when placed into water, reducing effectiveness because many species of mosquito feed near the bottom. Dunks are most effective when submerged just below the water surface (Aly 1983). Additionally, if dunks are placed into habitat that has abundant natural detritus, these natural materials can dilute and block the crystals from interacting with the midgut, causing a need for more dunks to be used. Snails can also consume Bti baits without adverse effects, rapidly degrading them and reducing the time for larval mosquitoes to feed (Aly 1983).

Mosquito feeding preferences and habitat association

Most of the approximately 3,500 mosquito species in the world are opportunistic blood feeders (McBride 2016) and rely on olfactory senses to find blood meals (Carey et al. 2010). Most species feed on vertebrates and may specialize on broad taxonomic groups, such as mammals or birds. However, some species are very host specific and within that group some are primarily human feeders including *Ae. aegypti* and *An. gambiae* (McBride 2016). Host preference is explained as a trait to optimize reproductive fitness and often, the most selective species are the most important for disease transmission due to parallel evolution (Nasci 1984; Takken and Verhulst 2013).

Host finding behavior also varies by mosquito species. Some blood-seeking mosquitoes move very short distances (10 m) from habitat edges and favor certain habitats. These habitat associations are not affected by host distribution or nectar availability although oviposition sites likely play a role in mosquito distribution as well (Reiskind et al. 2017). Specialization can also occur. For example, *Ae. fulvus pallens* has been recorded feeding on many different mammalian species, but has been suggested to feed primarily on armadillos in Florida (Edman, 1971). When the primary host is not available, alternative hosts may be used. For example, Irby and Apperson (1988) found that most of the blood meals taken by this species in North Carolina was from canines.

Seasonal changes can affect host use. For example, *Cx. tarsalis* feeds on birds in the summer and mammals in winter after birds migrate. Another species, *Cx. pipiens* prefers American Robins (*Turdus migratorius*). When these birds migrate south *Cx. pipiens* switches to mammals, including humans, increasing the risk of disease transmission (Takken and Verhulst, 2013).

Because of broad host use and adaptability to seasonal changes, the ability to use small temporary bodies of water that are free from fish, and the ability to produce eggs that can diapause, several mosquito species have become established outside their native range. Two of the most important are the yellow fever mosquito, *Ae. aegyti*, and the Asian tiger mosquito, *Ae. albopictus*.

Mosquitoes found in Oklahoma

Ae. aegypti can transmit several of the world's most important insect-borne, diseases including dengue, yellow fever, Chikungunya, and the Zika virus (Kuri-Morales, et al. 2017). It is also very anthropophilic (Lima et al. 2016), prefers to take blood meals from humans and frequently reproduces in manmade water containers (Santos and Meneses 2017). *Ae. aegypti* originates from tropical and subtropical Africa, but has spread around the world and can survive in subtropical habitats and urban areas (Romeo Aznar et al. 2013). *Ae. aegypti* produces highly resilient eggs, which can withstand desiccation for long periods (Dallimore et al. 2017), allowing it to spread.

In its native range, this species is a tree hole mosquito, but it has become particularly adapted to urban areas (Eisen and Moore, 2013; Romeo Aznar et al. 2013). These urban areas often have higher ambient temperatures than surrounding natural landscapes, which could result in decreased development times (Townroe and Callaghan 2014). *Ae. aegypti* is most abundant when rainfall is highest, but the availability of water containers is heavily influenced by human activity, so infestation varies by location and rainfall may not always be a good predictor for presence of the species (Eisen et al.
2014).

Between 1995 and 2004 many more counties reported *Ae. aegypti* and another invasive species, *Ae. Albopictus*, then previously. This result is likely from more surveillance activities rather than rapid range expansion of the two species. When looking at range maps, counties without reports should not be considered free of the mosquitoes. This is because the species could still by be cryptic in those locations. This can also be applied to state level maps, when counties are highlighted. Additionally, locations with annual positive captures cannot always be considered to have established populations, because these areas could have repeated introductions during the warmer months (Hahn et al. 2016).

New mosquito introductions are continuing today, especially in areas that receive shipments of horticultural commodities or tires. This despite regulatory inspections. For example, it is known that *Ae. aegypti's* presence in the Netherlands is due to tire shipments from Miami, Florida (Dallimore, et al. 2017). Recently, *Ae. aegypti* has been recorded at higher elevations, including in Colombia at 2,302 meters and in Bolivia at 2,600 meters (Kuri-Morales et al. 2017). An individual was captured in England during 2016. This mosquito is believed to have arrived with horticultural cargo form Africa (Dallimore, et al. 2017).

Ae. aegypti is thought to have been transported to the Americas with the slave trade, beginning 375 years ago (Brathwaite et al. 2012; Lima et al. 2016). Over the course of slavery in the Americas, it was probably introduced hundreds if not thousands of times. This allowed for a genetic diversity in the populations. Attempts were made to eradicate *Ae. aegypti* from the United States during the 1960's using widespread application of DDT. Surveys from that time showed that the species was widely distributed across the south (Brathwaite et al. 2012; Eisen and Moore 2013; Hahn et al. 2016). Through the eradication efforts, *Ae. aegypti* was eliminated from all areas except the Gulf Coast and deep south. Since the end of the eradication program, the mosquito has re-invaded and has moved into new areas (Brathwaite et al. 2012).

Today it is considered established in Louisiana, California, Arizona, New Mexico, Texas, and Florida. These states are reported as the "usual" range of *Ae. aegypti* in the US. It commonly occurs in the areas around Fort Polk, LA and could be moved with military activity to other areas (Kraemer et al. 2015). The species is collected sporadically in other southern and mid-Atlantic states, as well as rarely in locations as far north as New Hampshire (Hahn et al. 2016). Oklahoma and Arkansas are reported as "temporary summer range" (Eisen et al. 2014), but with climate change and more recent sampling (Bradt et al. 2018), the species appears to be resident in southern Oklahoma. *Ae. aegypti* has been recorded in central Oklahoma in the past (Kraemer et al. 2015). Transition regions are defined as areas where the mosquito could survive several years but can be extirpated by climate events (Otero et al. 2006). Further investigation could reveal if the species is overwintering in central Oklahoma, or is arriving with the warming weather each year.

The limiting factor to northward expansion of *Ae. aegypti* is thought to be low winter temperatures (Eisen et al. 2014; Hahn et al. 2017). The developmental threshold for the different life stages are 14 C^{o} for eggs, 11.8 C^{o} for larvae, and 10.3 C$^{\circ}$ for pupae (Eisen et al. 2014). Eggs can survive the cold but the duration is variable, so temperature and length of cold exposure must both be considered (Otero et al. 2006). Some studies have suggested that increasing air temperatures and adaption to subterranean habitat may be allowing *Ae. aegypti* to move north in the United States (Lima et al. 2016; Santos and Meneses, 2017). Most regions in the United States have summer temperatures that would allow *Ae. aegypti* development. (Hahn et al. 2017).

Experiments have shown that larval *Ae. aegypti* are out-competed and displaced by *Ae. albopictus* (McHugh and Hanny 1990; McHugh and VandeBerg 1989; McHugh, 1991, 1992, 1993). However, it has been noted that *Ae. aegypti* competes better in hotter, dryer conditions compared to *Ae. albopictus* (de Valdez 2017). Although *Ae. aegypti* and *Ae. albopictus* are competitors, they can be found coexisting as adults due to differences in urban habit preference (Leisnham and Juliano 2009; Hahn et al. 2016).

Ae. albopictus

The Asian Tiger Mosquito, *Ae. albopictus*, is native to southeast Asia, and is one of the most invasive mosquito species in the world (Sherpa et al. 2018). It ranks in the top 100 list of the World's Worst Invasive Alien Species from the Global, Invasive Species

Database (Takken and Knols 2007). This species transmits a similar array of diseases as *Ae. aegypti,* including yellow fever virus, dengue fever, and Chikungunya fever. It has also been found positive for West Nile Virus (Noden et al. 2015a).

Ae. albopictus has increased its range through its close association with humans and its ability to survive during transport (Reiter 1998). It is believed to have come to the United States as dormant eggs in tires into Texas ports (Zhong et al. 2013). *Ae. albopictus* has been successful since its arrival in the United States, and has spread throughout the greater south-east (Erickson et al. 2010). It is not as anthropophilic as *Ae. aegypti* and will utilize tree holes, along with human containers and tires. (Erickson et al. 2010) The

Eastern Red Cedar (*Juniperus virginiana*), could play a role in the westward expansion of *Ae. albopictus* (Reiskind and Zarrabi 2011). It is hypothesized that the trees provide nutrients thought the needles and provide the adults thermal refuges.

In addition to using natural breeding locations, *Ae. albopictus* will use manmade containers. In Mississippi *Ae. albopictus* populations peaked in the late summer and early fall, and was at its lowest during the winter and early spring as a result of low temperatures (Erickson et al. 2010). Similar to its relative *Ae. aegypti*, cold winters limit its northern range.

It is often thought that *Ae. albopictus* excludes *Ae. aegypti* when it moves into an area. However, *Ae. albopictus* fairs better in cooler environments, while *Ae. aegypti* does better warmer environments. In Taiwan, *Ae. aegypti* and *Ae. albopictus* have the ability to occupy the entirety of the island but do not. Instead, *Ae. albopictus* is more prominent in the northern two thirds of the island while *Ae. aegypti* occupies the southern third (Erickson et al. 2010).

Cx. erraticus

Cx. erraticus is a permanent water mosquito (Noden et al. 2015) and can be found in creek edge and lake shore habitats (Robertson et al. 1993). Populations have been documented to decrease with rising water levels as a result of exposure to predators (Robertson et al. 1993). Lakes that shrink from evaporation can increase breeding habitat for the *Cx. erraticus* (Robertson et al. 1993). Adults can disperse up to 2 km, but typically only travel between 0.5-1 km (Estep et al. 2010) in search of prey and breeding sites.

Cx. erraticus is a vector of West Nile Virus (WNV), and potentially can also transmit Eastern Equine Encephalitis Virus (EEEV), and Venezuelan Equine

Encephalitis (VEE) (Cupp et al. 2004; Estep et al. 2010; Mendenhall et al. 2012; Skelsey et al. 2013). It has been found positive for WNV in the winter months, suggesting year round potential for transmission (Godsey et al. 2013). WNV positive *Cx. erraticus* have been recorded in Florida at rates of 0.64% (Hribar et al. 2004), in Kansas at a rate of 0.19% (Harrison et al. 2009), and in Louisiana at a rate of 1.12% (Unlu et al. 2014). This mosquito occurs as far north as Ontario, Canada (Hunter et al. 2015). Possible positive WNV *Cx. erraticus* have been recorded in Texas as well (Bolling et al. 2005). This species is known to feed on a many vertebrates, including mammals, birds, reptiles, and amphibians (Mendenhall et al. 2012, 2014).

Water birds and whitetail deer are most commonly used as blood meals (Mendenhall et al. 2012). The species typically feeds on birds until they leave late in the season then switch to mammals (Burkett-Cadena et al. 2012; Mendenhall et al. 2012). Timing of this shift is different every year (Burkett-Cadena et al. 2012) and is influenced by the severity of winter conditions. Increased global temperatures could cause changes in timing of host shifts and change disease transmission patterns (Burkett-Cadena et al. 2012).

Both WNV and EEE in birds can be transmitted to mammals by 'bridge' vectors that feed on both groups (Burkett-Cadena et al. 2012; (Bingham et al. 2016). A native mosquito, *Culiseta melanura* is the primary vector. Horses and game birds are commonly infected and have mortality rates averaging >80%. It has shown that there is a 30-40% mortality rate in humans for EEEV (Estep et al. 2010). *Cx. erraticus* typically shifts to mammals by August but can shift as early as

May and June. Each hour of winter below 7.2 C º seemed to delay bird-mammal, shift by 0.22 (~1/5) of a day. It is thought that colder winters delay bird breeding and thus delaying the host switch.

Anopheles quadrimaculatus

An. quadrimaculatus is a complex of approximately five species, which are distinguished by distribution and genetics (Levine et al. 2004). Larval *An. quadrimaculatus* are typically found in lake edges and flood plains (Robertson et al. 1993). *Anopheles sp.* can also be found on green algae mats on the margins of streams and rivers, potentially because of their unusual larval position of resting parallel to the surface (Kim et al. 2007b). *An. quadrimaculatus* populations can increase with the rise of water levels of lakes (Robertson et al. 1993).

Anopheles spp. feed on large mammals such as deer and pigs (Robertson et al. 1993) and are most active from 10:00 pm to 2:00 am (Chang et al. 2016). *An. quadrimaculatus* overwinters as inseminated females (Robertson et al. 1993). Historically, *An. quadrimaculatus* was the primary malaria vector in the US (Levine et al. 2004). WNV has also been found in *An. quadrimaculatus*, but its vector competency is considered low (Unlu et al. 2014).

Aedes vexans

Ae. vexans is multivoltine, floodwater mosquito, and is found throughout the world (Tiawsirisup et al. 2008). This species, sometimes called the inland flood water mosquito, is a pest of livestock and humans in the central United States (Horsfall et al. 1975). It uses transient waters that occur in low areas of grasslands and forests for breeding. *Ae. vexans* lays its eggs on soil that is subject to flooding that is dry at the time of oviposition (Horsfall et al. 1975).

Ae. vexans feeds on wide variety of hosts but mostly uses large mammals, which can make up to 92.4% of blood meals (Nasci 1984; Molaei and Andreadis, 2006). The primary blood meal for the species is thought to be white tailed deer which comprise up to 80% of blood meals (Tiawsirisup et al. 2008; (Anderson et al. 2018).

Ae. vexans is also an aggressive human feeder (Molaei and Andreadis, 2006) and can transmit Zika virus (Dibernardo et al. 2017). It has also been found with WNV in its system, but is not considered a major vector (Bolling et al. 2005; Cupp et al. 2007; Unlu et al. 2014).

Culex pipiens

Cx. pipiens is a native mosquito that is commonly found in unsanitary conditions, such as stagnant water (Kim et al. 2009). During the warmer months when bird populations are present, *Cx. pipiens* primarily feeds on avian hosts. Because of this host preference, *Cx. pipiens* are often captured in areas with higher amounts of vegetation (Eshun et al. 2016).

Cx. pipiens also has a preference for human dwellings (Witt et al. 2004). Additionally, it will diapause in shelters to survive unfavorable conditions. For example *Cx. pipeins* survives in much of Russia where temperatures can range from -22 Cº to -4 Cº in the winter but in shelters, the temperatures reach -11 Cº to -1.1 Cº (Ewing et al. 2016).

Cx. pipiens is a highly efficient vector of WNV (Witt et al. 2004; Tiawsirisup et al. 2008; Ritchie et al. 2014). *Cx. pipiens* has been shown to be able to vector Japanese Encephalitis (Kim et al. 2015). The species utilizes manmade larval habitats, such as stored water containers and can allow increases in numbers independent of rainfall events (Ewing et al. 2016; Santos and Meneses 2017)

Psorophora columbiae

Ps. columbiae is a native species that breeds in open grassy areas which occasionally flood (Moncayo et al. 2008). *Ps. columbiae* also breeds in cattle hoof prints and possibly wild deer and pig tracks and tire tracks (Meek and Olson 1976). *Ps. columbiae* only require four days to develop into adults under optimal, conditions (Davey and Meisch 1977).

Adults prefer to fly and rest in open areas and they disperse long distances from larval sites. *Ps. columbiae* feed on a wide range of hosts, including mammals, birds, and reptiles. This species can be present in such high numbers that they have been recorded as exsanguinating cattle (Moncayo et al. 2008) although a more likely explanation for cattle deaths is asphyxiation.

Ps. columbiae has been recorded carrying Venezuelan Equine Encephalitis Virus (VEEV), which is one of the most important mosquito-transmitted viruses in the Americas. However, populations from the U.S. are less susceptible to VEEV than in other areas (Moncayo et al. 2008). In addition, WNV-positive mosquitoes have been recorded in Texas (Bolling et al. 2005) and Louisiana, (Unlu et al. 2014).

Uranotaenia

Uranotaenia sapphirina are amphibian feeders (Goddard et al. 2017) These mosquitoes are commonly found in areas around Oklahoma but are rarely collected in traditional mosquito traps (Noden et al. 2015).

CHAPTER II

MOSQUITO SURVEY OF CAMP GRUBER TRAINING CENTER AND COMPARISON WITH OKLAHOMA MILITARY BASES

Abstract

Army National Guard training requires personnel to visit wild areas for one weekend a month and two weeks a year. During this time, the soldiers may be exposed to mosquitoes and be at risk of mosquito-borne pathogens. Our survey investigated the mosquitoes at the Camp Gruber Military Training Base, and in the adjacent town of Braggs, OK. Thirty traps, ten BG Sentinel Traps, and twenty modified CDC light traps, using CO2 as attractant, were placed every other weekend (Friday morning-Sunday morning) for 23 collection periods between mid-April and late October. A total of 10,405 mosquitos from 7 genera and 26 species were collected, representing about 40% of the 62 species known from Oklahoma. The majority (98.4 %) of mosquitoes were collected from CDC traps. CDC traps most commonly collected Culex spp. (73% of total). BG traps collected 40.2% of the Aedes spp. The most abundant species were in the genus Culex, of which some can transmit West Nile Virus. A single adult Aedes aegypti was captured on the base, along with a larva that was genetically similar. This capture, along with the capture of Asian tiger mosquito, Aedes albopictus highlights the need for regular season-long monitoring to protect troops during training. Information generated from this was used to create a management plan for the base.

Introduction

Medically important arthropods, particularly mosquitoes, have long been significant pests of humans. Today, mosquito-transmitted diseases cause the biggest disease burden of any group of pathogens (Gubler 2011). Diseases like malaria have hindered developing countries in the tropical, and sub-tropical regions, and despite advances in medicines, the pathogen still causes approximately one million human deaths per year (Reiter 2001). Recently, the prevalence of other mosquito-borne diseases has dramatically increased after decades of being relatively obscure. For example, Chickungunya and Zika virus produced hundreds-of-thousands of disease cases in Mexico, Central and South America. Zika virus was highly prevalent in Brazil in 2015 and 2016 (Santos and Meneses 2017).

Currently, mosquito-borne diseases are most severe in developing counties that lack public health infrastructure, and usually lack clean water management systems (Reiter 2001). Mosquito-borne disease occurrences are also increasing because of growing human populations in developing countries. In addition, climate change producing increased average temperatures, increase mosquito generations per year, allow more feeding days, and improve survival of some tropical species. The increasing speed of commercial transportation has also allowed mosquitoes and their associated pathogens to spread faster and farther than in the past (Reiter 2001). Imported and newly rediscovered mosquito populations have raised alarms in developed countries, causing both concern and increased need for surveillance (Bradt et al. 2017; Dallimore et al. 2017). Large-scale rapid movement of people and equipment by military forces can also contribute to these introductions and the spread of mosquito-borne disease.

Military forces have been disrupted by arthropod-borne diseases throughout history, especially when troops are deployed abroad (Kitchen et al. 2009; Pages et al. 2010; Reeves et al. 2013; Reeves and Bettano, 2014). For example, American soldiers were ravaged by yellow fever, spread by introduced mosquitoes during the SpanishAmerican war in Cuba (Gibbins et al. 2012). Soldiers of all nationalities suffered from the effects of malaria during the Korean Conflict and Vietnam War (Gibson, et al. 1974: Kim et al. 2007). Recent conflicts in Iraq exposed soldiers to phlebotomine sand flies that transmit Leishmania (Hyams et al. 1995; Coleman et al. 2006).

Troops can also face vector borne diseases at home, especially during field training exercises (Yevich et al. 1995; Witt et al. 2004; Murphree et al. 2009; Rossi et al. 2015; Harris et al. 2017). For example, a small group of troops on maneuver at Fort Chaffee Arkansas became ill from a disease transmitted by ticks, leading to the discovery and description of Ehrlichiosis chaffiensis (McCall et al. 2001). The cases associated with Fort Chaffee and the identification of the new disease organism were unusual, because often symptoms do not develop until soldiers return home, complicating epidemiological investigations (Warner et al. 2001). The nature of modern conflicts means that military forces must be prepared for deployments on short notice, to any location (Rueda, et al. 2011). For example, the global war on terror requires that small contingents of United States armed forces be ready for deployment almost anywhere in the world (Moore et al. 1999).These deployments expose troops to new vectors and pathogens and can result in the unintentional movement of these organisms. The military proactively attempts to prevent the movement of organisms with their equipment by decontaminating it both before departure from an area of operation and often upon its return as well (Cofrancesco et al. 2007). Unfortunately, cleaning is not always perfect and this combined with the speed of deployments could allow mosquitoes and other vectors to be moved through military actions (Moore et al. 1999).

If mosquitoes or other vectors manage to survive movement to a base, they could establish on military grounds. Often, active duty troops returning from deployment are ‘in-processed’ at their home military base along with their unit’s equipment. Active military bases are full-time installations were military personnel work, train, and often live within the confines of the base. In addition, active bases have federal funds that allow for vector monitoring and management programs (McHugh et al. 1989; McHugh, 1992). These programs increase the chances of detecting introduced mosquitoes and can often respond rapidly to eradicate the introduced insects. In contrast, military installations that are used by reserve personnel, usually have only small permanent operating staff and few, if any, fulltime residents. Reservist bases have frequent influx and exodus of hundreds or even thousands of troops that disperse across a state once their exercise is done. In addition, training facilities have fewer personnel and less resources for vector monitoring and management. Thus, these training facilities may be at greater risk for the establishment of imported mosquitoes and their associated diseases.

In Oklahoma, Camp Gruber, near Braggs, OK, has received little evaluation of potential vectors or pathogens. The base is frequently used by Oklahoma National, Guard members for both weekend drills and two-week annual, training exercises in the summer months. The goals of this study were to: (1). assess adult mosquitoes and breeding areas on Camp Gruber, and (2). compare adult mosquito catches to mosquito faunas at four other more permanent military facilities in Oklahoma.

Thesis Objectives

1) Sample Camp Gruber, an Army National Guard training site, throughout the season to determine mosquito community and activity period;
2) Survey potential mosquito breeding sites to assess areas where mosquito management could be improved;
3) Create a mosquito guide for personnel using the Camp Gruber training facility.

Materials and Methods:

Study site

Camp Gruber Military Training Center is an Army National Guard training facility that is also occasionally used by other military and police task forces. It is open to public hunting during turkey and deer seasons. Camp Gruber is located in Muskogee County Oklahoma, and is approximately 45 km southeast of the city of Muskogee and it covers approximately 22,500 hectares (55,680 acres). The town of Braggs, OK that has a population of 257 residents is adjacent to the southern edge of Camp Gruber. The base has a cantonment area (250 Ha) with barracks, a store, and administrative offices, and firing ranges (185 Ha). The remainder of the base is a mixture of grassland and forest with streams and ponds. Greenleaf Lake (370 Ha) borders the southeast corner of the base.

Sampling- Adult Mosquitoes

Locations for sampling were selected based on previous reports of mosquito activity, troop activity, and training area access. Fifty locations were selected and approved by base personnel. Of the approved sites, thirty were sampled on each sampling trip because of scheduled activities, including training with live ammunition and movement of military equipment that prohibited access to trap sites during some scheduled sampling periods. During two scheduled sampling periods, access to thirty of the designated mosquito trap locations was not possible. During these two weekends, mosquito traps were placed at trap sites of a concurrent study of the American burying beetle, *Nicrophorus americanus* (W. Hoback pers. comm).

Two types of traps were used for sampling, modified CDC light traps and BioGents Sentinel traps. The CDC light traps were modified by removing the light source and using a carbon dioxide lure. The traps consist of a fan powered by a six-volt battery, a collection basket attached to the end of the fan assembly, and a rain guard on top. The carbon dioxide source was dry ice placed into a one liter insulated container that had holes drilled into the side and bottom to allow a slow release of carbon dioxide. Each evening, the container was filled with dry ice and this amount lasted until the trap was checked the next morning. The traps were suspended approximately 1-1.5 meters above the ground on trees or other structures using ropes and/or bungee cords. The batteries to power the traps were placed on the ground.

The BioGents (BG) Sentinel trap are large, compressible traps, that are made up of a modified laundry hamper basket, a black tube, a collection net, a fan, a BioGents supplied, human scented lure, and a twelve-volt battery for the fan. These traps were placed on the ground under vegetation, and the battery was placed inside the trap to decrease the chance of disconnection from the fan.

Sampling was conducted bi-weekly, usually every other weekend from late April through mid-October 2018, with one sampling period occurring on week days because of training activities on base. Traps were set on Friday, usually sometime between 10:00 and 13:00. Traps were checked between 05:30 and 11:00 each morning and on the first morning, dry ice was replenished for the CDC-Traps. On

Sunday the traps were checked and removed. During the first sampling week 20 traps (15 BG Traps and 5 CDC Traps) were available, thereafter thirty traps (10 BG Traps and 20 CDC Traps) were deployed each week. Between April and October 2018 there were a total of 23 collection dates.

There were a total, of 670 trapping events attempted. Of this total, 240 were BG Trapping events and 430 were CDC trapping events. Across events, two BG Traps and six CDC Traps failed, resulting in 662 valid trapping nights.

During the checking of traps, all captured mosquitoes were removed and placed into small containers, which were labeled with the trap type, trap location, and date of capture. These containers were placed into a mobile freezer set at approximately -8 ^{o}C. Upon return to the laboratory mosquitoes were transferred to a -20^{o} C freezer to until identification.

Mosquitoes were identified to species using a Labom Stereoscope (Luxeo 4Z Stereozoom Microscope) and keys by Darsie and Ward (2006). After identification, mosquitoes were placed into new containers by species, location of capture, and date of capture and returned to the freezer. Over the course of the sampling season several mosquitoes were captured that were heavily damaged or questionable in their identification due to similar characteristics with another species. In these cases, the specimens were examined and species were confirmed by Lisa Coburn and Drs. Justin Talley and Bruce Noden. If the specimen was too damaged for positive identification, it was only recorded in the total number captured but not in species totals. Male mosquitoes were identified if possible and recorded in overall number and species counts, while unidentifiable males were only recorded in overall counts Genetic analysis was conducted to confirm identification of *Aedes aegypti*. Polymerase chain reaction (PCR), was used with supplies from the Invitrogen: PureLink™ Ouick Gel Extraction Kit.

Because West Nile virus is spread by mosquitoes of the genes *Culex* and cases occur annually in Oklahoma, all female *Culex pipiens* and *Culex tarsalis* were shipped to the Army Public Health Center in Maryland were PCR was used to identify the presence or absences of the virus.

To compare the mosquito communities at Camp Gruber with those of other military installations in Oklahoma, data from an earlier survey were examined. Bradt et al. (2017) sampled six different Oklahoma cities for mosquito species presence between May 28, 2016 and September 20, 2016. Four cities, Altus, Enid, Midwest City, and

Lawton have military installations that were sampled. In addition to CDC Traps and BG Traps, CDC Gravid traps baited with water infused with decomposed Bermuda grass were used. Traps were deployed between 14:00 and 17:00 and were collected between

08:00 and 11:00 the next day

Larval Sampling at Camp Gruber

During adult sampling at Camp Gruber, water bodies and containers holding water within 100 m of each trap were sampled. Samples were collected with a mosquito dipping cup, which was dipped into the water three times at each trapping location each week. Larval, mosquitoes were stored in 70% ethanol after collection. All fourth instar larvae were identified using larval, dichotomous keys (Darsie and Ward 2005) and a Labom stereoscope. After identification the larvae were placed back into 70% ethanol and their species, collection location and date were recorded. Identifications were confirmed by OSU diagnosticians. One sample that contained a specimen identified as *Aedes aegypti* was tested using PCR and confirmed.

Aedes aegypti Confirmation Assay**:**

The larvae and adult thought to be *A. aegypti* with identity markings rubbed off during collection was tested by dissecting the head using sterile tweezers and placing it in a sterile vial. The vial was labeled with date and location of collection. The positive control used was *A. aegypti* Liverpool strain continuously reared in the laboratory. One day prior to extraction, using a genomic DNA extraction kit (GeneJET, Genomic DNA Extraction Kit, Thermoscientific, Grand Island, NY), 20µl of ProK and 180ul of Digestion solution were added to the sample tube containing the head of the unknown mosquito larvae and another tube containing the legs of the unknown adult, and each sample was incubated in a shaker overnight at 56^{o}C. The next day, 200µl of lysis solution and 400µl of 50% ethanol were added and the sample was vortexed and extraction was completed following the manufacturer protocol. Extracted DNA samples were stored in a freezer at -20^{o}C for further processing.

The extracted DNA was tested using primers that amplify a 361bp region of the ND4 mosquito gene (Costa et al. 2005): ND4-Forward primer (5'-ATTGCCTAAGG CTCATGTAG-3') and ND4 Reverse (5'- TCGGCTTCCTAGTCGTTCAT- 3'). The initial denaturation step occurred at 94°C for 2 min, followed by 35 cycles at 94°C for 1 min, 56°C for 30 s, and 72°C for 1 min, and a final elongation step at 72°C for 7 min in a BioRad C1000 Touch thermal cycler (BIO-RAD, Hercules, CA). The PCR products were visualized using agarose gel electrophoresis in 1x TAE buffer with 2% agarose gel stained with ethidium bromide under ultraviolet light. Results were photographed and printed for verification and documentation. DNA was extracted from the gel using an Invitrogen PureLink Quick Gel Extraction Kit (ThermoFisher Scientific, Waltham, MA) and sent to

Oklahoma State University Core Facility to be bi-directionally sequenced. Resulting consensus sequences were compared with GenBank submissions using default conditions on NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (highly similar sequences (megablast)) where the highest percent sequence identity was used to determine species similarity.

Results

Camp Gruber

Sampling of adult mosquitoes at Camp Gruber in 2018 produced 10,405 individuals. Over 98% of these mosquitoes (10,259). were identified to species using Darsie and Ward (2005), with confirmation of *Ae. aegypti* utilizing PCR techniques. The other 146 specimens were either too damaged to identify or were males that were not included in the count but were included in the overall total, count. Seven genera and 26 species were recorded (Table 1). The most commonly collected species was *Cx.*

erraticus with over 10 per trap night. Other species with more than one capture per trap night were *Ae. vexans* and *Ps. columbiae.* Modified CDC light traps captured 25 species including all species except *Toxorhynchites rutilus septentrionalis* while BG Traps caught only 13 species (Table 2).

Of the 10,259 mosquitoes identified, 72.3% (7,413) were *Culex*, the majority of which were *Culex erraticus* (97.4% (7,413)). The second-most abundant *Culex* was *Cx.*

pipiens with 163 (%%) as well as 32 *Culex tarsalis*. The second most commonly collected genus was *Psorophora*, which represented 13.3% of the total, with 1,361 individuals recorded. Of the six species collected, >65% were *Ps. columbiae* (905). *Psorophora discolor* was the second most common species from this genus with 273 recorded, representing 20.06% of the genus total. There were eight species of *Aedes* identified among 1,031 (10.05%) of all mosquitoes collected. The majority of mosquitoes in the genus were *Aedes vexans* (763) representing 74.0% of *Aedes* collected. The remaining 450 individuals belonged to three genera, *Anopheles* which included 377 *Anopheles quadrimaculatus*, *Ochlerotatus* with 19 individuals among four species, three specimens of *Toxorhynchites rutilus septentrionalis* and a single specimen of

Uranotaenia sapphirina

Of the 10,259 identified mosquitoes 10,095 (98.4%) were collected from the CDC traps and the remaining 164 (1.6%) were collected from the BG Traps. Between both trap types, an average of 15.45 mosquitoes were caught per trap per night. CDC Traps caught more, with 23.70 mosquitoes on average per trap night, and BG Traps averaged 0.69 mosquitoes per trap per night. The majority of mosquitoes collected with CDC Traps were *Culex spp.* (72.99%). *Psorophora spp*. were the second most common collected from the CDC Traps with 13.04% of the total, and *Aedes spp.* made up 9.56% of the CDC total, *Anopheles spp.* *Ochlerotatus spp.* and *Uranotaenia spp.* were also collected in CDC traps, while *Toxorhynchites spp.* was not. In contrast to CDC light traps, the BG Traps collected *Aedes spp. (*40.24% of the total), *Culex spp. Psorophora spp.* *Ochlerotatus spp.*, *Toxorhynchites spp. and Anopheles* spp. No *Uranotaenia spp.* were collected with the BG Traps.

<u>Larval, sampling at Camp Gruber</u>

Sampling of aquatic habitats in conjunction with adult trapping produced 743 individuals of which 323 could be identified to genus, and 312 to species. The remaining 420 larvae were not fourth instars or were already pupating. Larvae represented five genera and 14 species. The most diverse genus was *Psorophora*, with 5 species collected

(Table 3). Three species of *Aedes* were recorded along with two species of *Culex*, *Ochlerotatus*, and *Anopheles*.

Aedes aegypti Confirmation Assay:

Two unknown mosquito samples from Camp Gruber, one adult and one larvae, were tested by PCR for species identification and confirmed using NCBI Blast with 100% sequence identity with known sequences of *A. aegypti* (KX580042.1) while the positive control had 100% sequence identity with a known sequence of Liverpool strain (MF194022.1).

Table 1. Total numbers of collected and identified mosquito larvae from at Camp Gruber during 2018. The numbers are directly compared to the those of the adults collected for each species.

Species	Larvae	Adults
Ae. atlanticus	0	62
Ae. epaticus	0	17
Ae. vexans	93	763
Ae. triseriatus	0	21
Ae. albopictus	19	164
Ae. canadensis	0	1
Ae. fulvus pallens	0	2
Ae. aegypti	1	1
An. punctipennis	1	7
An. crucitans	0	47
An. quadrimaculatus	1	377
Cx. pipiens	2	163
Cx. erraticus	0	7,218
Cx. tarsalis	0	32
Oc. hendersoni	0	11
Oc. nigromaculatus	0	2
Oc. trivattatus	0	2
Oc. sollicitans	0	4
Oc. epaticus	140	0
Oc. zoosophus	4	0
Ps. columbiae	39	905
Ps. ciliata	1	54
Ps. cyanescens	0	99
Ps. Ferox	4	28
Ps. discolor	0	273
Ps. mathesoni	5	2
Tx. r. septentrionalis	0	3
Ur. sapphirina	0	1
Total identified	310	10,259

Seasonal trends

Mosquito species differed by season of activity. In 2018, *Aedes* peaked later than *Psophora* and *Anopholes* (Figure 1). For *Culex*, August was the month with the most adult activity and thus, the greatest potential for transmission of West Nile virus (Figures 2 and 3).

Figure 1. Monthly abundance of *Ae. albopictus*, *Cx. pipiens*, and *Cx. tarsalis* on Camp Gruber during 2018. Numbers of both Aedes albopictus and *Culex tarsalis* both peaked in August, one of the hottest and driest months. This is in contrast to the numbers of *Culex pipiens*, whose numbers were increasing as the sampling season ended.

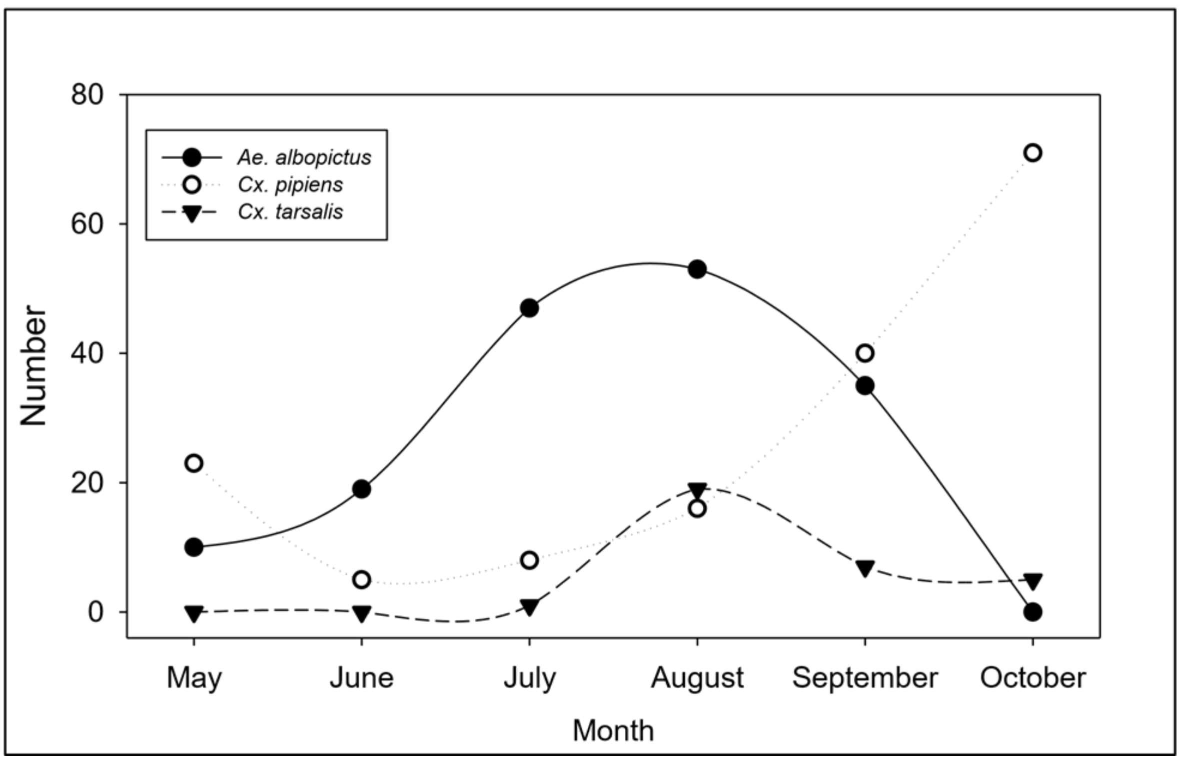


Figure 2. Monthly abundance of *Ae. vexans*, *An. quadrimaculatus*, and *Ps. columbiae* at Camp Gruber during 2018. Similar to other species, *Anopheles quadrimaculatus* and *Psorophora columbiae* both peaked in the month of August. *Aedes vexans* numbers reached their highest point later in the season during September.

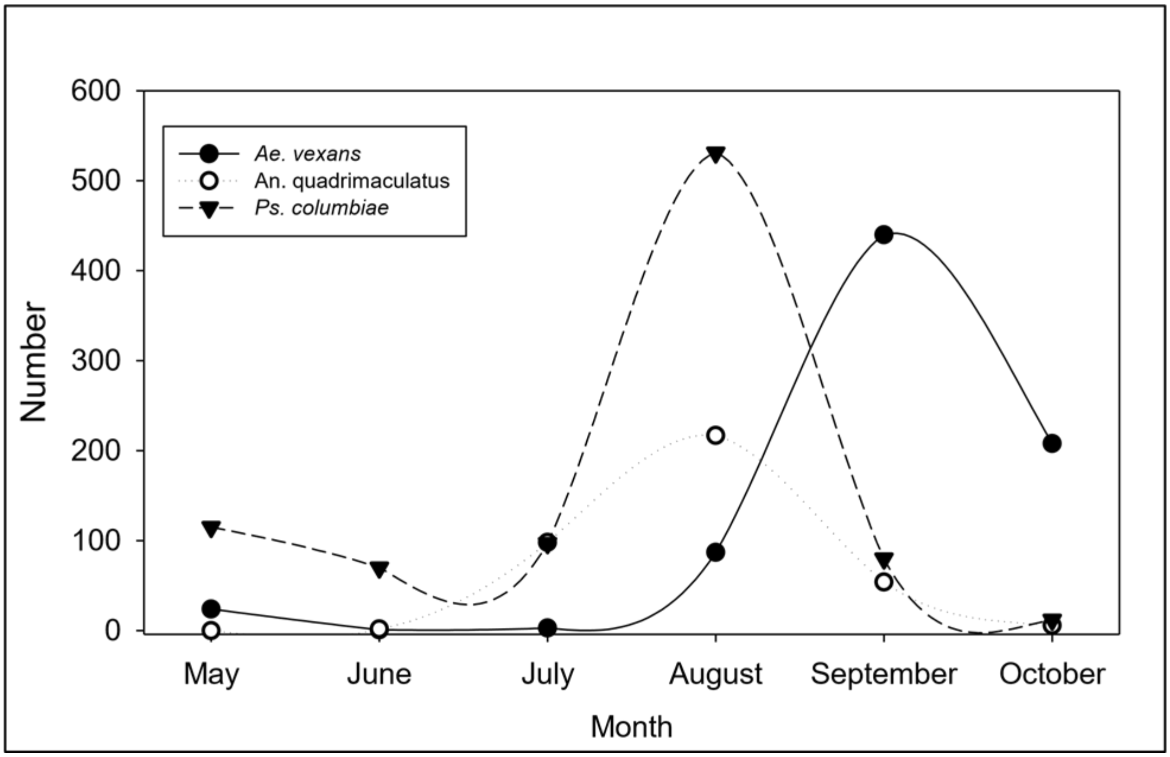
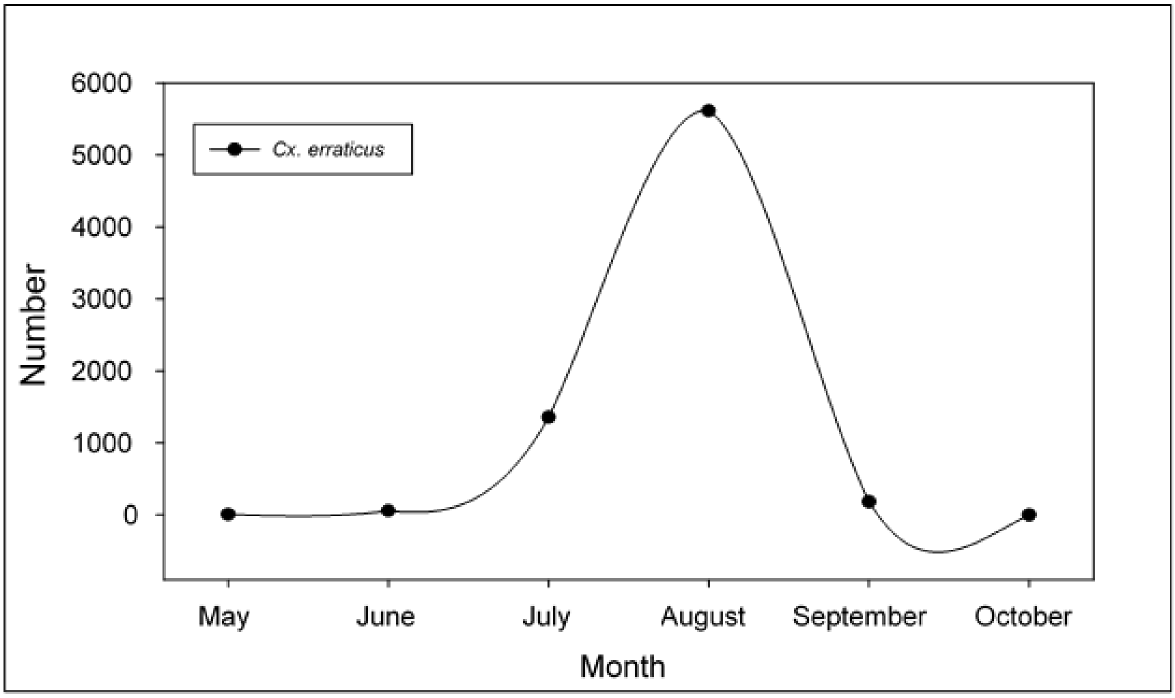


Figure 3 Monthly abundance of *Cx. erraticus* on Camp Gruber in 2018. Again, the month of August saw the highest numbers for this species. However, unlike some of the other species, the number of *Culex erraticus* increased into the thousands, making this the most abundant mosquito collected.



Comparison of Mosquitoes Among Military Installations in Oklahoma

More permanent Oklahoma military installations, three Air force bases, Vance, Tinker, and Altus, and one Army base, Fort Sill were sampled for mosquitoes in the summer of 2016 using CDC light traps, BG Traps, and oviposition traps. Altus Air Force base, in Altus OK, had four genera and 18 species. Both Tinker Air Force base in Enid OK, and Vance Air Force base, in Midwest City had five genera and 13 species. Fort Sill in Lawton, OK, had 5 genera and 24 species.

Table 2. Mean captures of mosquitoes by trap night captured at Oklahoma military installations in 2016 (Vance, Tinker, Altus, Fort Sill). and 2018 (Gruber). Data are reported an average captures per trap night using modified CDC Traps, BG Traps, and Gravid traps Totals are genera/ species. Numbers are reported as fractions of the number total of a species collected divided by the total number of traps nights. Numbers <1 indicate that the total number of that species collected was less than the total number of traps nights conducted.

Species	**Gruber**	**Vance**	**Tinker**	**Altus**	**Fort Sill**
Ae. aegypti	0.0015	0	0	0	0
Ae. albopictus	0.2470	0.0465	2.0400	0.0500	1.4783
Ae. atlanticus	0.0934	0	0	0	0
Ae. canadensis	0.0015	0	0	0.0250	0
Ae. epactius	0.0256	0	0	0	0
Ae. fulvus pallens	0.0030	0	0	0	0
Ae. sollicitans	0.0060	0.1163	0.0800	5.4000	0.1522
Ae. triseriatus	0.0316	0	6.4800	0	0.1522
Ae. vexans	1.1491	0	0.0200	0.0250	0.2609
Ae. zoosophus	0	0	0	0	0.0217
An. barberi	0	0	0	0	0.0870
An. crucians	0.0708	0	0	0	0
An. perplexens	0	0	0.0200	0.1000	0.0435
An. pseudopunctipennis	0	0.0233	0	0	0.1957
An. punctipennis	0.0105	0.0233	0.0800	0.0750	0.0870
An. quadrimaculatus	0.5678	0.1860	0.4600	0.0250	0.1957
C. inornata	0	0	0.0200	0	0
Cx. coronator	0	0	0.1000	0	0.0217
Cx. erraticus	10.8705	0	0	0.1000	0
Cx. nigripalpus	0	0.0698	1.0200	0.0250	0.1304
Cx. pipiens	0.2455	0.8605	1.6000	2.8000	8.3913
Cx. restuans	0	0	0.0200	0.1500	0.1522
Cx. salinarius	0	0.1163	0.1200	0	0.4348
Cx. tarsalis	0.0482	0.6512	0.5400	1.4000	1.3261
Cx. territans	0	0	0.0200	0.0750	0.0652
Oc hendersoni	0.0166	0	0	0	0
Oc nigromaculis	0.0030	0	0	0	0
Oc trivittatus	0.0030	0	0	0	0

Ps. ciliata	0.0813	0.0233	0	0.0750	0.0870
Ps. columbiae	1.3630	0.1395	0.5000	0.3000	7.2174
Ps. cyanescens	0.1491	0.2326	0.0200	0.1000	0.1739
Ps. discolor	0.4111	0	0	0	0
Ps. ferox	0.0422	0	0	0	0.0435
Ps. howardii	0	0	0	0	0.0435
Ps. mathesoni	0.0030	0	0	0	0
Tx. R. septentrionalis	0.0045	0	0	0	0
Ur. Sapphirine	0.0015	0	0	0	0
Totals: 8/41	7/26	4/12	5/17	4/16	4/22

Discussion

Over the course of the mosquito sampling season at Camp Gruber, the capture rates between species, location, and during different parts of the season, varied considerably.

Given the nature of the area encompassed by Camp Gruber's borders, the high capture rate of species considered to be floodwater mosquitoes, such as *Aedes vexans* and *Psorophora columbiae* does not seem out of the ordinary. Large open, low lying areas of grasses are common in the undeveloped areas and training areas in certain parts of the base, providing good habitat for such mosquitoes (Horsfall et al. 1975; Meek and Olson 1976). Additionally, the many dirt roads, which are not always well maintained and therefore are littered with potholes and deep ruts, which increases habitat space for these mosquitoes after a rain event (Meek and Olson 1976). After such events during the sampling season it was very common to find larval, mosquitoes in the divots of the roads.

The large numbers of both of these species collected, could have possible implication for the mosquito borne disease threat. Some evidence exists to support *Psorophora columbiae*'s capacity as a vector for Venezuelan Equine Encephalitis Virus (Moncayo et al. 2008). *Aedes vexans* has been shown to be a potential vector of the Zika virus, however it is not an efficient one (Dibernardo et al. 2017). This species has also demonstrated a capacity to transmit West Nile Virus between rabbits (Tiawsirisup et al. 2008). Studies have indicated that *Aedes vexans* prefers mammalian blood meals, and the white tailed deer, of which there are many at Camp Gruber, is a preferred source. This species also will feed on birds occasionally, which are probably the source of the WNV in their system (Molaei and Andreadis, 2006). These species could pose a threat to Camp Gruber personnel because of their potential capacity to transmit the virus and the virus's presence on the base.

By far the most numerous species collected was *Culex erraticus*, making up over 70% of the total mosquitoes captured. This species is commonly associated with the fringes creeks and rivers (Robertson et al. 1993). The area of the base where the vast majority of these species was collected, was very near the largest body of water in the area, Greenleaf Lake. The trap site in this location was positioned near a drainage ditch that often had standing water. This drainage system was not connected to the main body of the lake regularly. Additionally, this site was only a short distance (55m) from the edge of an inlet of Greenleaf lake. Upon investigation of the shore of this small inlet, we observed evidence of animal activity that could have facilitated the mosquito's ability to reproduce in very high numbers. On the shores of this inlet there are stands of cattail water plants (Typhaceae). In the area just behind these plants, much of the damp soil was disturbed. The suspected cause for this disturbance is wild pigs, of which many groups inhabit areas in and around the base. The rooting activity of these animals created small pockets of isolated water behind the stands of cattails. It seems that the combining factors of the rooting activity of the pigs, and their presents providing ample blood meals for the production of eggs, allowed for a short period of exponential reproduction. It cannot be certain that this is was the exact course of events that lead to the surprisingly high numbers of *Cx. erraticus*, over 4,300, captured on a single weekend. In other parts of the world, wild pigs have been implemented in the facilitation of mosquito breeding in other studies (Nogueira-Filho et al. 2009).

Culex erraticus has demonstrated a capacity to transmit pathogens-causing disease, such as Eastern Equine Encephalitis Virus. This species is predominantly bird feeding, but transitions to mammals after the birds have left with the changing season (Mendenhall et al. 2012; Bingham et al. 2016). *Culex erraticus* has also been shown to be a vector of WNV (Skelsey et al. 2013). The combination of the species host shifting nature and the presents of WNV within the bases borders, could magnify the threat that this species poses to personnel during the latter part of the summer and early fall as the seasons change.

In addition to an increase in *Cx. erraticus*, there was also an increase in *Anopheles quadrimaculatus* numbers. This species was captured in higher numbers than normally seen on a sampling weekend, in that same area. This species is known to feed on a varied of mammals, and was once the primary malaria vector in the United States (Edman 1971; Levine et al. 2004). This species has been demonstrated as an incompetent vector for Zika Virus (Dodson et al. 2018). One cannot say definitively why this *Anopheles spp.* did not see the same explosive growth that the *Culex* species. It could be that the newly provided habitat, possibly from the wild pig activity, was not suitable for the *Anopheles*, or that the wild pigs were not an attractive blood sources for them. The former of the two seems more likely, based on the feeding habits of both species (Edman, 1971). An alternative reason could be that the *Cx. erraticus* larvae were better competitors that the *Anopheles quadrimaculatus* larvae. It has been reported that this species population numbers tend to increase when the level of water is decreasing, and that *Culex erraticus* numbers tend to increase with lowering water levels in a body of water, and the number increase was during August, one of the hottest months (Robertson et al. 1993). The area had also not seen much precipitation during this period of time

Another area experienced a large spike in the number of *Cx. erraticus* captured during a sampling weekend. This location was not actually located within the boundaries of Camp Gruber, but nearby in the small town of Braggs. This trap was placed in the vicinity of the gas station on the north side of town, closest to the base. The trap was located approximately 110 meters from the base fence. This area is characterized by a think stand of trees, that back up to the edge of the bases southwestern border. This area is also frequented by the wild pig population that resides in and around the base. We observed for ourselves on more than one occasion, the movement of wild pigs in this area. The higher than normal numbers of this species collected at this site occurred in July, which was another very dry month for the base. There is a small pond located just over 180 meters away from the trap site. In addition to this pond, there are some remnants of building foundations located on the base. One set of foundations is about ~280 meters away and the other two sets being ~400 meters away. These deteriorating foundations are characterized by deep pits in the ground outlined by what is left of the foundations. It is feasible that these structures hold large amounts of water after a

rain event, which could persist for long periods of time. Both the pond and the foundations are within the flight range of *Cx. erraticus*, giving the possibility that either or both are the source of the large increase in numbers seen in mid-summer at this site (Morris et al.
1991)

Several species. that had either high capture rates during the season or are of medical importance were evaluated at in terms of seasonality by month. These species were, *Ae. albopictus*, *Ae. vexans*, *Anopheles quadrimaculatus*, *Culex erraticus*, *Cx. tarsalis*, *Cx. pipiens*, and *Psorophora columbiae.* All of these species peaked in August, except for *Cx. pipiens*, which peaked at the end of October. August was a particularly dry month for Camp Gruber. This leads us to believe that the rainfall is not the primary driver of the increase in mosquito numbers for the majority of the species listed previously. Instead, it could be that a high availability of blood meals for the females of these species is the main driving factor in the increase of these populations.

Camp Gruber has several small bodies of water and a couple of small creeks that traverse its grounds. It is also largely undeveloped land that has a mix of habitats, such as grassland, savannah, and forest. This leads to the base being able to sustain a large population of deer, wild pigs, and even a local population of elk. As well as these large ground fauna, there are a multitude of smaller animals, including many different bird species. All of these species could serve as blood meals for many of the mosquito species (Edman 1971). As the summer progressed, the temperatures continued to rise and the rainfall amounts remained low. This helped to concentrate the animals living within the base around the remaining water sources, both ponds and the remnants of the creeks. This theory is supported by information given to us by Camp Gruber environmental personnel, who were part of a previous study that tracked the movement of some individual wild pigs with collars over a period of time (unpublished data). If, in fact, the fauna of the base was more concentrated, remaining near what water was left, this could have made them a very reliable and constant source of blood for gravid mosquitoes. This along with the receding waters of the ponds and creeks could create more habitat for these mosquitoes to breed in and might have led to the population increases we saw in late summer at Camp Gruber.

The collection of the historically invasive, but long since naturalized, species *Aedes aegypti*, was a surprise. Not reported the area for almost 70 years (Eisen and Moore 2013; Hahn et al. 2016; Bradt et al. 2017), This was the first time that the species had been confirmed on a military base in Oklahoma (McHugh and Hanny, 1990). In recent years, this species has been discovered far from its native ranges, being moved with commodities, most likely due to its anthropophilic nature, giving it a higher chance of being transported accidentally (Dallimore et al. 2017). Two different individuals of the species were collected at separate times during the season. The first recorded collection of the species, was an adult female, which was found near a vehicle facility 2.63 km east from the area referred to as cantonment. Again, this capture was extremely unexpected, as the species had not been reported in that part of the state for a long period of time. It is important to note that the most current suitable range map from 2017, published by the Center for Disease Control, does put the Camp Gruber area within the northern range where the species could persist if present. It is not unheard of for invasive species to by moved with military equipment (Cofrancesco et al. 2007). Upon investigation, we were informed that a shipment of construction equipment had arrived from another military post, Fort Polk, Louisiana, approximately a week before the capture of this single *Aedes aegypti*. Several front end loaders and back hoes were included in this shipment of heavy machinery . We were allowed access to this vehicle facility to investigate any standing water that could be found, including tires, drainage areas, and the scoops of the construction equipment. Mosquito larvae were collected from all of these water collecting points. It is not known if the water in the construction equipment scoops was residual from Louisiana, if it had collected from a rain event on location, if it had collected along the journey, or a combination of any of the former scenarios. This adult was collected in September, which was also a very dry month for the Camp Gruber area.

The second example of *Aedes aegypti* collected was a larva. This individual was collected in mid-June, prior to the adult. It was collected from the spare wheel well of the trunk in an abandoned car. It was identified later in the year after the adult was confirmed with PCR. This information shed new light on the situation, with the presents of an immature indicating a breeding population being present before the discovery of the adult. This larva's identity was also confirmed using PCR, and the same Blast sequences, KX580042.1, aligned with 100% homology for both individuals. This gives more evidence that the two individuals possibly came from the same area and population. Genetics have been used to investigate possible resident populations for this species in other parts of the world (Lima et al. 2016). The location where this larva was found, is approximately 520 meters from the area where the adult was collected at the previously discussed vehicle facility. This distance is farther than the typical, 250-300 meters that this species normally travels in search of breeding sites. While it is not impossible for a female to have traveled this farther distance, it could also indicate that there are breeding areas between the two trapping sites, or that there are multiple areas where the species is reproducing separate from other groups.

There was a disparity between the number of mosquitoes collected from the two trap types. CDC Traps collected 98.40% of the total, with just 1.6% collected from BG Traps. This disparity could have been caused by a few factors. Firstly, BG Traps were deployed fewer times than the CDC Traps at 238 and 426 trapping events respectively. This gives a ratio of 56% more trap deployments when comparing CDC Traps to BG Traps. During the first couple of sampling weekends, the 12 volt batteries for the BG Traps were not reliable. This led to the batteries operating with insufficient power to function properly on occasion. Finally, it could simply be that the BG Traps did not have the same attractive power, using their human scented lure, that the CDC Traps did with their CO_2 bait

The make-up of the mosquito species collected with each of the two trap types, was noticeably different. The primary mosquitoes collected by the BG Sentinel traps were *Aedes spp.* followed by *Culex spp*, representing 40.23% and 27.44% of the total collection numbers from the BG Traps, respectively. This is likely because the BG Traps are designed to target *Aedes spp.* (Ritchie et al. 2014). *Psorophora spp.* prevalence in these traps was comparable to *Culex spp.* at 26.89%

The CDC traps collected primarily *Culex spp*, with the genus making up 73% of the total, number of mosquitoes collected in these trap. *Psorophora spp.* were the second most prevalent in these traps, making up 13.05% of the total. These numbers were more representative of the overall numbers scene on the base, due to the high numbers collect with the CDC traps. The overall percentages of *Culex spp.* and *Psorophora spp.* collected from both traps was 72.25% and 13.26% respectively.

When comparing the results of the mosquito sampling at Camp Gruber to those from other military installation which were previously sampled, Camp Gruber had more genera collected as well as species. This could be the result of the much higher number of trap nights that occurred at Camp Gruber, the longer sampling season, or a combination of both. Camp Gruber had a higher diversity of *Aedes spp. Ochlerotatus spp.* and *Psorophora spp.* Only Fort Sill had a higher number of *Anopheles spp.* present. All of the previously sampled bases had a higher number of *Culex spp.* collected than did Camp Gruber. One genus, *Culiseta*, was only collected at Tinker Airbase, which did give it the highest genera diversity of all of the previously sampled military bases. This genus was also not collected at Camp Gruber.

Two genera were collected at Camp Gruber that were not collected at any of the other bases. These were *Toxorhynchites* and *Uranotaenia*. Both of these genera were represented by a single species each, *Tx. r. septentrionalis* and *Ur.*

sapphirina. The capture of both of this species is odd for separate reasons. *Toxorhynchites spp.* as adults do not feed on blood, but instead the females are nectar feeders like the males. The capture of this species could be considered accidental, because the adults would not be seeking a blood meal. However, an alternative reason for this occurrence could be based on the predatory nature of the larvae from this genus (Steffan and Evenhuis 1981). The three collected *Tx. r. septentrionalis*, were collected in BG Traps, which are designed to target *Aedes spp.* of mosquitoes (Ritchie et al. 2014). Many of the species in this genus are referred to as tree hole mosquitoes, because of their breeding site preference Species within these breeding habits are the primary prey for the larval, stages of *Toxorhynchites spp.* so it is possible that the presence of BG Traps indirectly brought in these mosquitoes because of the traps ability to attract *Aedes spp*. (Steffan and Evenhuis 1981).

A single *Uranotaenia sapphirina* was collected in a CDC Trap. This capture is not as unusual, as the *Tx. spp.* because this species' females feed on blood. However, they are considered specialist feeders which target amphibians and reptiles (Cupp et al. 2004). These mosquitoes locate frogs by their call, not the CO_2 produced through respiration. It is thought that the capture of the *Uranotaenia sapphirina* was the result of the trap being placed above a puddle of water, in which a frog was probably calling, bringing in this mosquito near enough to be pulled into the trap.

CHAPTER III

GUIDE TO MOSQUITOES AND THEIR MANAGEMENT AT CAMP GRUBER

Management Plan for Mosquitoes of Camp Gruber Military Training Center

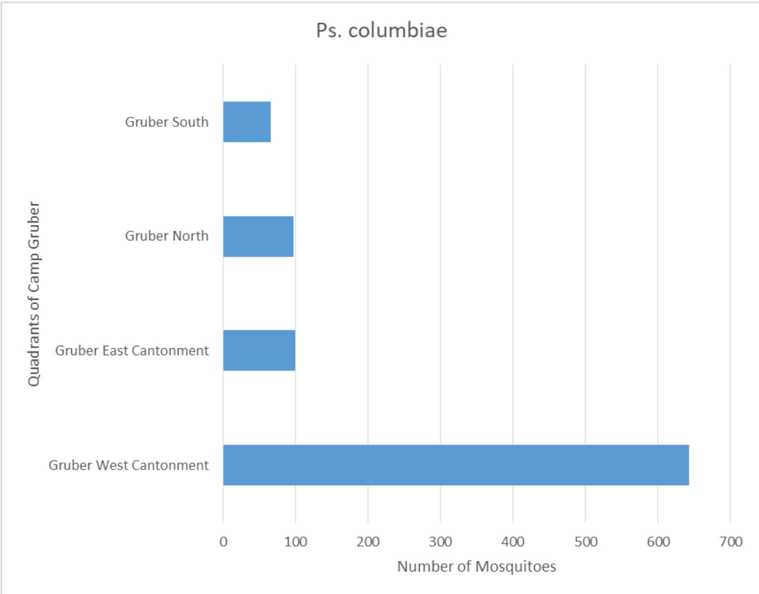
This guide provides information on the identification of problematic mosquitoes and methods of management for these species at Camp Gruber. This guide includes the most common species from each common genus, and species that were collected in small numbers but are known to be important disease vectors.

There were four species collected frequently in high numbers during the 2018 sampling season.

Psorophora columbiae:

This mosquito is sometimes referred to as the "Dark Rice Paddy Mosquito" because of its preference for flooded, grassy areas. Its breeding pattern falls under the "flood water" mosquito type, because it capitalizes on standing waters that occur after large rain events. This species was most commonly collected in the newly cleared and developed land on the east end of cantonment near the new barracks and the land just to the east. This species is large in size, and very dark in color. It is native to the Americas, is a strong flier, and is an aggressive day time biter. This mosquito is not known to be an important vector of any human diseases, but has been recorded transmitting diseases to cattle. Managing standing water and improving drainage systems are good options for control. Additionally, the application of pesticides via a vehicle mounted sprayer is also viable.

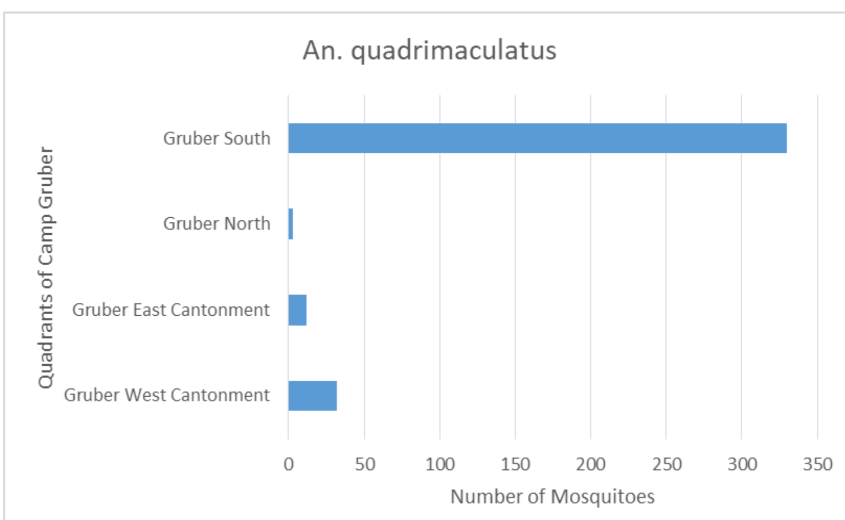
Photo courtesy of the Virginia Mosquito Control Association



Anopheles quadrimaculatus:

This mosquito is sometimes called the “Common Malaria Mosquito” because of its historical role as the primary vector for malaria when the disease was established in the United States. This species is native to North America and can be identified by the presence of very long palps beside is mouth. It also has very distinctive dappling of black and brown colors on its wings. This species commonly reproduces in the small tributaries and inlets around the edges of large bodies of water. At Camp Gruber, this species was captured almost exclusively in close proximity to Green Leaf Lake. Even though malaria is no longer the threat it once was in the United States, this species could still harbor and transmit the disease if it ever made a return. The only training area that is in the same area that these mosquitoes were collected is the Amphibious Landing Zone. If any troops were spending extended periods of time in this location, especially if they would be staying overnight, they could be exposed to a large number of these insects. Because the managing of all of the potential breeding sites around the lake’s edge would be impossible, the use of a collection trap such as the Mosquito Magnet® Patriot Plus, could be a viable option. These traps are commercially available and are known to collect and kill large quantities of mosquitoes. They are highly portable and can be moved as needed.

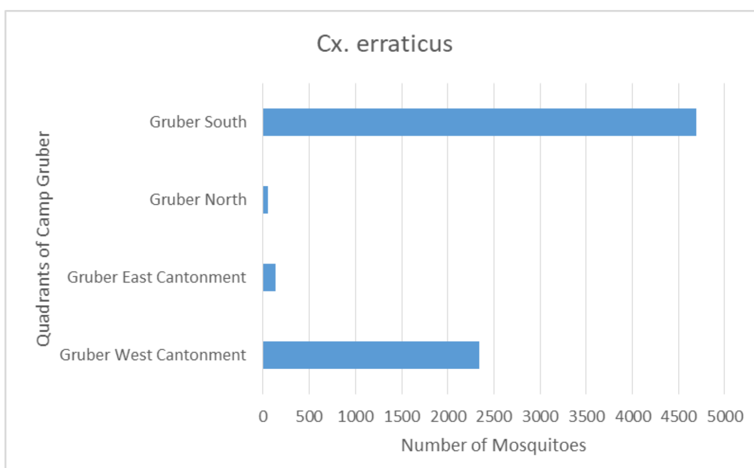
Photo courtesy of the Virginia Mosquito Control Association



Culex erraticus:

This is a small, dark brown, native species, which prefers similar habitat as the common malaria mosquito, *An. quadrimaculatus.* It has characteristic patches of white scales on its sides and it has dark mouthparts and palps. It was found in very high numbers near Green Leaf Lake. Additionally, large numbers of the species were collected near the gas station in Braggs, just on the other side of the stand of trees that separate Camp Gruber from the town. This could be because of a small pond that is located in the area or standing water in foundations of old buildings in the same area. This species is a known vector of Eastern Equine Encephalitis Virus and has been suggested as a possible vector for West Nile Virus. For management of this species, a mosquito magnet similar to the suggestion for *An. quadrimaculatus* could be used in the vicinity of Green Leaf Lake. For the tree stand between Braggs and Camp Gruber, mosquito dunks containing Bti (*Bacillus thuringiensis israelensis*) could be placed in both the small pond and the foundations. The ultimate goal should be filling the foundation in with soil to limit breeding sites for all mosquito species.

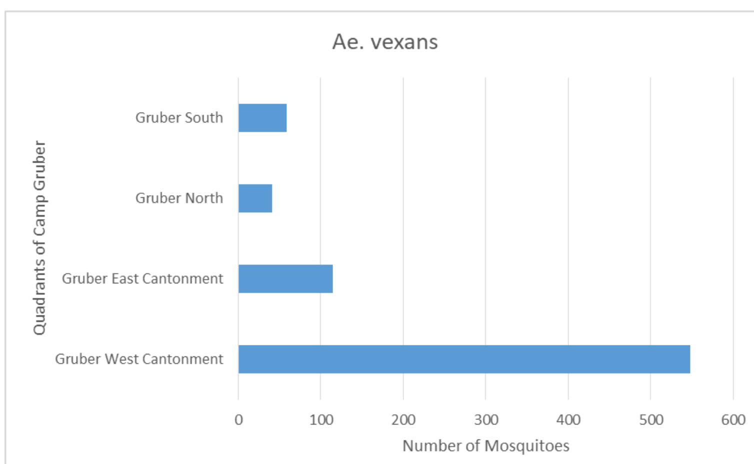
Photo courtesy of the Virginia Mosquito Control Association



Aedes vexans:

This species is known as the “Inland Floodwater Mosquito,” and can be found worldwide. As its common name suggests, it prefers flooded areas. These mosquitoes are primarily brown in color and have a distinctive pattern of a rounded “W” shape on the top of their abdominal segments. Similar to *Psorophora columbiae*, this species likes to breed in flat, flooded grasslands. This species was collected primarily in the cantonment areas, near the newly built barracks and in the areas just east of them. This species is an aggressive feeder with strong flying capabilities. It has been found to harbor West Nile Virus but is not an efficient vector. Management approaches for this species are similar to *Ps. columbiae*, where standing water should be eliminated and potential insecticide treatment of breeding areas.

Photo courtesy of the Virginia Mosquito Control Association

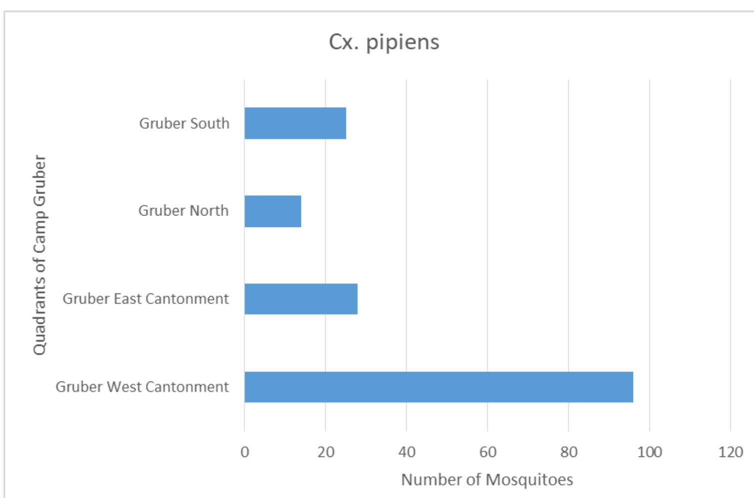


This following group of mosquitoes were not captured in large numbers, but their presence is a concern for potential disease risks for potentially exposed personnel

Culex pipiens:

This species is referred to as the "Common House Mosquito." It will readily enter human dwellings to find a blood meal. This species is typically a mix between pale pink and brown colors and is one of primary concerns for the transmission of West Nile Virus. This species likes to breed in stagnant water, commonly being found in poorly draining ditches, in residential areas. Most of the examples of this mosquito were collected from the western side of cantonment. Within cantonment, many of the large ditches hold water for a long period after a rain event. Once these ditches have become a series of isolated puddles, free of fish, they are prime breeding habit for *Culex pipiens*. The improvement of these ditches, through better angling to increase flow, or the addition of cement and or rocks, in the entirety of the system could help reduce breeding habitat.

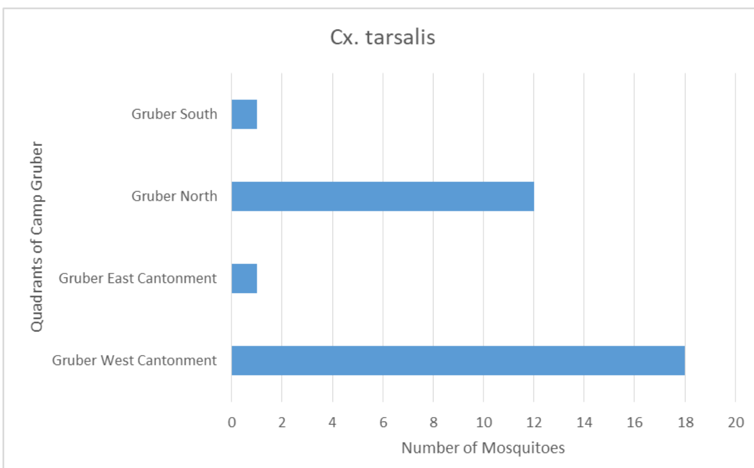
Photo courtesy of Identify US



Culex tarsalis:

This species is known as the "Western Encephalitis Mosquito," and is a native to North America. This mosquito is small and primarily a light brown color. They also have white bands around the joints of the legs and a small white band around the proboscis. *Culex tarsalis* prefer to breeding in standing, stagnant water. Similar to *Culex pipiens*, the majority of this species collected came from the western end of cantonment. This species is known to be a vector of St. Louis Encephalitis and West Nile Virus. The control recommendations for this species are very similar to those *of Culex pipiens*. The improvement of the ditch systems around cantonment would help reduce breeding.

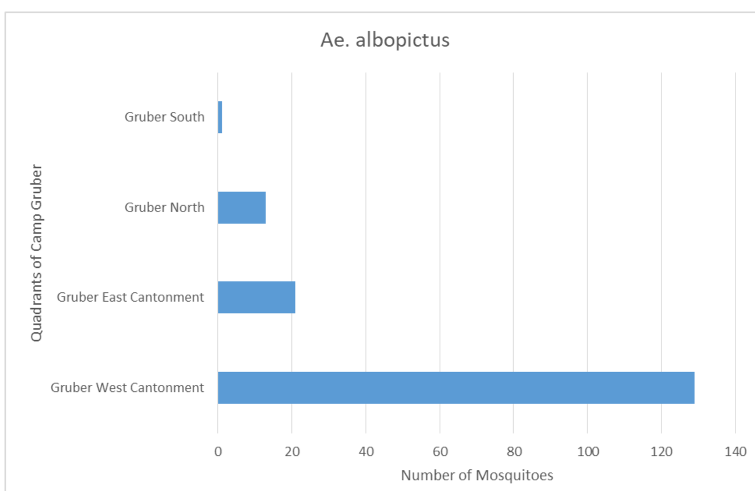
Photo courtesy of Identify US



Aedes albopictus:

This species is known globally as the "Asian Tiger Mosquito," and is native to South East Asia. It is known as a "tree hole" breeding mosquito, but will also utilize human containers, such as buckets and tires to reproduce. This species has very dark black coloration on most of its body, and two distinct white strips that run along the top of its back and white bands at the joints of the legs. This species is commonly found in relatively close proximity to humans. At Camp Gruber, most individuals were collected from the western half of cantonment. The highest number of individuals were collected at the range control building. This species is an important vector of several diseases, including Dengue, Chikungunya, and Zika. It has also been shown experimentally to transmit several other diseases. To control this species by managing the containers in an area remove water sources used for reproduction, or if neither of these can be done, placing mosquito dunks, small floating bate containing materials toxic to mosquitoes, in containers that hold water which cannot be removed.

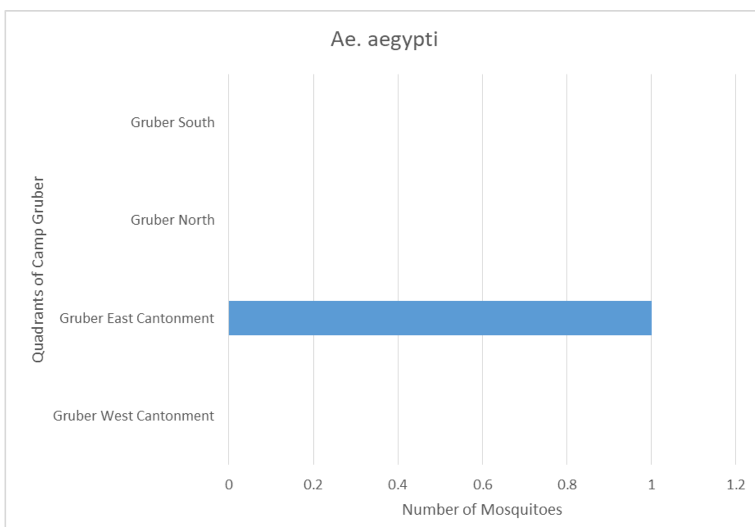
Photo courtesy of the Center for Disease Control



Aedes aegypti:

This species is referred to as the "Yellow Fever Mosquito," and is native to Africa. It is a small mosquito, similar in its characteristics to its relative *Aedes albopictus*, sharing the white stripes on the joints of the legs, but being more brown in color, and having two distinct white stripes, flanked by two white lyres on the top of its back in contrast to the single white stripe of *Aedes albopictus*. This species is a vector for several human pathogens, including Chikungunya, Yellow Fever, Dengue and Zika Virus. Additionally this species has a preference for human blood meals, and readily seeks out containers in close proximity to humans, for breeding sites. Management of these mosquitoes is similar to that *of Aedes albopictus*, with the elimination of water holding containers being essential. These mosquitoes will take advantage of very small breeding sites, such as bottle caps and pools of water in air conditioners, so vigilance is important when trying to exclude this species.

Photo courtesy of the Center for Disease Control



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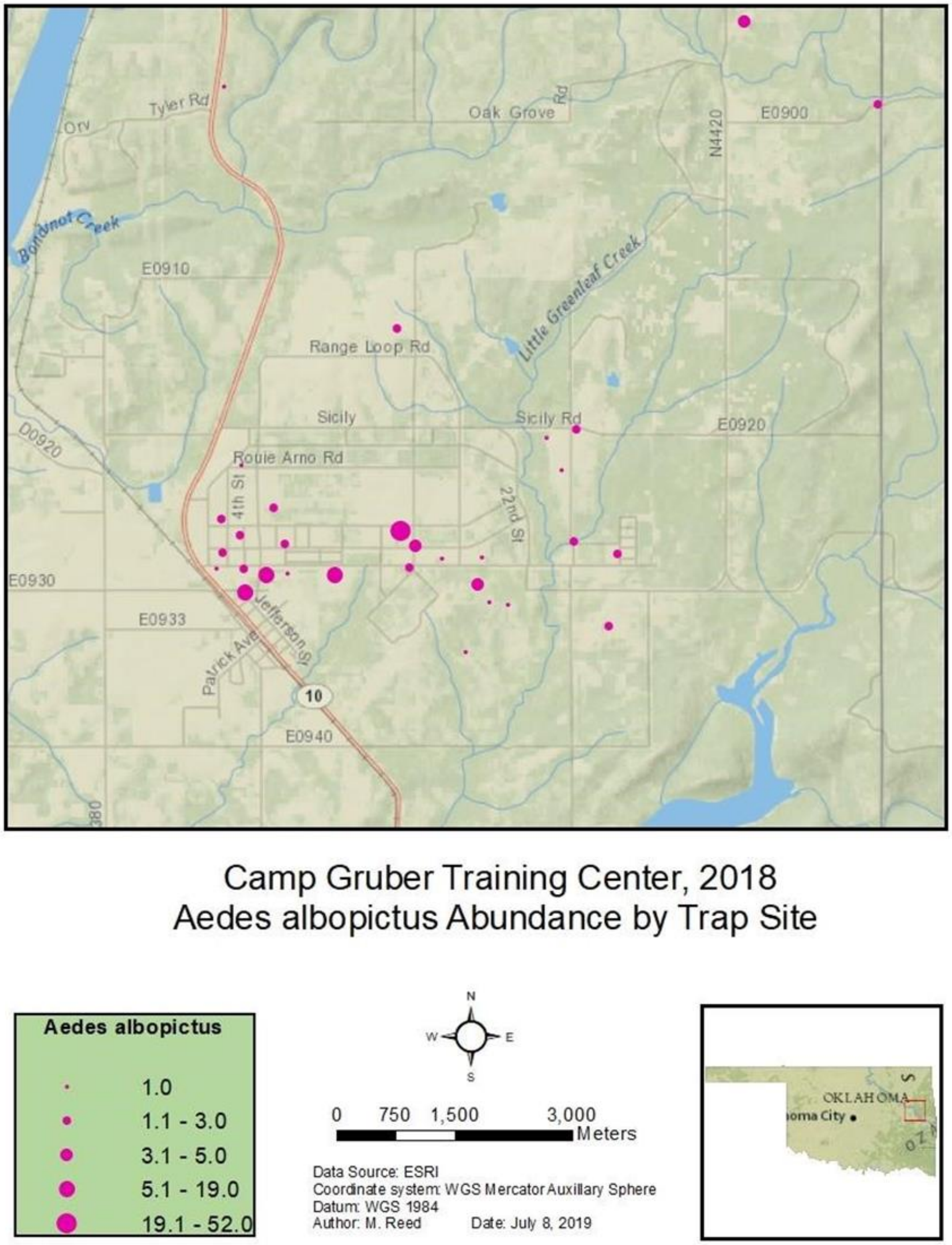
APPENDICES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Camp Gruber Training Center, 2018
Aedes albopictus Abundance by Trap Site
Aedes albopictus
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Author: M. Reed
Date: July 8, 2019
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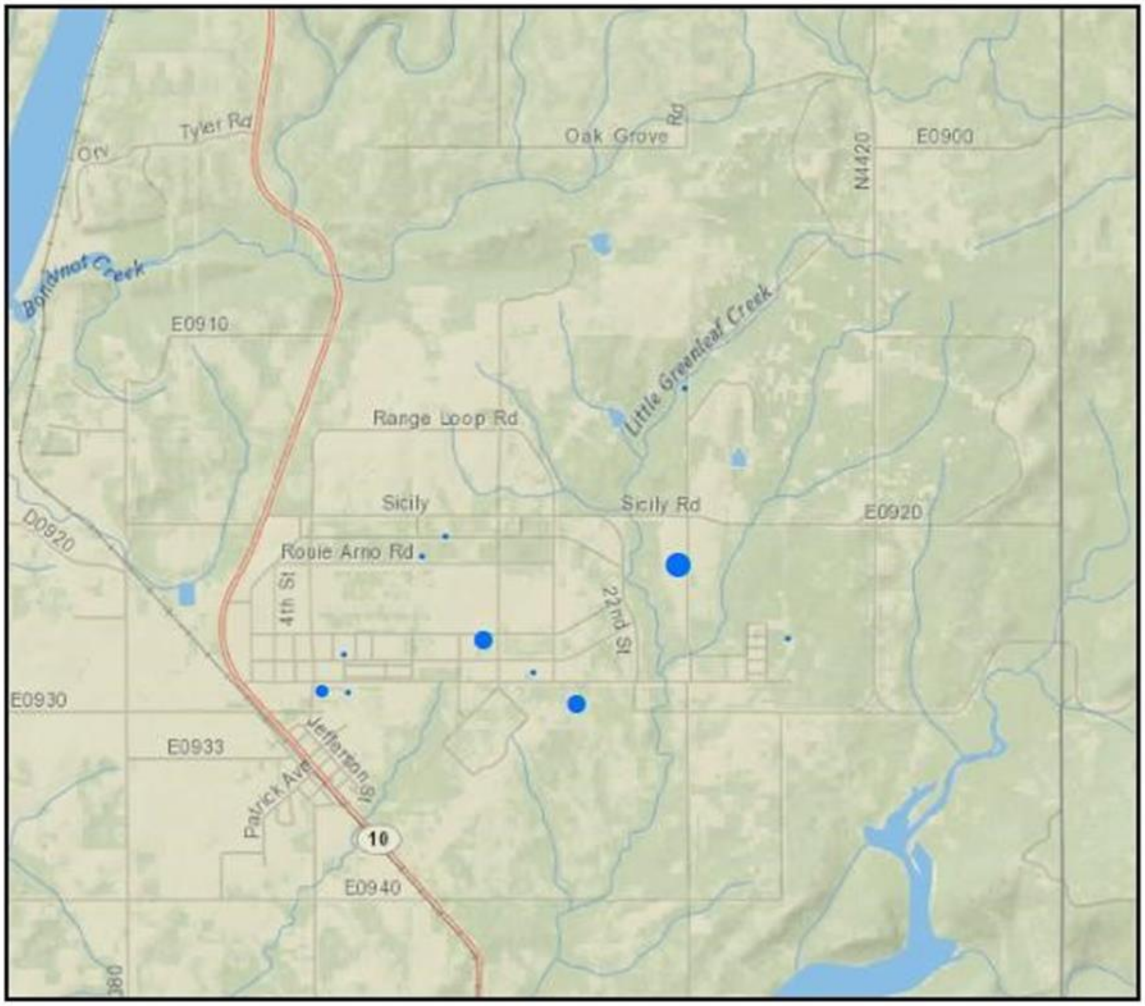

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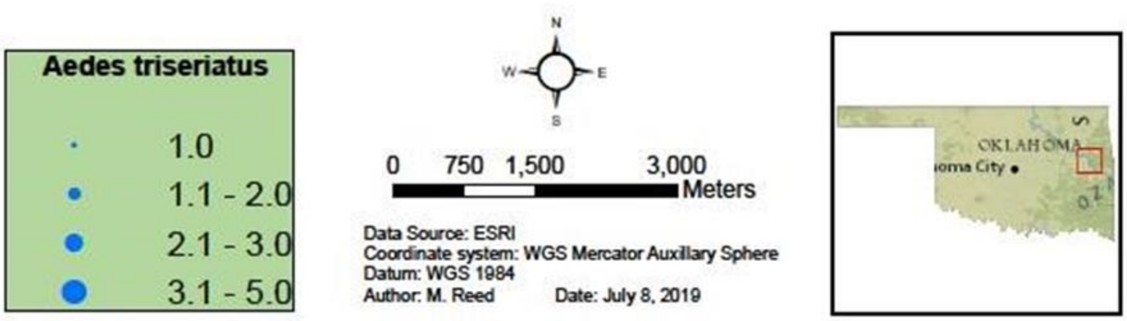
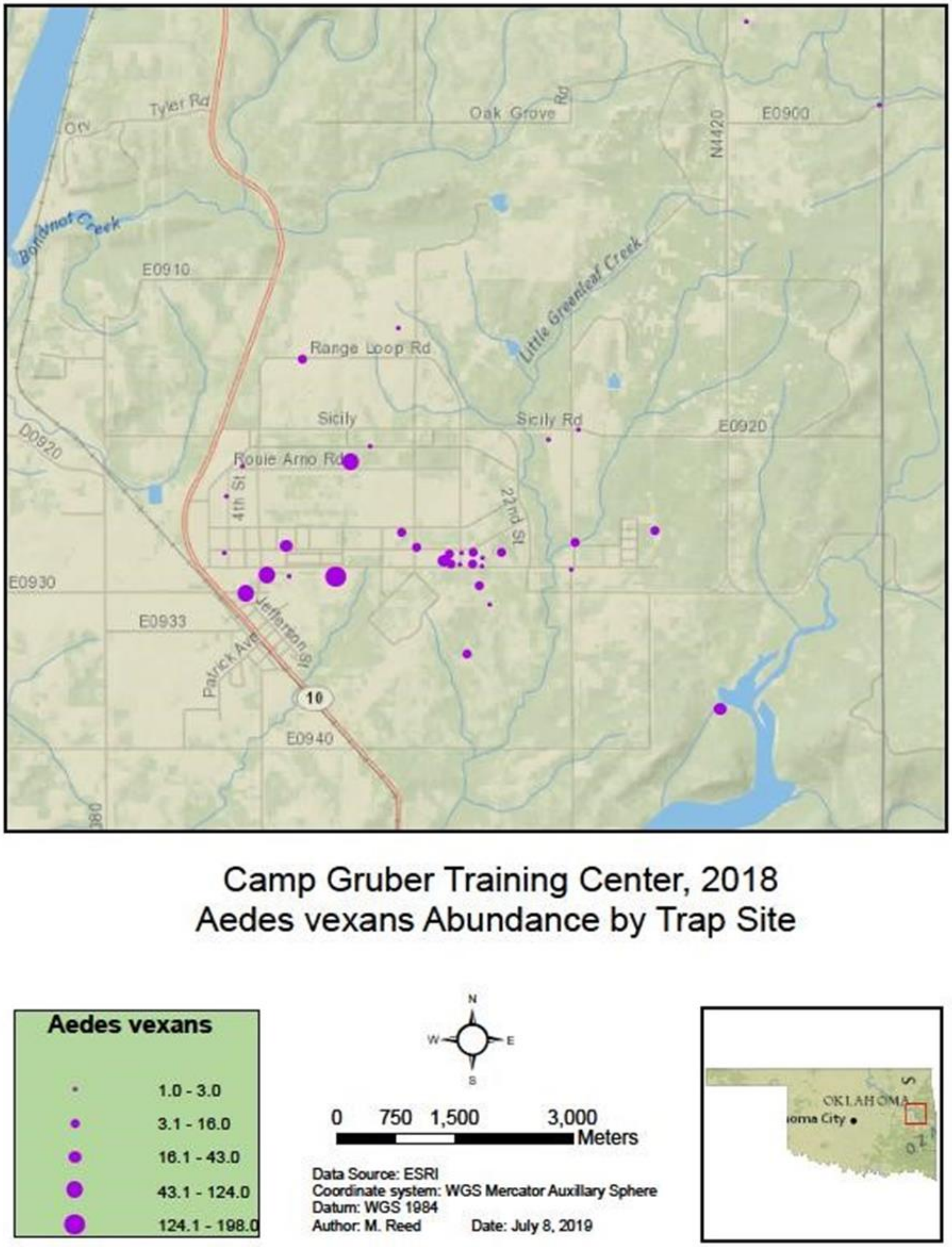
Aedes triseriatus Abundance by Trap Site

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Camp Gruber Training Center, 2018
Aedes vexans Abundance by Trap Site
Aedes vexans
1.0 - 3.0
3.1 - 16.0
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43.1 - 124.0
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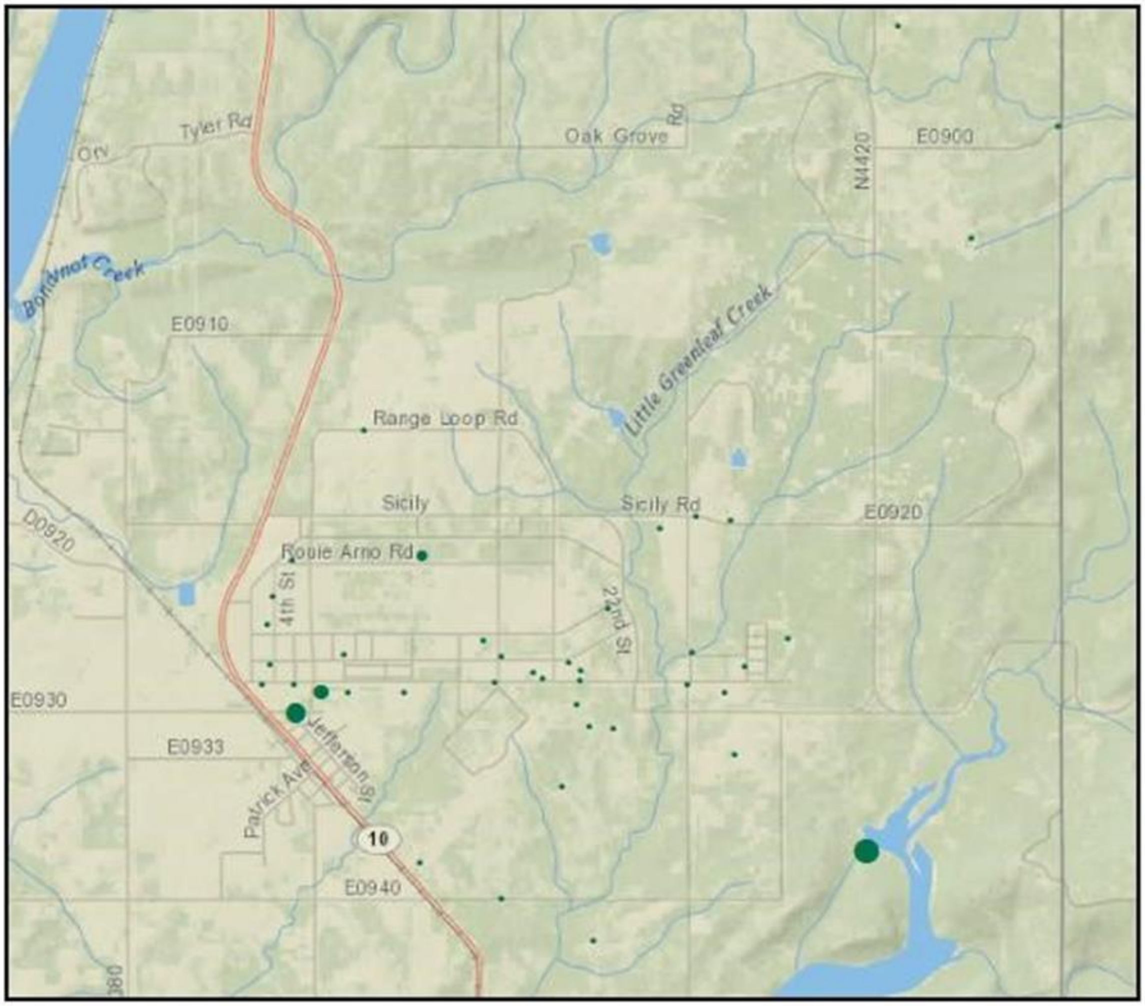

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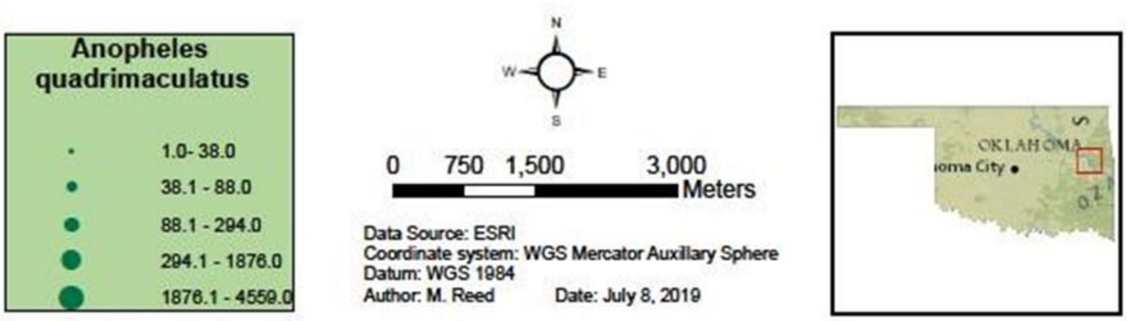
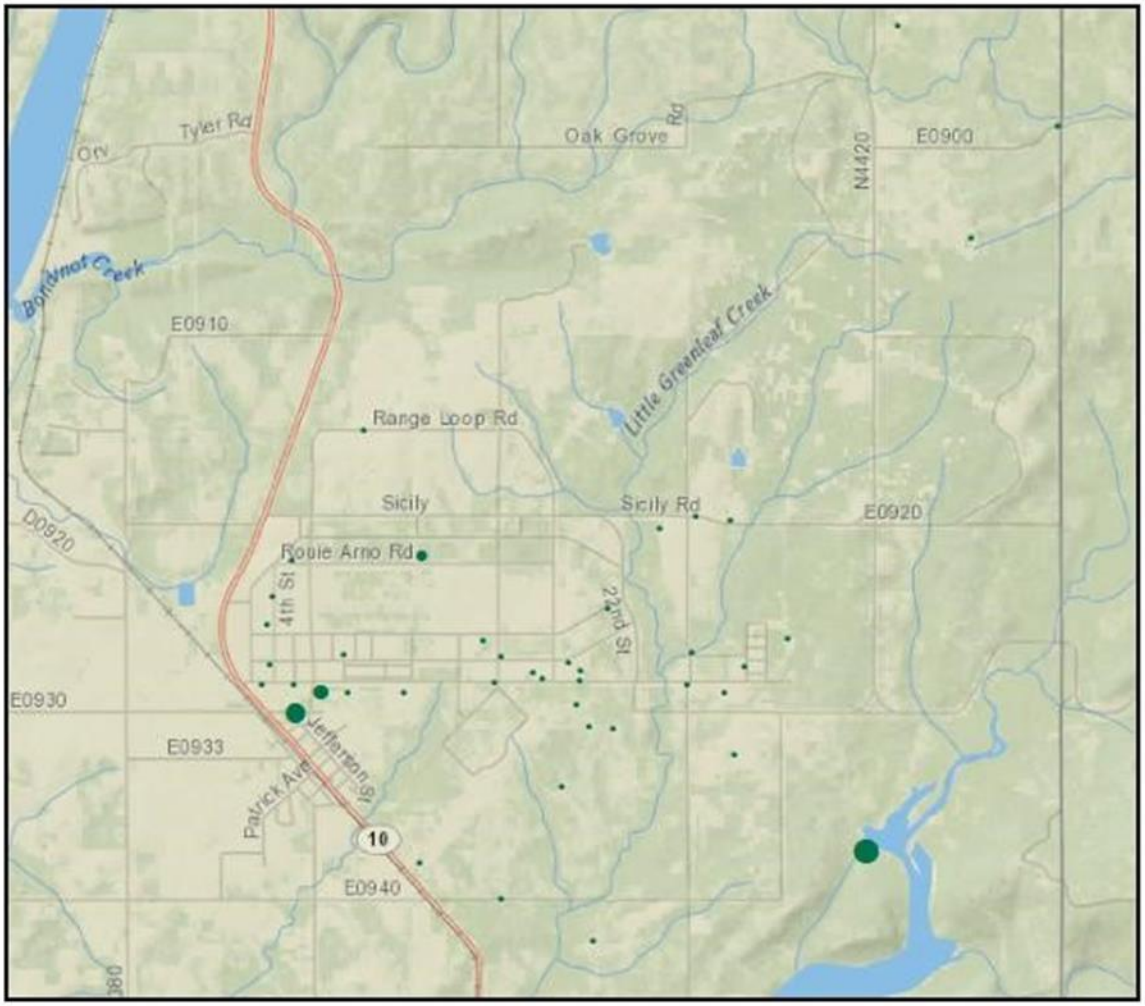
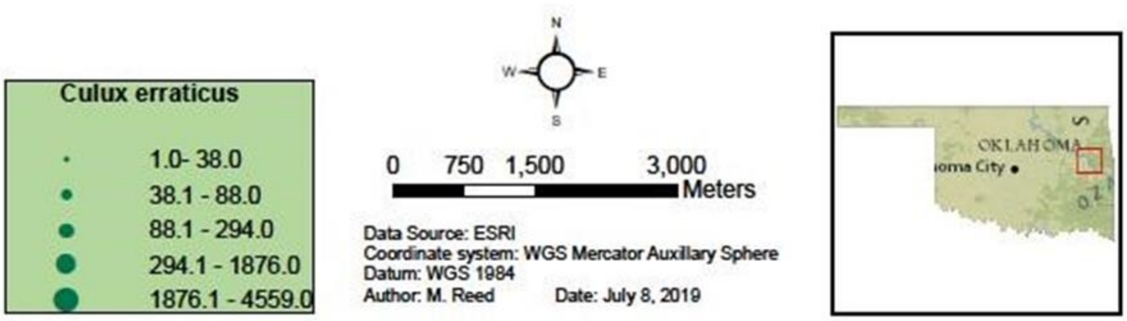
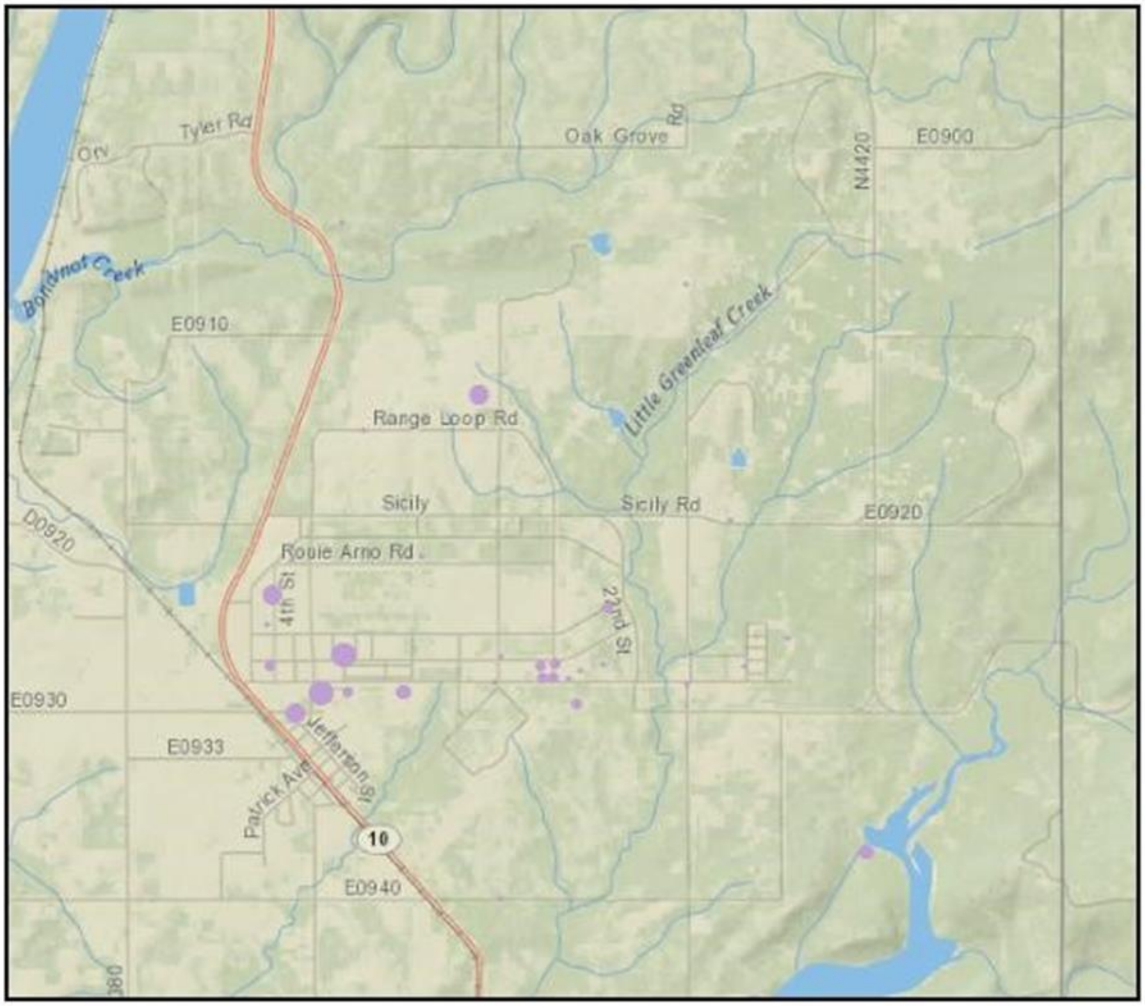
Anopheles quadrimaculatus Abundance by Trap Site





Camp Gruber Training Center, 2018
Culux erraticus Abundance by Trap Site





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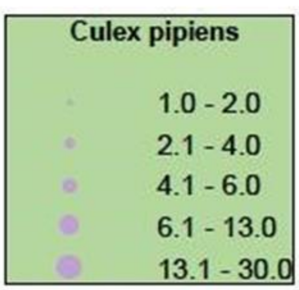
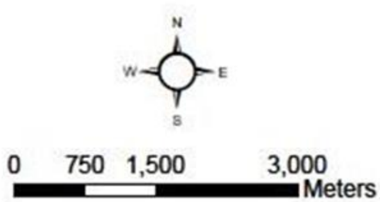
Culex pipiens Abundance by Trap Site





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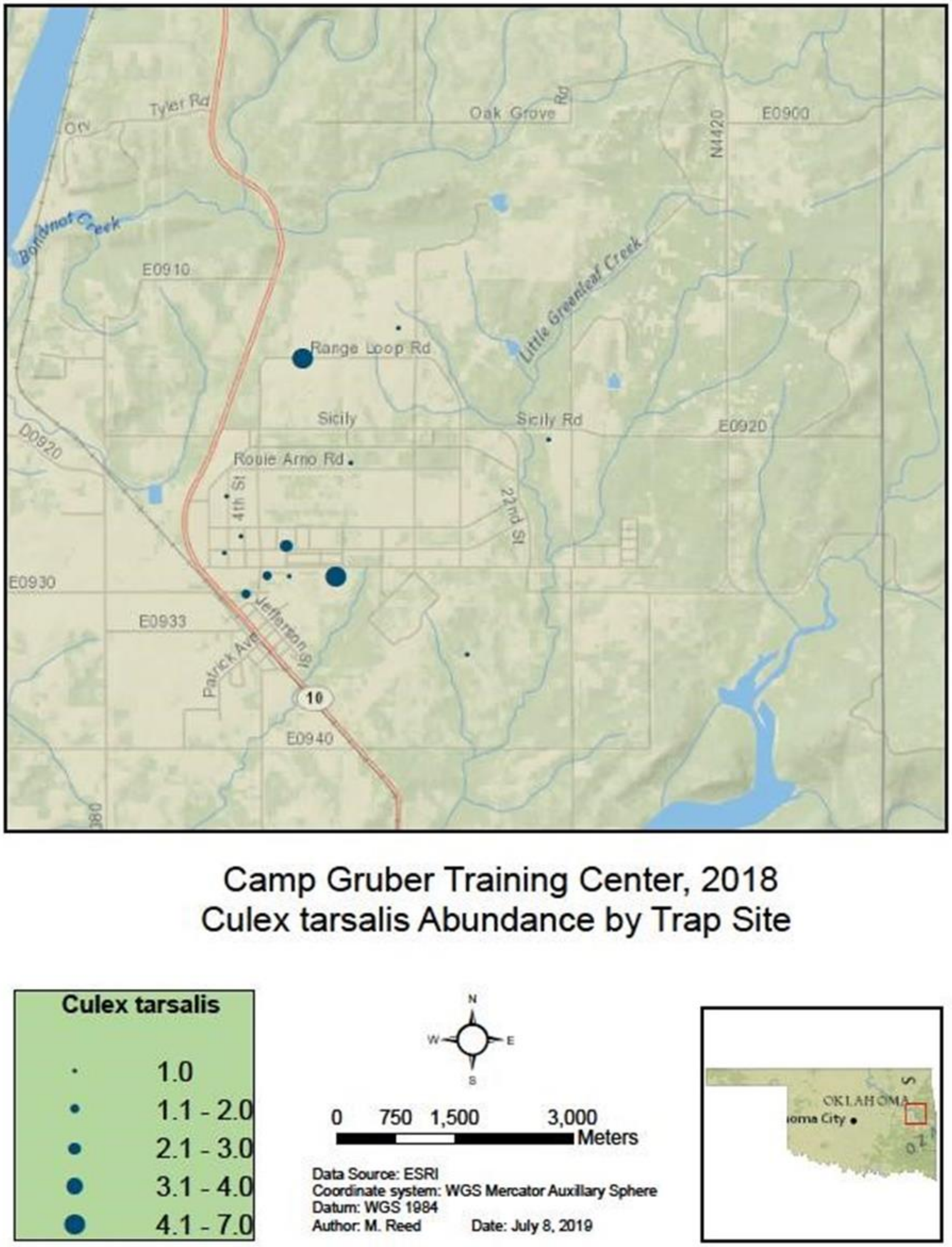
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Jefferson St
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Camp Gruber Training Center, 2018
Culex tarsalis Abundance by Trap Site
Culex tarsalis
1.0
1.1 - 2.0
2.1 - 3.0
3.1 - 4.0
4.1 - 7.0
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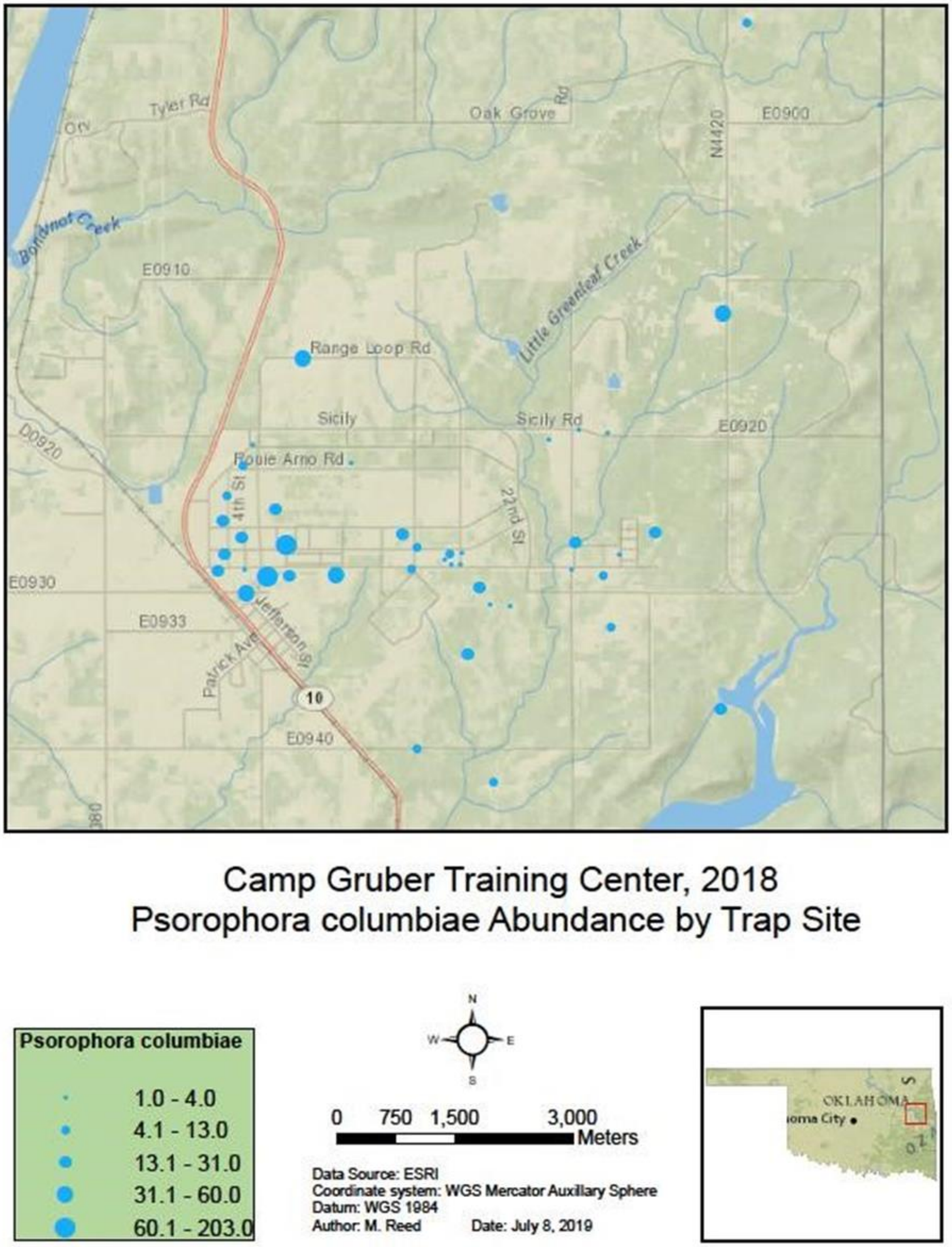
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Rouie Arno Rd
4th St
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Jefferson St
Patrick Ave
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Camp Gruber Training Center, 2018
Psorophora columbiae Abundance by Trap Site
Psorophora columbiae
1.0 - 4.0
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13.1 - 31.0
31.1 - 60.0
60.1 - 203.0
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Data Source: ESRI
Coordinate system: WGS Mercator Auxillary Sphere
Datum: WGS 1984
Author: M. Reed Date: July 8, 2019
OKLAHOMA

VITA

Thomas Magnum Hess

Candidate for the Degree of

Master of Science

Thesis: MOSQUITO SURVEY OF CAMP GRUBER MILITARY TRAINING CENTER AND COMPARISON WITH OTHER OKLAHOMA MILITARY BASES

Major Field: Entomology and Plant Pathology

Education:

Completed the requirements for the Master of Science in your Entomology and Pant Pathology at Oklahoma State University, Stillwater, Oklahoma in July, 2019.

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Professional Memberships:

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