

**THE INFLUENCE OF MOSQUITO PREDATORS ON
POPULATION DYNAMICS OF ENDEMIC AND EXOTIC
MOSQUITOES**

By

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ABSTRACT

The presence of predators can shape the population dynamics of prey. Here, I evaluated the influence of predators on mosquito populations by direct effects through predation, and indirect effects through sub-lethal responses. The predator under investigation was the backswimmer *Anisops wakefieldi*.

I first quantified the relationship between mosquito and predator populations in animal drinking water troughs, and correlated their abundance with water volume and environmental factors. Logistic regression indicated that the presence of mosquitoes was primarily affected by three factors; predator numbers, week of observation, and water volume. A translocation experiment was established to understand the pre- and post-treatment effects on mosquito survival after exposure to the predator *Anisops wakefieldi*. The presence of these predators in water troughs significantly decreased subsequent survival of mosquito prey within two days post-translocation.

A major hypothesis in invasion ecology suggests that native predators have less impact on exotic species relative to native prey species, enabling exotic species to establish and thrive in novel environments. This is the “escape from natural enemies” hypothesis. Contrary to this hypothesis *A. wakefieldi*, a native New Zealand predator, showed a greater preference, and consumed more of the exotic mosquito *Aedes* (*Ochleratatus*) *notoscriptus* compared to the native (*Culex pervigilans*) mosquito larvae.

Anisops wakefieldi exhibited a decelerating Type II functional response for both prey species, but consumed more exotic mosquito species at high prey densities and displayed higher attack rates. The effects of the preference of this predator on mosquito behaviour were examined. In the presence of predators the exotic species, *Ae. notoscriptus*, demonstrated significantly higher levels of “thrashing” behaviours, apparently making itself more obvious to the predators. In contrast *Cx. pervigilans* fulfilled the “threat sensitivity” hypothesis by altering its behaviour towards “resting”, known to be the best strategy for avoiding predators.

In addition to lethal effects, predators can substantially alter prey populations by means of sub-lethal influences. To further our understanding of how predators limit mosquito abundance, I developed an experiment based on adult mosquito oviposition. I predicted that the New Zealand native mosquito, *Cx. pervigilans* would likely avoid containers with *A. wakefieldi* or their kairomones. Contrary to our predictions, *Cx. pervigilans* appeared to ignore the presence of predators and their kairomones when choosing oviposition habitats and the number of egg rafts was not significantly affected by the density of predators.

Culex pervigilans eggs from the oviposition experiment were reared in two different laboratory conditions: (A) in clean water without any traces of predators, or (B) in water with the same treatments as in field. This experiment was established in order to have better understand on what happens to the next generation after exposure to *A. wakefieldi* predators or their kairomones. Sub-lethal effects were still apparent in the developing

larvae. I observed a significant reduction in the survival rate of *Cx. pervigilans* in the presence of predators and their kairomones, even when the eggs were only exposed briefly to water containing either predators or kairomones in the field, and were then reared in clean water without any traces of the predator. No effect was observed on the sex ratio of *Cx. pervigilans*, or the development times of each life stage.

Overall, this thesis has highlighted the importance of predators in influencing mosquito populations, not only through direct predation, but also in indirect and sub-lethal ways. This study may have implications for the dynamics of other predator-prey systems. Despite this, we are only beginning to understand the complex interactions between predators and prey.

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CHAPTER ONE:

General Introduction

There is concern that the establishment of exotic and invasive mosquito species may increase the risk of infectious disease to humans occurring in New Zealand. **The overall goal of this thesis is to investigate the influence of predators on mosquito population dynamics.** To achieve this aim, I focused my study on the lethal and sub-lethal effects of the native predator, *Anisops wakefieldi*, on the endemic mosquito, *Culex pervigilans*. I also examined the interaction between this predator and the exotic mosquito, *Aedes notoscriptus*.

1.1 Introduction to mosquitoes

Mosquitoes are known as the most important disease vectors in the world because of their ability to carry various pathogenic agents such as viruses, protozoa and helminthes. Mosquitoes have a worldwide distribution; they occur throughout tropical and temperate regions, and their range extends northwards into the Arctic Circle, they are only absent from Antarctica. *Anopheles*, *Culex*, *Aedes*, *Ochlerotatus*, *Psorophora*, *Haemagogus*, *Mansonia* and *Sabethes* are a few of the most important genera that can be vectors for a variety of diseases. There are a total of 41 genera in the family Culicidae, containing 3450 known species and subspecies of mosquitoes. Culicidae can be divided into three subfamilies; Toxorhynchitinae, Anophelinae and Culicinae (Service 1996, Service 2004).

Anopheles species are well known vectors for malaria, Filariasis (*Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*) and other arboviruses. Certain *Culex* species can transmit *Wuchereria bancrofti* and a variety of arboviruses. Arboviruses are a class of virus transmitted to humans

or animals by arthropods such as mosquitoes and ticks. The first two letters of the words **arth**ropod and **borne**, make up the 'arbo' that now designates this group of viruses as arthropod-borne. *Aedes* species are important vectors of yellow fever, dengue, encephalitis viruses and many other arboviruses, and they are also vectors of *Wuchereria bancrofti* and *Brugia malayi*. Filariasis and encephalitis viruses are also transmitted by mosquitoes that are closely related to *Aedes* (*Ochlerotatus*) species. *Mansonia* species transmit *Brugia malayi* and sometimes *Wuchereria bancrofti* and a few arboviruses. *Haemagogus* and *Sabethes* mosquitoes are vectors of yellow fever and other arboviruses in Central and South America, while the genus *Psorophora* contains some troublesome pest species in North and South America. However, many species of mosquito do not carry disease but are a biting nuisance and pose other problems to humans and animals (Service 1996, Service 2004).

1.1.1 Mosquito lifecycle

Mosquito pass through four distinct stages in their lifecycle; eggs, larva, pupa and adult (Figure 1.1). Eggs are laid either singly or in rafts, within 2 to 3 centimeterson the water surface or undersides floating vegetation (Service 2000; 2004). Some mosquitoes may lay their eggs on vegetation in the water. Within 1 or 2 hours after the eggs are laid, the endochorion of the shell changes from a soft white layer to a hard dark one (Fay 1964). According to Weissman-Strum and Kindler (1963), it is possible to divide the process of hatching into two distinct stages. The first stage is the breakdown of the water impermeable barrier which is most likely due to

the action of the pro larvae and the second stage is the influx of water to the larvae with concomitant swelling and emergence from the shell.

There are four stages, or instars, in the mosquito larvae. Normally, the third and fourth instar larvae are used in efficacy studies because of their stable biology and susceptibility (Busvine 1971). The pupae have comma-shaped bodies divisible into two distinct regions. The front region consists of the head and thorax (cephalothorax) and is greatly enlarged. It bears a pair of respiratory trumpets to take in oxygen. The second region is the abdomen which has freely-movable segments with a pair of paddle like appendages at the tip. Feeding does not take place during the pupal stage (Blackmore and Lord 2000).

When maintained on a carbohydrate restricted diet, most adult female mosquitoes are likely to produce eggs after ingesting a blood meal (Klowden 1993). Adult mosquitoes emerging from habitats with insufficient nutrients may lay eggs that are programmed to go into early diapause. Oogenesis is initiated in about 20% of the mated population even when as little as 0.1µl of blood was ingested (Pumpuni et al. 1992). Large females ingest more than twice the blood volume of small females and their fecundity quadruples (Briegel 1990).

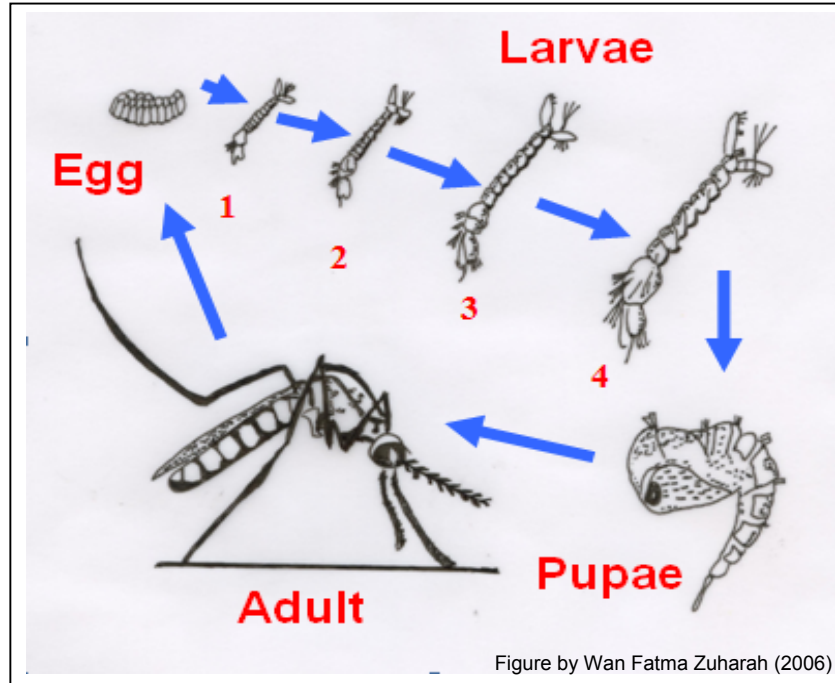


Figure 1.1: Four distinct stages of the mosquito life cycle. There are four instars of larval development in mosquitoes and one non-feeding stage in the pupa.

Most mosquito species survive winter, or overwinter, in the egg stage, awaiting the spring thaw, when waters warm and the eggs hatch. A few important species spend the winter as adult, mated females, resting in protected, cool locations, such as cellars, sewers, crawl spaces, and well pits. With warm spring days, these females will seek blood sustenance and begin the cycle again. Only a few species can survive overwinter as larvae (Service 2004).

1.2 Mosquitoes in New Zealand

Before the arrival of humans, New Zealand had only 12 indigenous species. Since the arrival of humans in New Zealand, approximately 800 years ago, the abundance of exotic species has increased rapidly. The invasion rates have increased since European settlement and the arrival of goods and passengers (Derraik 2004a). As of 2004, it has been estimated that New Zealand has 12 endemic and 4 exotic mosquito species (Russell 1995, Snell 2005). The endemic mosquito species in New Zealand are *Coquillettidia (Austromansonia) tenuipalpis*, *Coquillettidia (Coquillettidia) iracunda*, *Culex (Culex) asteliae*, *Culex (Culex) pervigilans*, *Culex (Culex) rotoruae*, *Culiseta (Climacura) novaezealandiae*, *Culiseta (Climacura) tonnoiri*, *Maorigoeldia argyropus*, *Ochleratatus (Nothoskusa) chatamicus*, *Ochleratatus (Ochleratatus) antipodues*, *Ochleratatus (Ochleratatus) subalbirostris* and *Opifex fuscus*. The four exotic species are *Culex (Culex) quinquefasciatus*, *Aedes (Ochleratatus) (Finlaya) notoscriptus*, *Ochleratatus (Halades) australis* and *Ochleratatus (Ochleratatus) camptorhynchus* (Derraik 2004a).

Since European settlement, 3 out of the 4 exotic mosquito species are believed to have established in New Zealand. *Culex quinquefasciatus* Say, native to the warmer parts of Africa, was first collected in New Zealand in 1848. *Aedes (Ochleratatus) notoscriptus* was first collected in Auckland in 1916 during World War I. The third exotic mosquito species, *Ochleratatus (Halades) australis* was discovered only three decades ago in 1962. These mosquitoes were accidentally transported aboard sailing vessels and dispersed along the North Island's east coast and the South Island. *Aedes*

(Ochleratatus) notoscriptus was more successful than *Culex quinquefasciatus* in colonizing New Zealand, probably because it can adapt to low temperatures and has radiated into varied and widely dispersed larval niches. The latest exotic mosquito found in New Zealand is *Ochleratatus (Ochleratatus) camptorhynchus*, originating from Australia, it was discovered on the east coast of the North Island in 1998 (Derraik 2004a; 2004b, Laird 1990, Laird 1995, Weinstein et al. 2007).

There is a possibility of new mosquito species entering New Zealand by accidental introduction. At least 30 other exotic culicid species have been intercepted at national entry posts, including the Asian tiger mosquito, *Aedes albopictus*, which is a vector of dengue fever (Derraik 2006). In May and September 1929, a new mosquito species *Anopheles maculipennis*, was reported to have reached Auckland from Indonesia aboard a shipping vessel. The possible introduction of *Anopheles* species, a malaria vector, represents a real danger in temperate country such as New Zealand (Laird 1990). However, no cases of malaria transmission have yet been reported in New Zealand as *Anopheles maculipennis* is not yet been established in New Zealand. MAF Biosecurity New Zealand has carried out over 11 years of the eradication programs involved helicopter and ground treatment, sampling and trapping using using S-methoprene granules and *Bacillus thuringiensis*. Due to these eradication programs, that the later exotic mosquito species may soon be declared.

1.3 Successful factors of mosquito invasions in New Zealand

Human activities have been causing dramatic and unpredictable changes for the Earth's ecosystem. As a result, a growing number of disease outbreaks have occurred, including mosquito borne disease. The New Zealand landscape has suffered dramatic changes since the arrival of humans, leading to extensive habitat destruction, extinction of native species and the introduction of exotic species (Cook et al. 2002, Derraik and Slaney 2007). Williams et al. (2005) predicted that climate changes in Australia and New Zealand will lead to the presence of new mosquito species such as *Culex gelidus*.

There are several other characteristics of exotic mosquito species that contribute to their success in non-native habitats. For example; rapid growth, short lifespan, high fecundity, the ability to utilize a broad range of habitats, association with human activity and fewer natural enemies are several factors that will determine their likelihood of establishment (Griswold and Lounibos 2005). New Zealand's species poor mosquito fauna and high level of anthropogenic environmental disturbance make it particularly vulnerable to invasion by exotic mosquitoes (Derraik 2004a). The apparent underutilization of larval breeding habitats may also encourage exotic mosquitoes to establish in New Zealand (Crump et al. 2001, Laird 1990, Laird et al. 1994).

1.4 Risks of mosquito borne diseases for New Zealand

The human population in New Zealand has not yet experienced any outbreaks of mosquito borne diseases. However, their potential involvement in local disease transmission has been recently highlighted. Exotic mosquito

species have demonstrated vector competence for human arboviruses (Derraik and Slaney 2007, Weinstein et al. 1997, Weinstein et al. 2007). The rapid modern movement of people, the consequent routine arrival of infected people, exotic arthropods, and increasing international trade and commerce are likely to increase New Zealanders chances of contracting mosquito borne disease (Derraik 2004a, Derraik 2004b, Derraik and Calisher 2003, Laird 1990, Spurr and Sandlatt 2004, Weinstein et al. 1997).

All of the four exotic species of mosquitoes in New Zealand are known as vectors for mosquito borne diseases in other parts of the world. *Culex (Culex) quinquefasciatus* is known to be a West Nile Virus vector overseas (Derraik 2004a). This mosquito is believed to have originated in America. As of 2004, Spurr and Sandlatt (2004) reported that at least 30 bird and 16 mammals, especially introduced species in New Zealand, are susceptible to the West Nile Virus. This mosquito can cause irritation and discomfort because they feed on mammals (including humans). Given that New Zealand has many proven avian and mammalian hosts of the West Nile Virus, the risk of infection in this country is quite high, provided that this virus can overwinter here. *Culex (Culex) quinquefasciatus* has also been proven to be a vector for Murray Valley encephalitis virus in the laboratory and Ross River Virus (Lindsay et al. 1993, McLean 1953, Weinstein et al. 2007).

The natural Australian habitats of larval *Ae. notoscriptus* are tree hole and rock pool environments. However, after they became established in New Zealand, this mosquito has made the shift from natural to artificial containers. The establishment of *Ae. notoscriptus* was likely facilitated by a lack of competition from native species, and mosquito larval habitats that were

considerably underutilized (Laird 1990). Because of their establishment, infection by the avian Whataroa virus has a possibility of occurring in humans. However, no human cases of infection with Whataroa virus has been recorded even though this mosquito is capable of harboring the virus under laboratory conditions (Lee et al. 1982, Weinstein et al. 2007). This mosquito is also known to be a vector for Yellow fever, Dengue fever, Murray Valley encephalitis, Ross River Virus, Japanese encephalitis and Barmah Forest virus (Derraik and Calisher 2003, van der Hurk et al. 2003, Watson and Kay 1998, Weinstein et al. 2007).

Due to high number of viraemic people entering New Zealand, the abundance of possums, and the presence of *Oc. camptorhynchus*, the transmission of Ross River Virus within New Zealand is possible. In 2001, annual infection rates of Ross River Virus and Barmah Forest virus in Queensland ranged between 48.4- 423.5 and 3.8- 40.4/ 100,000 respectively. It has been estimated that more than 100 viraemic travelers may enter New Zealand from Queensland each year (Kelly-Hope et al. 2001). Ross River virus is the most common mosquito-borne pathogen affecting humans in Australia and seems likely to occur sooner rather than later in New Zealand (Derraik and Calisher 2003).

One of the exotic mosquitoes, *Oc. australis*, has not been considered a significant threat, in the public health context, other than as a potential vector of Ross River virus. This is because they do not have a sufficiently close relationship with humans. As an adult, it is autogenous and capable of oviposition without a blood meal (Weinstein et al. 2007). Very little research

has been done on New Zealand's endemic mosquito species, thus their vector capability is largely unknown.

1.5 Mosquito predators in New Zealand

Mosquito larvae are prey for a wide variety of aquatic organisms. Invertebrate predators directly or indirectly influence mosquito population dynamics. Backswimmers and Dysticid beetles are the most common and abundant predators (Graham 1939). Predators in the family Corixidae are occasionally found in water bodies. Backswimmers, *Notonecta* spp., are highly predaceous and can consume large numbers of mosquito larvae (Gilbert and Burns 1999). Their presence in water bodies can significantly reduce oviposition by adult mosquitoes (Blaustein et al. 1995). Diving beetle and damselfly larvae are often observed in water bodies and have been known as aquatic predators of mosquitoes in New Zealand. However, their effects on mosquito population dynamics have not been studied previously (Lester and Pike 2003).

Flightless predator morphs are well-suited to keep stock-trough water containers relatively or totally free from mosquito larvae (Laird 1990). Young (1970) stated that flightlessness and polymorphism might have considerable practical value in limiting mosquito populations.

1.6 Predator-prey interaction in shaping population dynamics

Ecological communities are collections of species that co-occur in space and time and interact with one another. The main objective of studies

of ecological communities is to better understand the pattern within and among these collections of species and the processes that generate those patterns. Many metrics have been used to search for patterns in community 'structure', including the number of species, absolute and relative abundances, the type of species present (e.g. guilds, trophic levels) and their phenotypic properties (e.g. body size, morphological attributes, physical tolerances, behavioral responses). Many processes can shape patterns in these metrics of community structure, including the supply of energy and materials, environmental tolerances (e.g. abiotic limits, disturbance), direct interactions among species (e.g. interference competition, predation, mutualism, disease, parasitism), indirect effects of direct interactions transmitted through intermediate species (e.g. exploitative competition), migration and dispersal and historical contingencies (McPeck and Miller 1996).

More recently, community ecologists have recognized the way in which controphic species can indirectly interact with each other when embedded in complex communities. Interference competition, exploitive competition, apparent competition or mutualism, indirect mutualism, intraguild predation and keystone predation are six mechanisms that illustrate the rich diversity of effects in shaping communities (Blaustein 1998, Blaustein and Chase 2007).

Apparent competition occurs when two (or more) prey species share a common predator, and the predator's numbers are limited by prey availability. The second prey species enables the abundance of the predator to increase by becoming an additional food source, and as a result reduces

the density of the target species (Holt 1977). This situation also occurs when there is competition for enemy free space, in which the prey are confined to using certain sources (e.g. refuges, food type) in the presence of predators. The species can limit each others' numbers if more than one prey is present and both utilize enemy-free resources (Blaustein and Chase 2007). This interaction occurs when predator and prey share the same trophic level. However, the roles of controphic species of mosquito larvae in affecting mosquito populations have received little attention.

1.7 Lethal effects of predators on mosquitoes

The functional response has been utilized extensively as a tool to assess the potential of natural enemies to control prey or pest species (Lester et al. 1999). This is the relationship between the density of prey in a certain area and the average number of prey consumed by each predator in that area. The functional response of the target prey is dependent on the predator, life-stage and species of an alternative prey. Prey factors that influence prey selection are suggested to include size, mobility, defense or avoidance characteristics, palatability, abundance, and the extent of overlapping prey and predator habitats.

The presence of alternative prey in same water body can affect the number of target prey eaten by predators. One obvious effect is that the pursuit, capture and consumption of alternative prey reduce the encounter time available for predators towards the target prey (Chesson 1989). But if an alternative prey species is more abundant, predators may develop a

search pattern resulting in a preference for the more common species (Murdoch and Oaten 1975, Tschanz et al. 2007).

1.8 Sub-lethal effects of predators on mosquitoes

Prey behavioral responses to predators may have important effects at the community level. For example, predators often have important impacts on prey activity rates, such as influencing feeding, growth rates, competitive abilities and changing the local prey density and community structure. In the presence of predators, prey communities may increase, decrease or not alter their movement rates (Wooster and Sih 1995). These are all 'sub-lethal' effects of predators that can have a major role in structuring communities.

Aquatic arthropods can respond to the presence of predators by changing their behavior and life history traits. Behaviors by aquatic arthropods can be assigned to specific anti-predation responses, such as non-aggressive behavior, by reducing the time spent foraging, and increasing their use of water currents to drift away (Stibor 1992). In other words, the prey will seek refuge and focus more on their own productivity rather than fighting against the predator. Predators can dramatically reduce prey population growth and fecundity rates, resulting in a significant reduction of fitness beyond the direct mortality effects usually considered in studies of predator-prey interactions (Peckarsky et al. 1993). Higher activity levels are associated with a higher predation rate, since the probability of encounter between prey and predators increases. Lower activity levels may also result in fewer encounters with food which in turn reduces energy gain to the prey

species (Mikolajewski et al. 2005). Thus, investigations of the sub-lethal consequences of behavioral responses of prey to predators are essential to further understanding of the total impact of predators on prey populations and communities.

Mosquito adult fecundity is a function of the amount of food eaten, but their potential fitness is determined by the number of offspring that survive to adulthood. Therefore, in situations where juvenile mortality is high, the adult oviposition strategy becomes very important (Kindlmann and Dixon 2001). For example, females of the mosquito species *Culiseta longiareolata* avoid ovipositing in pools that contain the backswimmer, *Notonecta maculata*, which preys on its larvae (Kiflawi et al. 2003). Faced with a choice between an equal number of pools with and without predators, about 90% of females oviposit in the pools that have no predators.

1.9 Thesis aim and structure

The aim of this thesis is to understand the mechanisms of how a common native predator influences mosquito populations in New Zealand. I examined lethal and sub-lethal effects that may directly or indirectly influence mosquito population abundance in the presence of the mosquito predator, *Anisops wakefieldi*. Functional response studies reflect lethal effects. To examine sub-lethal effects, I tested oviposition habitat selection, life history changes and predator avoidance behavior. I also investigated the effects of predators on mosquito populations in the field. The field sampling survey was conducted in water troughs in Queen Elizabeth II

Park, Waikanae, to observe the relationship between predators, mosquito larvae and other associated variables (such as water volume, temperature, wind speed etc.). My thesis consists of six main chapters. Each chapter is written as a stand-alone manuscript, and some repetition may occur in the information contained in the introduction and discussion.

In **Chapter Two**, the aim in this study was to examine the relationship and interactions between predators and mosquito populations in animal drinking troughs. This study was conducted every week during the southern hemisphere summer for two consecutive years. I sought to understand the relationship between mosquito predators and immature mosquitoes in natural conditions. I also considered associated water volume and environmental factors to include in the model. In order to examine the effectiveness of *A. wakefieldi* in controlling the *Cx. pervigilans* community, I ran a separate experiment where I transferred this predator between troughs with different densities of mosquito larvae.

Based on 'the escape from natural enemies' hypothesis, I tested this hypothesis by examining the New Zealand native predator *A. wakefieldi* consuming native (*Cx. pervigilans*) and exotic (*Ae. notoscriptus*) mosquito larvae in **chapter three**. First, I established the experiment by offering *A. wakefieldi* predators *Cx. pervigilans* (native) and *Ae. notoscriptus* (exotic) prey and estimated their functional responses. I also examined the predator's preferences and prey switching behavior when these two mosquito species persisted together in the water body. The avoidance behavior of mosquitoes when facing the predator was also investigated in order to understand the strategies that may help mosquitoes successfully establish.

In **Chapter Four**, the aim of this experiment was to assess the influence of the notonectid predator *A. wakefieldi* on *Cx. pervigilans* mosquitoes when choosing their oviposition habitat under the field. Here, I established a number of artificial container habitats with four treatments of varying predator densities; control (without *A. wakefieldi*), free-roaming *A. wakefieldi*, *A. wakefieldi* in cages and *A. wakefieldi* kairomones (with a kairomone concentration of varying numbers of *A. wakefieldi* that were present in the water for 24 h prior to the experiment, but removed at the start of each trial). The purpose of this experiment was to assess the influence of predators on mosquitoes when choosing their oviposition habitat. Oviposition habitat selections by adult mosquitoes can play a very important role in determining the success rate of their progeny.

Culex pervigilans eggs that were successfully oviposited in the containers used in experiments in chapter four were brought back to laboratory in order to establish an experiment for **Chapter Five**. The aim of this study is to investigate the survival rate and subsequent life history of the mosquito *Cx. pervigilans* (Bergorth) under the influence of its major predator *A. wakefieldi* (White). In this chapter, I also examined the mosquitoes' life history including the successful rate of egg hatching, survival rate, and development time in two different water conditions; (1) eggs which were reared in the same treatments used in chapter four and, (2) eggs which were reared in clean water without any traces of predators or their kairomones. The purpose of these experiments is to investigate the subsequent and indirect effects of *A. wakefieldi* predators on mosquito progeny.

Lastly, in **Chapter Six**, I integrated all the findings from the previous chapters and combine all the current results to come out with into a general discussion. Overall, this thesis suggested that the successful establishment of mosquito communities can be explained by a complex synthesis of two different components; (1) lethal effects- by direct predation, prey preferences and prey switching behavior, (2) sub-lethal effects- by life history changes and avoidance behavior in mosquito larvae after exposure to predators or their kairomones. I did not find any evidence for a change in oviposition behavior. I hope this entire thesis will provide good baseline knowledge and add new information as to how predators influence the mosquito population dynamics in New Zealand.

CHAPTER TWO:

The influences of aquatic predators on mosquito abundance in animal-drinking troughs in New Zealand

In part published as: Zuharah WF and Lester PJ. The influence of predators on the mosquito community structure. *Journal of Vector Ecology*. 35(2): (In Press, See appendix D)

(In Press in *Journal of Vector Ecology*, accepted on 10 May 2010)

2.1 ABSTRACT

The occurrence and abundance of mosquito populations may be associated with the abundance of predators. My aim in this manuscript was to examine the relationship between aquatic predators and populations of mosquitoes in animal-drinking water troughs in Waikanae, New Zealand. I also investigated the effects of water volume and environmental factors (temperature, rainfall, wind speed, humidity and pressure) in order to further understand factors influencing mosquito and predator populations. Logistic regression indicated that the presence or absence of mosquitoes was primarily affected by three factors; predator abundance, week of observation, and water volume. Pearson's correlation indicated the presence of predators had a positive correlation with water volume ($r^2 = 0.176$, $p < 0.05$). Otherwise, the presence of mosquito larvae in water troughs was negatively correlated with water volume ($r^2 = -0.159$, $p = 0.022$) and wind speed ($r^2 = 0.142$, $p = 0.041$). I established a translocation experiment in which predators or mosquitoes were moved between troughs in order to examine the prey survival rate after the exposure to *A. wakefieldi* predators. The survival rate of mosquitoes was statistically similar, between 0- 0.1%, irrespective of the number of predators translocated (1-9) or the initial mosquito density (20- 70 larvae). My results suggested that *A. wakefieldi* predators may have the potential to become one of the most promising biological control tools for the control of mosquito populations by means of altering mosquito population dynamics.

CHAPTER 2: THE INFLUENCES OF AQUATIC PREDATORS ON MOSQUITO ABUNDANCE

Keywords: Environmental factors · Mosquito population · Predator · Translocation · Water trough · Water volume.

2.2 INTRODUCTION

Artificial containers such as bottles, cans, tires, pots and animal drinking troughs are major sources of habitat for mosquito populations. However, aquatic predators also occur in many of these habitats including animal drinking troughs and have been suggested to have a major influence on the abundance of mosquitoes (Laird 1990). Mosquito species may coexist and share the same habitat with predators, with some predators appearing to have little influence on mosquito populations (Lundkvist et al. 2003). Different mosquito species may be differently affected by predators (Griswold and Lounibos 2006), with the differential consumption of various mosquito species by predators resulting in the coexistence of some mosquito species (Griswold and Lounibos 2005).

A variety of aquatic insects in the orders Coleoptera, Hemiptera, Odonata and Diptera are known to attack and eat mosquito larvae. In general, most predators are polyphagous and consume a broad range of prey species (Shaan et al. 2007). In New Zealand the common mosquito predators seen in natural and anthropogenic water bodies are the notonectid backswimmer *Anisops* spp. (Hemiptera), diving beetles in the genus *Rhantus* (Coleoptera), and damselfly larvae (Odonata) (Graham 1939). Several studies have focused on notonectid predators because of their effectiveness in controlling mosquito populations (Scott and Murdoch 1983, Murdoch and Scott 1984, Murdoch et al. 1984, Koivisto et al. 1997). Notonectid predators clearly have the potential to alter mosquito communities via direct or indirect effects (Gilbert and Burns 1999).

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The indirect effects of the presence of notonectid predators in water bodies can include a reduction in oviposition by adult mosquitoes (Chesson 1984, Blaustein et al. 1995), while direct effects occur primarily through predation.

New Zealand currently has only 16 mosquito species, with 12 indigenous species and four exotic species (Derraik 2004a, 2004b, Laird 1990, Laird 1995). No outbreaks of mosquito-borne disease have been recorded. However, out of 12 indigenous mosquito species, *Culex pervigilans* and *Culiseta tonnoiri* have been identified as vectors of the avian arbovirus (Whataroa Virus) (Maguire et al. 1967). *Culex pervigilans* is New Zealand's most abundant and widespread species, and has the potential to be a vector of human pathogens. *Culex pervigilans* is often found in artificial containers including animal drinking troughs (Graham 1929, Graham 1939, Belkin 1968, Laird 1990, Laird 1995). There are other species which are of much greater concern such as *Aedes notoscriptus* and *Coquillettidia iracunda*, with the former also being found in drinking water troughs in New Zealand (Laird 1995).

Environmental factors may affect the abundance of predator and mosquito populations. A backtrack simulation study of wind speeds sufficient for mosquito transport from Papua New Guinea to Australia was research by Ritchie and Rochester (2001). Winds at 36 and 72 km/h were capable of carrying mosquitoes from New Guinea to the Northern Peninsula of Australia. Slow velocity winds are also important as they allow female mosquitoes to place their eggs calmly without disturbance in water containers (Service 2000). Temperatures can also strongly influenced predation rates. For example, Anderson et al. (2001) showed that tadpoles raised in warmer water experienced

a higher probability of capture by notonectids predators than those reared in cooler conditions.

My aim in this study was to examine the relationship and interactions between predators and mosquito populations in animal drinking troughs. I translocated predators between troughs in order to better understand their influence on mosquito populations. I also examined the effects of water volume and environmental factors on predator and mosquito populations.

2.3 MATERIALS AND METHODS

2.3.1 Study site

My study was undertaken at Queen Elizabeth II Park, Waikanae, New Zealand (41°06'31.43 S, 175°13'12.91 E). This area contains a variety of habitats, including coastal and inland sand dunes as well as pastoral areas for animal farming. A total of 13 water troughs within an area of 4.8km² were sampled. Troughs ranged in volume from 0.32- 0.84m³ were sampled at weekly intervals. Sampling continued for two southern hemisphere summers from December to February of 2007/2008 and 2008/ 2009.

2.3.2 The relationship between predator and mosquito populations

During each sampling occasion predators such as *Anisops wakefieldi* (backswimmers), *Rhantus rhantus* larvae (diving beetles) and *Onychohydus hookeri* (water beetles) were visually observed and counted. Predators were inspected and identified *in situ*, as they were easily identified to genus level. To estimate mosquito densities I used a conventional dipping technique (Russell 1993, Service 1995). The water surface was stirred using a stick before the sample was taken. A total of ten litres of water was sampled by dipping at equal intervals around the water trough. The water samples were strained through 0.10 mm mesh and the filtered water was then returned to the trough. Mosquitoes collected in the strainer were placed separately in containers with 500 ml of water and brought back to laboratory for identification. If any predators were caught in the strainer, they were released back into the trough. Mosquito larvae were identified using a key by Snell (2005). The dipper and strainer were thoroughly rinsed with distilled water before sampling the next water trough.

The water volume for each trough was recorded. Water volume was used rather than water depth because there were different shapes of water trough located in the field. Environmental data (temperature, wind speed, rainfall, pressure, and humidity) were provided by New Zealand National Institute for Water and Atmospheric Research from Paraparaumu Aero Station, which is approximately 4.5km from my study site.

I analyzed the data with logistic regression using R version 2.10.1 (Ihaka and Gentleman 1996). The presence and absence of mosquitoes served as the

response variable. The predictor variables were predator, water volume, week of observation, temperature, rainfall, wind speed, and humidity. This logistic regression approach was used because prior work has suggested that solely the presence of a predators or kairomones (chemical cues that are released by the predator) can influence mosquito communities through altering mosquito behaviours such as adult oviposition (Blaustein et al. 2004, Blaustein et al. 2005). I then used Pearson's correlation tests in SPSS 15.0 (2006) to examine the relationship between the environmental factors and water volume, and the number of predators and mosquito in water troughs. If, as in my study system, the presence or absence of predators do not influence mosquito behaviour, such as oviposition, an analysis of the influence of predator abundance is more appropriate.

2.3.3 Predator translocation experiment

In order to examine the effectiveness of the predator *A. wakefieldi* in altering mosquito populations, I transferred these predators between troughs. *Anisops wakefieldi* was used in this study because it was the most common predator species at my study site. I transferred between one and nine predators, and third instar *Cx. pervigilans* mosquito larvae, in each water trough as follows; (A) zero predators (control) with 20, 40, or 70 mosquito larvae, (B) one predator with 10, 15, or 20 mosquito larvae (C) three predators with 20, 30, or 40 mosquito larvae (D) nine predators with 30, 50, or 70 mosquito larvae.

Experiments were replicated four times. Data were recorded two days after each transfer.

In order to test the effects of predator density and prey number on mosquito survival rates in the translocation experiments, I ran a two-way ANOVA in SPSS 15.0 (2006). The percentage of surviving prey served as the dependent variable, whereas type of treatment and initial number of prey offered were fixed factors. Survival rates are expressed as a percentage and were therefore transformed using arcsine square root prior to analysis to satisfy the assumptions of ANOVA.

2.4 RESULTS

2.4.1 The relationship between predator and mosquito populations

The only mosquito species that was observed to utilize animal drinking troughs during the entire study was *Culex pervigilans*. The three predator species observed in same habitat were *Anisops wakefieldi* (91.8%), *Rhantus rhantus* larvae (6.6%), and *Onychohydrus hookeri* (1.6%). The total number of *Cx. pervigilans* larvae collected in 2007/2008 was 3293, with only 715 individuals in 2008/2009 over the same sampling duration. The total number of predators was high in 2007/2008 with 916 individuals; but, only 419 individuals were recorded in 2008/2009.

In 2007/2008 the highest numbers of predators (300) was observed after the first week of sampling during summer but densities declined closer to autumn (Figure 2.1A). In the same trough in the following year (2008/2009), the situation

changed with a high abundance of mosquitoes. However, only two predators were observed for the entire study period (Figure 2.1A). In most troughs the pattern of population dynamics of mosquitoes and predators were similar to that shown in Figure 2.1B.

The model resulting from the logistic regression indicated that with each increase in the abundance of predators the probability of mosquito population existence also increased; however, it is not statistically significant (Figure 2.2). For each increase in the abundance of predators, the odds of mosquitoes being present (vs. absent) positively increased by the factor of 1.006. The probability of mosquito presence significantly decreased with increasing water volume and week of observation ($P < 0.05$; Table 2.1).

Predator abundance increased significantly with increasing water volume in both years of the study ($r^2 = 0.176$, $P = 0.011$; Table 2.2 and Figure 2.3), but was not correlated with any other environmental variable nor the abundance of mosquitoes ($P \geq 0.232$). In comparison, mosquito abundance decreased significantly with increasing water volume ($r^2 = -0.159$, $P = 0.022$; Table 2.2) and wind speed ($r^2 = 0.142$, $P = 0.041$), but was not correlated with any other environmental variable ($P \geq 0.220$).

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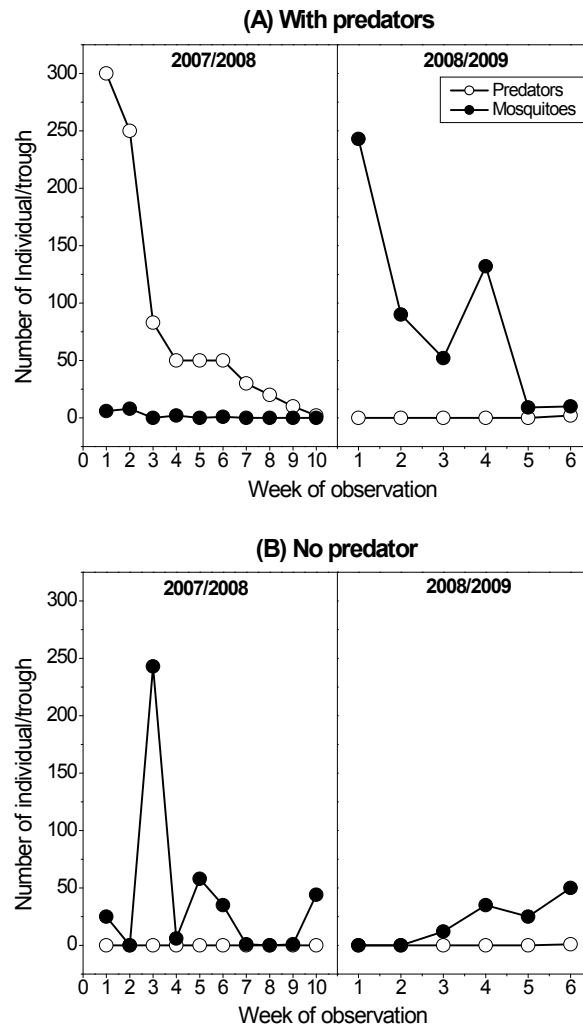


Figure 2.1: Examples of the relationship between predators and mosquitoes in water troughs at Queen Elizabeth II Park, Waikanae, New Zealand. These graphs show two of the 13 troughs examined in the study, one trough with predators (A) and one without predators (B). The open circles represent predators and closed circles represent mosquitoes.

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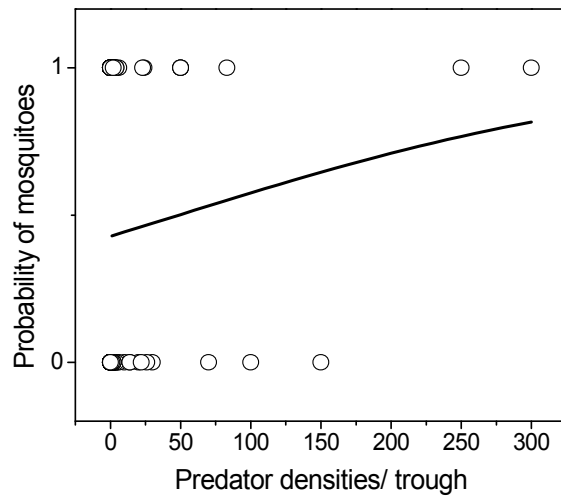


Figure 2.2: Logistic regression of the presence or absence of mosquitoes in association with predator abundance.

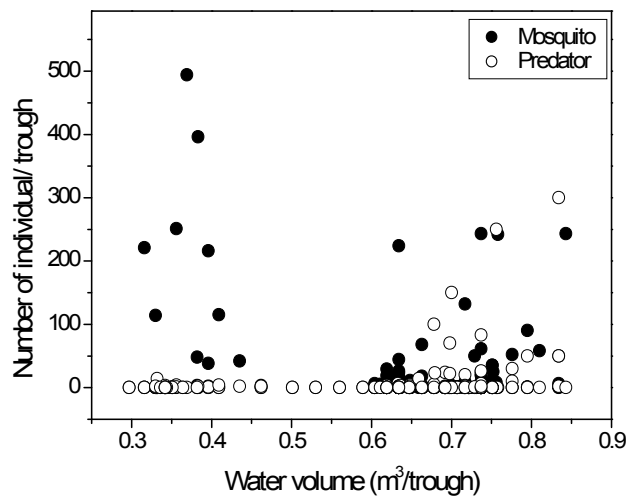


Figure 2.3: Abundance of mosquito larvae and predators recorded in animal drinking troughs compared to cumulative water volume.

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Table 2.1: Logistic regression analysis for the predictor variables included in the model that effect the presence or absence of mosquito populations in the water troughs. $df = 202$ (** $= P < 0.001$, ** $= P < 0.01$, * $= P < 0.05$).

	Estimates	Std Error	z-value	P-value
Intercept	4.937	1.561	3.163	0.002**
Predator	7.217^{e-03}	6.343^{e-03}	1.138	0.255
Water Volume	-2.194^{e-06}	1.114^{e-06}	-1.969	0.049*
Water trough	5.201^{e-02}	6.036^{e-02}	0.862	0.389
Temperature	1.196^{e-01}	7.143^{e-02}	-1.675	0.094
Wind speed	3.939^{e-02}	2.376^{e-02}	1.658	0.097
Rainfall	2.658^{e-02}	2.062^{e-02}	1.289	0.197
Humidity	-1.477^{e-02}	9.551^{e-03}	1.289	0.197
Week	-1.976^{e-01}	5.084^{e-02}	-3.887	<0.001***

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Table 2.2: Results of Pearson correlation tests between *Anisops wakefieldi* or mosquito populations with environmental factors (temperature, rainfall, wind speed, humidity and pressure) and water volume. Data were collected from animal water troughs during the Southern Hemisphere summer (December to February 2007/08 and 2008/09) for two consecutive years. Significant values are in bold with $df= 207$. Data represent Pearson's correlation (2-tailed).

	Predator	Mosquito
Mosquito	-0.052, 0.454	
Water volume	0.176, 0.011	-0.159, 0.022
Temperature	-0.020, 0.773	-0.085, 0.220
Rainfall	-0.041, 0.558	0.049, 0.484
Wind speed	-0.063, 0.365	0.142, 0.041
Humidity	-0.023, 0.741	0.064, 0.357
Pressure	0.083, 0.232	0.022, 0.748

2.4.2 Predator translocation experiment

The translocated predators had a major effect on mosquito populations. The maximum number of mosquito prey left alive was one mosquito in two days post-treatment. Even at the highest number of 70 prey, with the largest water volume of 0.72m^3 , the predator successfully consumed all of the prey (Figure 2.3). The two-way ANOVA showed that the mosquito larvae survival rate in treatments with predators was significantly lower than in the control treatment without predators ($F = 443.92$, $df = 3$, $P < 0.001$). There was no significant effect of initial mosquito number on mosquito survival rate ($F = 0.87$, $df = 6$, $P = 0.524$), and the interaction between treatments and mosquito abundance was also non-significant ($F = 0.53$, $df = 6$, $P = 0.780$). Post-hoc comparisons showed that treatments with predators (1, 3, and 9 predators) were not significantly different from each other ($P \geq 0.704$), but had a significantly lower prey survival (Tukey, $P < 0.005$) than in the control treatment without predators. These results clearly demonstrate the ability of this predator to reduce the number of mosquitoes in water troughs within only two days. In the control treatments, where mosquito larvae were present in the water troughs without predators, the mortality rate of mosquitoes was less than 5%.

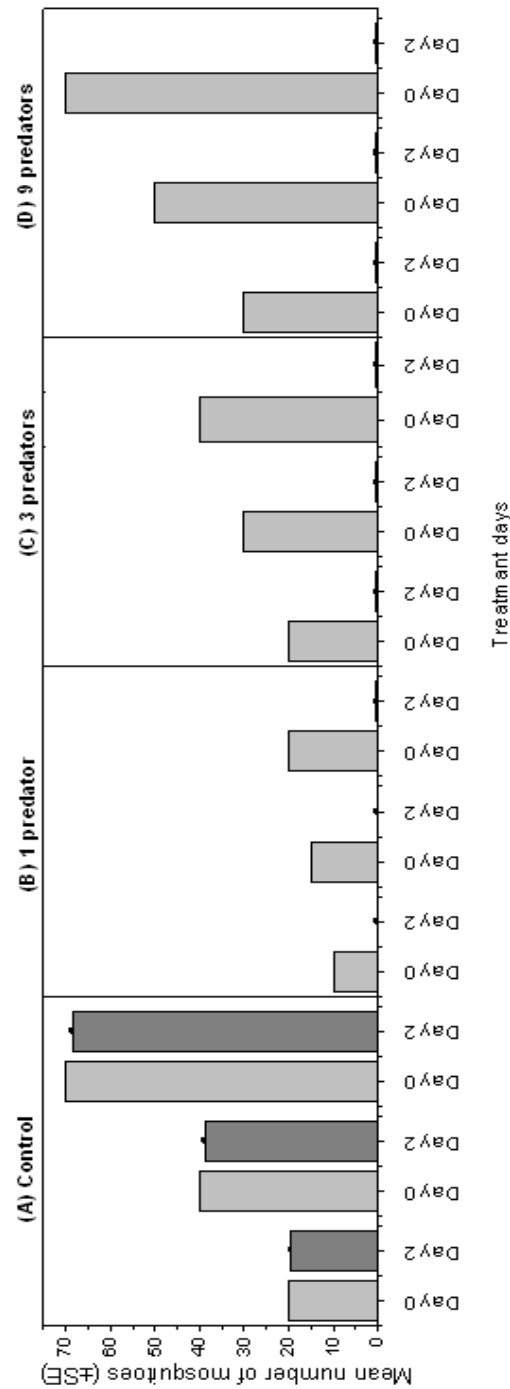


Figure 2.4: Results of the pre- (day 0) and post-treatment (day 2) experiment after mosquito prey were exposed to (A) control (with the absence of predators), (B) one free-roaming predator, (C) three free-roaming predators, and (D) nine free-roaming predators.

2.5 DISCUSSION

The only mosquito observed in this study was *Cx. pervigilans*. *Culex pervigilans* is the most common mosquito in New Zealand, utilizing many water containers and habitat types (Graham 1939, Belkin 1968, Laird 1990, Laird 1995). It has been found to prefer organically rich water bodies (Graham 1939, Dumbleton 1968, Urbinatti 2001), but has wide tolerance for a variety of water qualities (Graham 1929, Derraik and Slaney 2004). The predators *A. wakefieldi*, *R. rhantus* and *O. hookeri* are also widely distributed throughout the North Island of New Zealand (Brown 1943, Winterbourn et al. 2006). At my sampling site *A. wakefieldi* was the most abundant predator observed; only 2% of all predators were *R. rhantus* and *O. Hookeri*. In my study, most of the water troughs utilized by predators had low densities of mosquitoes. Some of the water troughs that were utilized by large numbers of predators in 2007/08 had few predators and large numbers of mosquitoes in 2008/2009. There are at least two hypotheses to explain these results. Firstly, predators may consume large numbers of mosquitoes and control their population numbers. Secondly, mosquito adults may have an ability to detect the presence of a predator and will not oviposit in troughs with predators.

To test the hypothesis that the predators consumed a large number of mosquitoes, I undertook a translocation experiment in which *A. wakefieldi* and mosquitoes were placed together in the same environment. My translocation experiment showed that within two days, all mosquitoes were consumed by predators. Other laboratory studies confirm that a single *A. wakefieldi* predator in

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500ml of water can kill about 18 individual mosquitoes within 24 hours (Zuharah and Lester, In Press B). In my study, it appears that even in large water volumes *A. wakefieldi* are voracious predators. Both adults and nymphs of *A. wakefieldi* prey upon mosquito larvae. This predator is believed to be most effective against immature stages of third and fourth instar mosquitoes (Hazelrigg 1963, Lee 1967, Ellis and Borden 1970, Bay 1974). Additionally, *Anisops* spp. prefers mosquito larvae over other prey when given a choice (Hazelrigg 1963, Ellis and Borden 1970).

My second hypothesis to explain the relationship between predators and mosquitoes was that mosquito adults may have the ability to detect the presence of a predator and consequently do not oviposit in troughs with predators. Predators may therefore regulate prey abundance without directly killing mosquitoes (Brown et al. 1997). Stav et al. (1999) suggested that the distribution and abundance of a mosquito species is dependent on their ability to selectively oviposit according to predator abundance. Other mosquitoes such as *Culiseta longireolata* can detect chemicals from *Notonecta* predators for periods of up to eight days after their removal from the pool (Blaustein et al. 2004) and this period is as low as two days for *Culex* species (Blaustein et al. 2005). It thus seems reasonable to expect that the mosquitoes would have shown a response to this predator. With increasing predator densities, the concentration of kairomones also increase and may result in reduced oviposition (Eitam and Blaustein 2004). A reduction in oviposition activities by mosquitoes may be expected with increasing numbers of predators, due to the ability of these prey species to

detect the presence of a predator above a threshold density (Angelon and Petranka 2002) or a specific concentration of kairomones (Eitam and Blaustein 2004). In a separate experiment I examined the oviposition of *Cx. pervigilans* in response to the presence of *A. wakefieldi* and kairomones. Unlike work elsewhere (e.g. Blaustein et al. 2004, 2005), I observed no response of mosquitoes to the presence of these predators (Zuharah and Lester 2010).

It thus appears that the primary effects of predators were through predation, rather than sub-lethal effects such as a change in oviposition behaviour. This is in accordance with the voracious nature of these predators (Zuharah and Lester, In Press B). However, my logistic data indicated a positive relationship between mosquito and predator densities, though this relationship was only significant at the $p < 0.10$ level. Adult *Anisops* predators frequently disperse and may fly up to 1.6km between habitats (Brier 1998). It is possible that these predators periodically move between habitats and are more likely to stay longer in habitats with high numbers of mosquitoes. Further work is needed to confirm such a hypothesis.

The abundance of mosquitoes was observed to be dramatically different between the two years of this study. None of the environmental factors I examined correlated with the abundance of predator and mosquito populations. Wind speed correlated only with the abundance of mosquitoes. Mosquitoes need 10- 15 minutes to oviposit therefore, slow velocity winds are important as they allow female mosquitoes to place their eggs without disturbance in water containers (Service 2000). The lack of any other relationship between mosquito

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abundance, and that of predators, with environmental variables may be a result of averaging. The environmental data analyzed are the average conditions over each weekly period of sampling; but within any one week there may have been conditions suitable for brief periods of oviposition or predator movement.

Water volume was highly correlated with predator and mosquito abundance in water troughs. Larger water troughs may be advantageous to *A. wakefieldi* colonies because they are less likely to dry out or overflow (Wilton 1968, Frank and Curtis 1977). A larger space offered by bigger water troughs can increase larval survivorship and reduce mortality risks such as cannibalism (Sota 1998, Sunahara et al. 2002). I also found that mosquito populations were negatively correlated with water volume. Female mosquito may oviposit in all types of habitats even if the water has evaporated. Browne (1994) observed female mosquitoes ovipositing in full and nearly empty containers, suggesting that these mosquitoes are incapable of assessing water depth. Similarly *Cx. pervigilans* appear incapable of assessing water depth (Derraik and Slaney 2004). The positive relationship between water volume and predators, and the negative relationship between depth and mosquitoes, is in accordance with the observations that predators influence mosquito populations.

My results indicate that the predator, *An. wakefieldi*, can control *Cx. pervigilans* densities even in relatively large volumes of water. However, the fluctuating in environmental changes from year-to-year, and even within a year, may indirectly affect the effectiveness of this predator in controlling mosquito population.

CHAPTER THREE:

Are exotic invaders less susceptible to native predators? A test using native and exotic mosquito species in New Zealand

In part published as: Zuharah WF and Lester PJ. Are exotic invaders less
susceptible to native predators? A test using native ad exotic mosquito
species in New Zealand. *Population Ecology*.

(In Press in *Population Ecology*, accepted 10 July 2010)

3.1 ABSTRACT

A major hypothesis in invasion ecology is that native predators have less impact on exotic prey species relative to native prey species. Here I tested this hypothesis by examining the New Zealand native predator *Anisops wakefieldi* consuming native (*Culex pervigilans*) and exotic (*Aedes notoscriptus*) mosquito larvae. *Anisops wakefieldi* exhibited a decelerating Type II functional response for both prey species, but at high prey densities consumed more of the exotic mosquito *Ae. notoscriptus*. A significantly higher attack rate was observed for the native predator feeding on exotic species. In the presence of both prey species, the predator showed preferences towards *Ae. notoscriptus* and demonstrated switching behavior towards this exotic species. The preference of the native predator towards the exotic mosquito appeared related to behavioral differences between the two prey species. I tested the behavioral response of both mosquito species in four conditions; (1) control (without predators), (2) free-roaming predators, (3) caged predators, and (4) kairomones only. Resting activities at the water surface and wall positions were the most frequently behaviors exhibited by *Cx. pervigilans* in the presence of predator. In contrast the exotic species *Ae. notoscriptus* demonstrated significantly higher levels of “thrashing” behaviors with vigorous lateral movement of body, apparently making itself more obvious to the predators. The behaviors showed by *Cx. pervigilans* fulfilled the “threat sensitivity hypothesis”. No evidence here supported the idea that exotic mosquito species are less prone to the influence of native predators and the “escape from natural enemies” hypothesis seems not apply in my

study. In fact, I observed that this invader was more susceptible to the predator.

Keywords: Behavior · Escape from natural enemies · Exotic · Native · Threat sensitivity · Predation

3.2 INTRODUCTION

Is the establishment of exotic mosquitoes facilitated by a lack of predation from native predators? The “escape from natural enemies” hypothesis (Elton 1958) is one of the famous explanations for successful establishment by non-native species, which was first noted by Darwin in 1859. This hypothesis suggests that escape from predation, parasitism, and herbivory, may permit non-native species to survive, grow and reproduce at higher rates in their new habitat range (Elton 1958, Blossey and Notzold 1995, Maron and Vilá 2003). Escaping from key natural enemies enables non-native species to grow explosively and become more abundant in the community into which they were introduced (Maron and Vilá 2003). For example, Russell et al. (2001) reported the native freshwater fish (fly-specked hardyhead, *Craterocephalus stercusmuscarum stercusmuscarumon*) consumed fewer exotic than native mosquito species. Liu and Stiling (2006) also found that insect herbivore richness is higher and caused greater damage to native plants than to introduced plants, which may allow introduced plants to establish successfully in a new environment.

A key factor for the population dynamics of predator-prey systems is the functional response. The functional response is the relationship between prey density and a predator’s consumption rate. It describes the rate at which predator kills its prey at different prey densities and can thus help determine the efficacy of a predator in regulating prey populations (Murdoch and Oaten 1975). Functional response curves enable a prediction of predation intensity and predator behavior over a range of conditions, providing a baseline for

predicting stability of predator–prey interactions (Griswold and Lounibos 2005). Holling (1959) classified three main types of functional response. Type 1 responses involve prey consumption increasing with the prey density to a plateau, type 2 responses are decelerating rates of prey consumption to a plateau, and in type 3 responses a sigmoidal relationship of prey consumption is observed to a plateau. It is important to differentiate between type II and type III functional responses at relatively low prey numbers (Murdoch 1969, Lester et al. 2005, Pervez and Omkar 2005). Most of the functional response analyses of mosquito predators were suggested to be fitted by type II response curves, including the two dipteran predators *Toxorhynchites rutilus* and *Corethrella appendiculata* (Griswold and Lounibos 2005).

The long-term stability of predator and prey populations is thought to be through mechanisms of density-dependent predation and prey switching (Holling 1965, Murdoch and Oaten 1975, Hassell and Comins 1978). Prey switching involves two or more prey species and one predator species. When all prey species are in equal abundance, predators may indiscriminately select between prey species. But if one prey species is more abundant, predators may develop a search pattern resulting in a preference for the more common species (Murdoch and Oaten 1975, Tschanz et al. 2007). This effect of prey switching based on density-dependent prey availability may stabilize the coexistence of two or more prey species.

Why should functionally similar prey species be different in their susceptibility to a predator? In aquatic ecosystems there are several

potential mechanisms that limit the effects of a predator on prey species. These mechanisms include the chemical detection of a predator by the prey (Blaustein et al. 2004, Eitam and Blaustein 2004). Similarly, variability in prey behaviour may differentially expose one species to predators. Other possible mechanisms includes habitat complexity that may reduce the incidence of encounters with some prey but not others (Alto et al. 2005), and abiotic changes such as the oxidation of sediments or chemical changes in water that may limit the susceptibility of prey to predators (Bay 1974).

One of the main responses exhibited by prey is to altering their behaviour in the presence of predators in an attempt to increase their survival (Sih 1980; 1986, Van Buskirk 2000, Kesavaraju et al. 2006). The “threat sensitivity hypothesis” states that prey species alter their avoidance response according to the magnitude of the threat: with increasing predation risk prey exhibit increasing avoidance behaviour (Helfman 1989). The result of predation on prey behaviour thus may be different from one organism to the next. Prey may reduce their feeding and foraging activity, migrate to less favourable habitats, and reduce mating efforts or alter their life history (Bishop and Brown 1992, Peckarsky 1996, Boersma et al. 1998, Moses and Sih 1998, Peacor and Werner 2000, Smith and Belk 2001, Turner 2004, Mirza et al. 2006). Aquatic insects frequently receive warning of predation risk by chemical cues released by predators known as kairomones. Kairomones that indicate the presence of a predator may also be released by injured prey (Dodson et al. 1994, Kats and Dill 1998, Kusch et al. 2004).

In reference to “the escape from natural enemies” hypothesis, I examined the functional response of predator *Anisops wakefieldi* consuming larvae of the exotic mosquito *Aedes notoscriptus* and the native mosquito *Culex pervigilans*. By examining each prey species singly, the preferences for a particular prey species can be predicted by estimating the attack constant and handling time in the experiments (Cock 1978). The attack rate and handling time by *A. wakefieldi* for each prey species was also determined using the Rogers (1972) equations and by actual observation. I examined the effects of alternative prey and prey switching by *A. wakefieldi* predator when *Ae. notoscriptus* and *Cx. pervigilans* were presented together. I also examined the hypothesis that *Cx. pervigilans* and *Ae. notoscriptus* will alter their behavior in the presence of free-roaming *A. wakefieldi*, caged predators and kairomones remnant from the predator treatments. Finally, I investigated how these prey behaviorally mediate their anti-predator response in different water volumes. I made the following predictions for both mosquito larvae species in order to escape from a predator: (1) prey larvae that encounter the predator directly or its kairomones will likely minimize their movement in the water, and (2) prey larvae will modify their behavior to minimize capture by predators. The evolutionary history of predator and prey relationships may be critically important in determining prey susceptibility to predators, especially in scenarios with native and exotic prey species. Due to coevolution, I might expect that the native mosquito species *Cx. pervigilans* might behave more adaptively than *Ae. notoscriptus* to the presence of the predator.

3.3 MATERIALS AND METHODS

3.3.1 Predator and prey colonies

Predator and prey colonies

Predatory backswimmers (*Anisops wakefieldi*) were captured from water troughs in Queen Elizabeth Park II, Waikanae, New Zealand (40°57'S, 174°58'E). These predators are commonly found in such water containers in New Zealand (Laird 1990). All *A. wakefieldi* were at third and fourth nymphal instar stages with body sizes between 6 - 9mm. Methods used by these predators in finding and attacking the prey include behaviors such as flushing prey from the bottom, capturing mosquitoes as they hang beneath the water surface, and pursuit or semi-stalking through the water column (Toth and Chew 1972, Bay 1974).

Larvae of two species of mosquito, the exotic *Ae. notoscriptus* and native *Cx. pervigilans*, were utilized as prey. *Culex pervigilans* were also collected from Queens Elizabeth Park II and *Ae. notoscriptus* were collected in the Manawatu area (40°33'S, 175°24'E).

3.3.2 Functional response study

The functional response of backswimmers (*A. wakefieldi*) to two species of mosquito larvae, *Ae. notoscriptus* and *Cx. pervigilans* was examined. All experiments were conducted at $25 \pm 1^\circ\text{C}$ in photoperiod L : D; 14 : 10 h, using *A. wakefieldi* that were captured within a week prior to use in the experiment. Late third and early fourth instars mosquito larvae were used

for this experiment. Each predator was placed in seasoned water in a plastic tube measuring $19 \times 17.5 \times 17$ cm (height \times length \times width). Seasoned water is water that has been left standing 24 h before the experiment to standardize hunger levels (Murdoch 1973).

To determine the functional response, predators were offered 1, 3, 6, 10, 15, 20, 30, 40 or 50 mixed late third instar and early fourth instar larvae of *Cx. pervigilans* or *Ae. notoscriptus* in separate experiments. Five replicates were used in each experiment. Two hours after placing the prey in 500 ml of seasoned water the predator was introduced. The numbers of prey remaining alive were counted after 24 h. Control (prey only) treatments indicated the mortality rate without predators was low at approximately 1 - 2%. Prey were not replaced as they were eaten. Data were analyzed in two steps. In the first step, the shape (type) of functional response was determined as either a type II or III functional response. In type II functional responses the proportion of prey eaten declines monotonically with prey density. For type III functional responses, the proportion of prey eaten is positively density-dependent on prey up to the inflection point of the sigmoid curve, which is then followed by a monotonic decrease (Schenk and Bacher 2002, Allahyari et al. 2004, Lester et al. 2005, Pervez and Omkar 2005). To determine the shape of the functional response I used the method by Juliano (2001), which relates the proportion of prey eaten (N_e) to amount of prey offered (N_0). The polynomial function was fitted to the data that describes the relationship between N_e / N_0 and N_0 :

$$\frac{N_e}{N_0} = \frac{\exp (L_0 + L_1 N_0 + L_2 N_0^2 + L_3 N_0^3 + L_4 N_0^4)}{1 + \exp (L_0 + L_1 N_0 + L_2 N_0^2 + L_3 N_0^3 + L_4 N_0^4)} \quad \text{Eq. 1}$$

Where N_e = the number of prey eaten, N_0 = the initial number of prey available, and thus N_e / N_0 is the probability an individual is eaten. Maximum-likelihood estimates of the parameters L_0 to L_4 were obtained using PROC CATMOD in SAS® (SAS Institute Inc. 1999) to the dichotomous variable that equals 0 for surviving prey and 1 for consumed prey. The parameters L_0 , L_1 , L_2 , and L_3 (being the intercept, linear, quadratic and cubic coefficients respectively) were estimated using the method of maximum-likelihood. If $L_1 > 0$ and $L_2 < 0$, the proportion of prey consumed is positively density dependent, thus describing a type III functional response. If $L_1 < 0$, the proportion of prey consumed declined monotonically with the initial number of prey offered, thus describing a type II functional response (Juliano 2001).

Once the shape and type of functional response was determined, nonlinear least squares was used to estimate the parameters associated with the response. As no prey were replaced during the experiment, the “random-predator” equation by Rogers (1972) was used as a description of the Type II functional response:

$$N_e = N_0 \{1 - \exp [a (T_h N_e - T)]\} \quad \text{Eq.2}$$

where T_h is the time required to handle a prey item, a is the instantaneous searching rate or attack coefficient, and T is the total time the prey and predator were exposed to the each other. Previous studies (e.g., Colin et al.

1981, Carter et al. 1984) have used the random prey equation by Rogers (1972) because this model allows an analysis of the functional response despite depletion of prey by predators.

I ran independent t-tests in order to compare the differences between the number of prey (*Cx. pervigilans* and *Ae. notoscriptus*) that were consumed by *A. wakefieldi*. Differences between the handling time and attack rates by *A. wakefieldi* of these two prey species were also examined using t-tests.

I examined if different estimates were obtained for the attack rate and handling time when I used the Rogers (1972) equation compared with actual observations of *A. wakefieldi* feeding on the two species of mosquito larvae, *Cx. pevigilans* and *Ae. notoscriptus*. I used the same experimental containers with seasoned water as described above. Mosquito larvae were introduced into 500 ml of water and kept for 2 h before introducing *A. wakefieldi*. Only one mosquito larva was introduced to one *A. wakefieldi* at any one time. The duration of attacks, from initial attack until prey release was recorded. I compared the values of attack rate and handling time of *A. wakefieldi* using paired t-tests with values from actual observations and values estimated from the “random-predator” equation by Rogers (1972) as described in Eq. 2.

3.3.3 Attack rate and handling time using two methods

I examined if different estimates were obtained for the attack rate and handling time when used the Rogers (1972) equation compared with actual observations of *A. wakefieldi* feeding on the two species of mosquito larvae, *Cx. pevigilans* and *Ae. notoscriptus*. I used the same experimental containers with seasoned water as described above. Mosquito larvae were introduced into 500 ml of water and kept for two hours before introducing *A. wakefieldi*. Only one mosquito larva was introduced to one *A. wakefieldi* at any one time. The duration of attacks, from initial attack until prey release was recorded. I compared the values of attack rate and handling time of *A. wakefieldi* using paired t-tests with values from actual observations and values estimated from the “random-predator” equation by Rogers (1972) as described in Equation 2.

3.3.4 Prey preferences and prey switching

I examined the prey preferences of *A. wakefieldi* when offered the two species of mosquito larvae *Ae. notoscriptus* and *Cx. pevigilans*. To examine prey preference experiments were conducted using a total of 100 prey in 1000 ml of seasoned water. The size of the plastic container used in this experiment was 19 × 17.5 × 17 cm (height × length × width). Each predator was offered mosquito prey at ratio of 0 : 100; 20 : 80; 40 : 60; 50 : 50; 60 : 40; 80 : 20; 100 : 0 (*Ae. notoscriptus* : *Cx. pevigilans*). Each treatment was replicated 5 times. The prey densities chosen represent a range of conditions

which a Notonectid predator might reasonably experience near the water surface (Chesson 1989). After 24 h exposure the predator was removed and any remaining prey were counted and identified to species under a microscope. This experiment was maintained at a temperature of $25 \pm 1^\circ\text{C}$ in photoperiod L : D 14 : 10 h. Prey preferences were determined using Manly's α (Manly 1974) with Chesson's (1982) alteration to account for prey depletion:

$$\alpha = \frac{\ln(N_{Ae} - C_{Ae}) / N_{Ae}}{\ln((N_{Ae} - C_{Ae}) + \ln(N_{Cx} - C_{Cx}) / N_{Cx})} \quad \text{Eq. 3}$$

where N is the initial number and C is the number consumed of *Ae. notoscriptus* (A_e) and *Cx. pervigilans* (C_x). Using the attack constant from the functional response experiment I can predict the preferences (α) for each predator with this multiplicative model:

$$\alpha_a = \frac{\alpha_{Ae}}{\alpha_{Ae} + \alpha_{Cx} - (\alpha_{Ae}\alpha_{Cx})} \quad \text{Eq. 4}$$

where α_a is the predicted preference for *Ae. notoscriptus*, α_{Ae} and α_{Cx} are attack constants for *Ae. notoscriptus* and *Cx. pervigilans*, respectively. A t-test was performed to determine any significant differences in the preference of predators for these two mosquito species.

Prey switching is defined as predator's preference for a specific species of prey as that prey increases in abundance. In nature a predator may show strong preferences for the most abundant prey and weak

preferences for rare prey. The prey switching model was first described by Murdoch (1969) as the relationship between the proportion of prey offered in environment and the ratio of prey consumed by predator. It was described by this equation:

$$E_1/E_2 = c(N_1/N_2) \quad \text{Eq.5}$$

where N_1 and N_2 are the abundance of prey species 1 and 2 in the environment and E_1 and E_2 are the abundances of the same prey species consumed by the predator. $c = 1$ when the two prey species are attacked at the same rate. If c increases with N_1/N_2 , prey switching is presumed to have occurred. The opposite of prey switching is when a predator eats disproportionately more of the rarest prey than would be expected by chance. From the equation above this would occur when c (the preference) increases as N_1/N_2 (amount in the environment) increases. Data were analyzed using logistic regression based on the proportion of two prey species offered to *A. wakefieldi*.

3.3.5 Predator avoidance behavior

In this experiment I examined the avoidance behavior of *Ae. notoscriptus* and *Cx. pervigilans* larvae in four different treatments: (1) control, without any predators; (2) when prey were placed with a free-roaming predator; (3) when prey were with a caged predator; and (4) when prey were placed in water which had predator kairomones but no predators. All treatments above were

conducted in 200 ml and 500 ml of water volume, in order to test for different behaviours depending on water volume. All experiments were conducted at $25 \pm 1^{\circ}\text{C}$ in photoperiod L : D 14 : 10h. Experiments were replicated six times. Experiments were conducted in seasoned water in a plastic tube measuring $19 \times 17.5 \times 17$ cm (height \times length \times width).

First, *A. wakefieldi* were placed in plastic cylinders within the containers (4 cm in diameter \times 6 cm in height) I then added *Ae. notoscriptus* larva or *Cx. pervigilans* larva. After 5 min of acclimation time I released the predator from the plastic cylinder. For the control treatment no predator was used and the prey remained alone. For the caged predator treatment the *A. wakefieldi* remained in the plastic cylinder in a vertical position with the open side covered with mesh. Behavior was recorded after 5 min acclimation time. For the kairomone treatment the water contained only remnant kairomones without the actual predator. To get kairomones into the water a predator was released in 500 ml of water and fed with 10 mosquito larva for 48 h prior to start of the experiment. The predator and remaining mosquito larva were then removed from the water.

The activity and position of mosquito larva were recorded every 30 s for either 30 min or until all the prey were captured (Juliano and Reminger 1992). To classify mosquito behavior I used the method described by Juliano and Reminger (1992) with four activities: (1) resting- larva neither feeding nor moving; (2) browsing- larva propelled along the surface of the container by the movement of their mouthparts; (3) filtering- larva floating in the water column propelled by the movement of their mouthparts; (4) thrashing-

vigorous lateral movements of the larval body, propelling themselves through the water. Four positions were recorded: (1) surface- spiracular siphon of the larva in contact of the water-air interface; (2) bottom- larva within 1 mm of the bottom of the container; (3) wall- larva within 1 mm from any surface of the container walls; (4) middle- larva more than 1 mm from any surface of the container and not in contact with the water surface.

The behavioral data were analyzed using multinomial logistic regression in SPSS 15.0 (2006). I score the behavior categories from 1 - 4 for activities and 5 - 8 for positions as follows: (1) resting; (2) browsing; (3) filtering; (4) thrashing; (5) surface; (6) bottom; (7) wall; and (8) middle, which were then modeled as being dependent on prey species (*Ae. notoscriptus* and *Cx. pervigilans*), and treatments (control, free-roaming predator, caged predator and kairomones).

3.4 RESULTS

3.4.1 Functional response study

The logistic regression results showed that the linear parameter L_1 was negative for the interaction between the predator and both *Cx. pervigilans* and *Ae. notoscriptus*, indicating that type II functional responses were observed (Table 3.1). T-tests showed that the functional responses with the prey *Ae. notoscriptus* and *Cx. pervigilans* were significantly different ($t_{88} = 1.88$, $P = 0.002$; Figure 3.1). The average maximum number of prey consumed by *A. wakefieldi* was 18 ± 0.01 (\pm SE) *Cx. pervigilans* and 31 ± 0.01 *Ae. notoscriptus* per day. This result indicated that *A. wakefieldi* were

better able to catch and consume on *Ae. notoscriptus* compared to *Cx. pervigilans*, or that *Ae. notoscriptus* were comparatively more preferred.

The attack rate and handling time were estimated using Rogers (1972) equation. The attack rate estimated by this model was significantly lower at 4.35 ± 0.14 for *Cx. pervigilans* than for 5.69 ± 0.10 for *Ae. notoscriptus* ($t_8 = 2.41$, $P = 0.043$). The handling time needed by *A. wakefieldi* when feeding on *Cx. pervigilans* and *Ae. notoscriptus* was $20.41 \text{ min} \pm 1.08$ and $17.97 \text{ min} \pm 0.21$, respectively. This difference in handling time was significantly different ($t_8 = -7.13$, $P < 0.001$).

I examined differences in attack rate and handling time by estimating these variables via the “random-predator” equation (Roger 1972) and by actual observation. No significant differences were detected in handling time by *A. wakefieldi* for *Cx. pervigilans* and *Ae. notoscriptus* when using these two methods. However, significant differences were observed between these two different methods for estimating attack rates for both mosquito species (Table 3.2). I found that the attack rate values for *A. wakefieldi* were significantly higher in the observations compared with Roger’s equation for both prey species.

Table 3.1: Maximum likelihood estimates of the logistic regression of the proportion of *Culex pervigilans* and *Aedes notoscriptus* eaten by the predator *Anisops wakefieldi*

Parameter	Estimates		SE		P	
	Cx. <i>pervigilans</i>	Ae. <i>notoscriptus</i>	Cx. <i>pervigilans</i>	Ae. <i>notoscriptus</i>	Cx. <i>pervigilans</i>	Ae. <i>notoscriptus</i>
L ₀	4.68	8.45	1.28	1.11	0.0002	<0.0001
L ₁	-0.191	-0.32	0.14	0.04	0.17	<0.0001
L ₂	0.0019	0.0031	0.005	0.001	0.69	<0.0001
L ₃	-2.92E ⁻⁶	3.81E ⁻⁶	0.00005	9.33E ⁻⁶	0.95	0.68

Note: L₁ values are coefficients from the logistic regression assessing the type of response, indicating a type II response. n= 5 for all treatments.

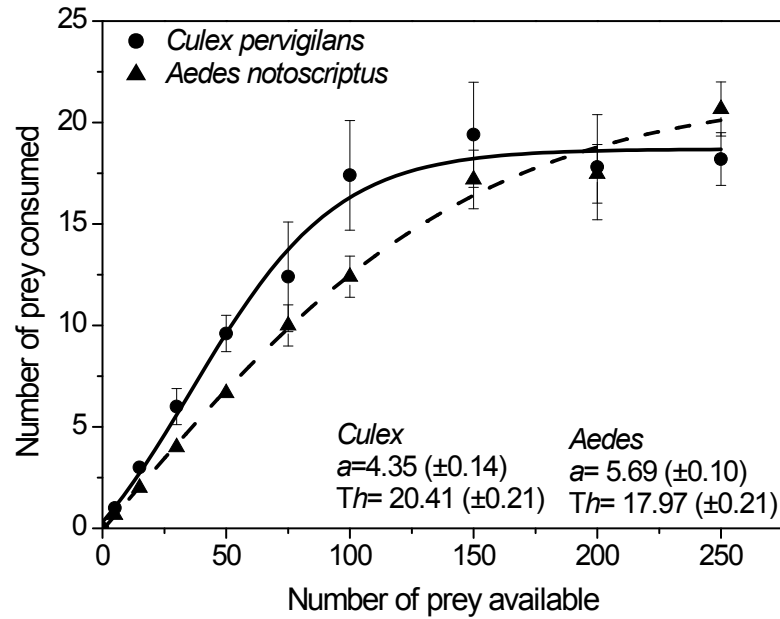


Figure 3.1: Type II functional responses displayed by *A. wakefieldi* while consuming late 3rd and 4th instar mosquito larvae of native *Cx. pervigilans* and exotic *Ae. notoscriptus*. Data are means with 95% confidence intervals.

Table 2. Comparisons between the attack rate and handling time from actual observations and Rogers (1972) equation using paired sample t-test. Significant values are in bold. *df* = degree of freedom.

	Mean	SE	<i>t</i>	<i>df</i>	Significance
Attack Rate					
1) Actual observations of <i>Culex pervigilans</i> – Rogers' equation for <i>Culex pervigilans</i>	0.254	0.102	2.483	4	0.068
2) Actual observations of <i>Aedes notoscriptus</i> – Rogers' equation for <i>Aedes notoscriptus</i>	0.428	0.822	5.209	4	0.006
Handling time					
1) Actual observations of <i>Culex pervigilans</i> – Rogers' equation for <i>Culex pervigilans</i>	-0.506	0.257	-1.965	4	0.121
2) Actual observations of <i>Aedes notoscriptus</i> – Rogers' equation for <i>Aedes notoscriptus</i>	-0.054	0.062	-0.878	4	0.429

3.4.2 Prey preferences and prey switching study

Predator preference, estimated using Manly's α , showed a significant difference in preference between *Ae. notoscriptus* and *Cx. pervigilans* ($F_{6, 21} = 144.08$, $P < 0.05$). *Anisops wakefieldi* consumed more *Ae. notoscriptus* even in treatments even when there were proportionally fewer *Ae. notoscriptus* relative to *Cx. pervigilans* (Figure 3.2).

Switching behavior was observed in this experiment as *A. wakefieldi* consumed more *Ae. notoscriptus* when their population in the pool became more abundant ($r_{135} = 1.85$, $P < 0.05$). Fig 3.3 illustrates that switching behavior was observed when the prey ratio crossed the ratio available line at $N_1 / N_2 = 1$ and then lay below the available ratio line. This result indicated that *A. wakefieldi* demonstrated a switching reaction, consuming the prey species that were more abundant.

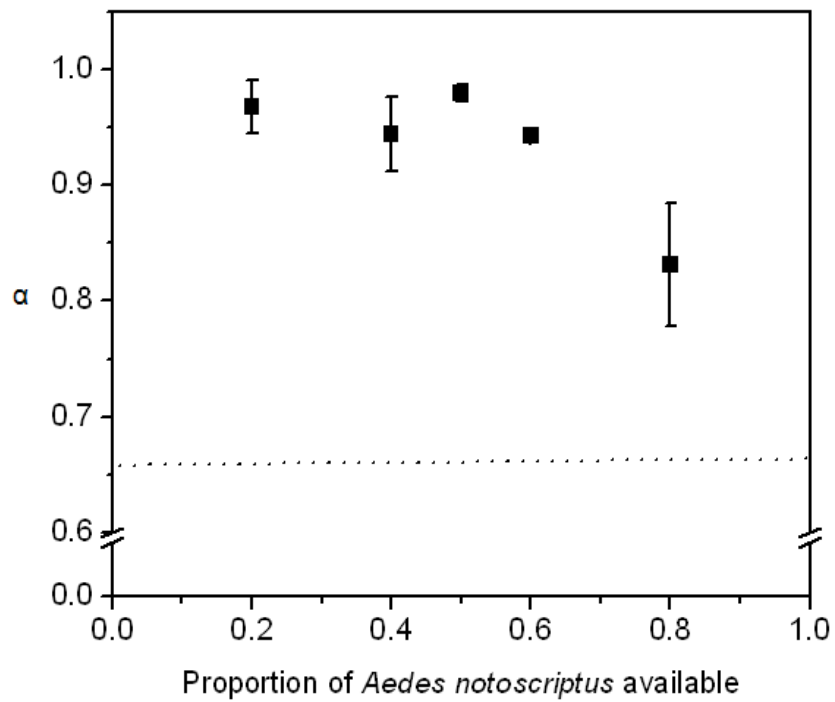


Figure 3.2: The preference of *A. wakefieldi* for exotic *Ae. notoscriptus* larvae compared to native *Cx. pervigilans* larvae, indicated by Manly's alpha (α) (\pm SE). The dash line indicates no preferences for either mosquito larvae, at $\alpha = 0.667$. α = Manly's α used to determined prey preferences by predator.

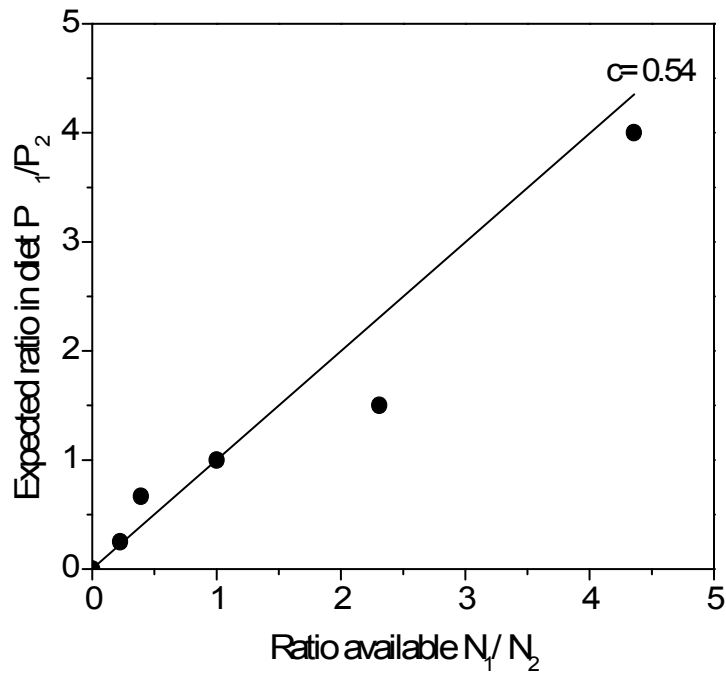


Figure 3.3: Switching behavior by *A. wakefieldi* when two prey species were offered: native *Cx. pervigilans* (N_1) and exotic *Ae. notoscriptus* (N_2). The solid line ($c = 0.54$) indicates the expected ratio in the case of no switching.

3.4.3 Predator avoidance behavior

The multinomial logistic regression likelihood ratio test showed significant effects of species ($X^2 = 201.08$, $df = 1$, $P < 0.01$), types of treatment ($X^2 = 36.76$, $df = 3$, $P < 0.01$), activities ($X^2 = 1421.20$, $df = 7$, $P < 0.01$), and water volume ($X^2 = 53.91$, $df = 1$, $P < 0.01$). In all 500 ml treatments *Cx. pervigilans* showed a high frequency of “resting” activity at “surface”, “wall” and “middle” positions, except for in the free-roaming predator treatment (Figure 3.4a). In contrast, *Ae. notoscriptus* displayed an approximately equally distributed frequency of behaviors. *Aedes notoscriptus* displayed a high frequency of “thrashing” activity with movement of their mouthparts in treatments with free-roaming predators and kairomones (Figure 4.4b). Table 3.3 shows the nominal parameter estimates from the model, in which the response of each factor is compared with a reference factor. The free-roaming predator treatment showed significant differences in behavior between *Ae. notoscriptus* and *Cx. pervigilans* (odds ratio = 0.084, $P < 0.001$). Similarly, *Cx. pervigilans* exhibited more “resting” behavior compared to *Ae. notoscriptus* (odds ratio = 197.94, $P < 0.001$). The Cox and Snell’s pseudo statistic showed that less than half the variation in prey behavior was explained by the model ($R^2 = 0.32$).

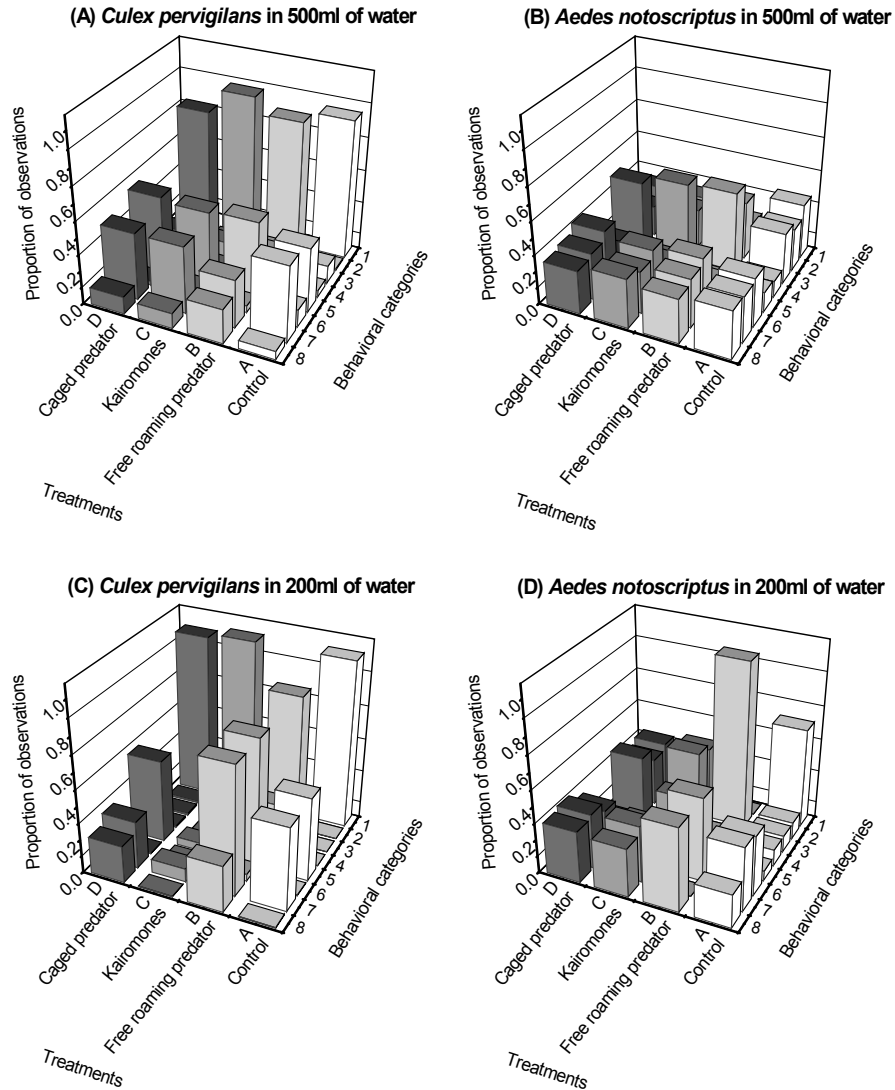


Figure 3.4: Native *Cx. pervigilans* and exotic *Ae. notoscriptus* behavior in 200ml and 500ml of seasoned water. The treatments were control (absence of predators); free roaming predators; caged predators; and water with predator kairomones. The mosquito behaviors were 'resting' (1); 'thrashing' (2); 'browsing' (3); and 'filtering' (4); 'surface' (5); 'bottom' (6); 'wall' (7); and 'middle' (8).

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Table 3.3: Results from multinomial logistic regression showing the nominal parameter estimates from the model. Mosquito species, type of treatments, activities, and position functions were analyzed in two different water volumes. The reference category was *Aedes notoscriptus*. Significant values are in bold.

Water volume		B	Std. error	Wald	df	Significance
In 500ml	<u>Treatments</u>					
	<i>Culex</i> Control	-1.468	0.165	79.603	1	<0.001
	<i>pervigilans</i> Free-roaming	0.079	0.27	0.084	1	<0.001
	predator					
	Kairomones	0.043	0.13	0.11	1	0.734
	Caged predator	0	.	.	0	.
	<u>Activities</u>					
	Resting	2.981	0.212	197.941	1	<0.001
	Thrashing	-1.102	0.412	7.171	1	0.007
	Browsing	-0.014	0.248	0.003	1	0.958
	Filtering	0.979	0.312	9.835	1	0.002
	<u>Position</u>					
	Surface	2.231	0.209	114.217	1	<0.001
	Bottom	-0.958	0.287	11.106	1	0.001
	Wall	1.411	0.915	52.161	1	<0.001
	Middle	0				

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In 200ml	<u>Treatments</u>					
<i>Culex</i>	Control	1.315	0.180	53.438	1	<0.001
<i>pervigilans</i>	Free-roaming	5.734	0.877	42.715	1	<0.001
	predator					
	Kairomones	-0.114	0.189	0.363	1	0.547
	Caged predator	0	.	.	0	.
	<u>Activities</u>					
	Resting	6.703	1.114	36.231	1	<0.001
	Thrashing	1.100	1.430	0.592	1	0.442
	Browsing	1.778	1.282	1.924	1	0.165
	Filtering	3.006	1.316	5.214	1	0.022
	<u>Position</u>					
	Surface	5.937	1.119	28.128	1	<0.001
	Bottom	-0.243	1.514	0.026	1	0.873
	Wall	4.675	1.114	17.618	1	<0.001
	Middle	0	.	.	0	.

In all 200 ml treatments *Cx. pervigilans* displayed “resting” activity at “surface”, “wall” and “middle” positions, except for the kairomones treatment where *Cx. pervigilans* larvae were “resting” at all positions tested (Figure 3.4c)

Aedes notoscriptus displayed an approximately equal probability of all behavioral activities except for in the free-roaming predator treatment, when larvae showed a high frequency of “thrashing” at the “bottom” and “middle” of the containers (Figure 4.4d). This “thrashing” behavior may have attracted predators towards *Ae. notoscriptus* larvae. The multinomial logistic regression indicated significant differences in behavioral categories 1 (resting) and 4 (filtering) at positions 5 (wall) and 7 (surface), indicating that *Cx. pervigilans* mosquito larvae are more likely to exhibit “resting” behavior (odds ratio= 36.23, $P < 0.001$) and “filtering” (odds ratio = 5.21, $P < 0.05$) when they were faced with predation by *A. wakefieldi*. In response to predators *Cx. pervigilans* also altered their positions at “surface” (odds ratio = 28.13, $P < 0.001$) and “wall” of the containers (odds ratio = 17.62, $P < 0.001$; Table 3.2). These behaviors seem to be defensive, and reduced their chances of being taken by the predator.

When comparing activities by *Cx. pervigilans* in 200 ml of water with those in 500 ml of water, I found that water volume had a significant effect on their activities (odds ratio = 609.75, $df = 7$, $P < 0.01$). *Culex pervigilans* in the free-roaming predator treatment displayed “resting” activities but also a very high frequency of positioning at the “wall” of containers in 200 ml compared to the 500 ml treatment. In contrast, *Ae. notoscriptus* in the free-

roaming predator treatments were more likely to display “thrashing” activities at the “bottom” and the “middle” of the containers. Different activity patterns by *Ae. notoscriptus* were observed even in the control treatment (in the absence of predators) in 200 ml compared to 500 ml, when larvae showed more “resting” behavior on the bottom of the containers.

3.5 DISCUSSION

I found no evidence to support the “escape from enemies” hypothesis. The native predator showed a preference for the exotic mosquito species, *Ae. notoscriptus*, over the endemic species, *Cx. pervigilans*. The predator consumed similar numbers of both mosquitoes except at high prey densities. The maximum rate of *Cx. pervigilans* consumption was attained with approximately 19 larvae. However, I observed no evidence of a plateau in prey consumption when *A. wakefieldi* were feeding on *Ae. notoscriptus*. The functional response equation predicted the plateau would occur only when densities of *Ae. notoscriptus* exceeded 50. A Type II functional response best described the predatory behavior of *A. wakefieldi* toward the two mosquito species. This functional response has also been found for a number of other insect and arthropod species (Murdoch 1969, Lester and Harmsen 2002, Beier et al. 2004, Griswold and Lounibos 2005). Also contrary to my expectations, the native predator preferentially consumed the exotic mosquito species. This preference was supported by the predators attack rate, in which the predator showed higher attack rates for the exotic mosquito species compared to native mosquito species. A study by Griswold

and Lounibos (2005) also found that dipteran predators, *Toxorhynchites rutilus* and *Corethella appendiculata* showed preferences and consumed more invasive mosquito species, *Ae. albopictus* than the native species, *Ochelarotatus triseriatus*.

The preference and attack rates of the predator seem likely to be related to mosquito behavior. Clearly there was a difference in behavior that was substantial enough to result in prey switching by the predator. The role of behavioral plasticity is one of the key factors mediating a species' invasion success (Sol et al. 2002, Sagata and Lester 2009) and prey frequently adjust their behavior according to the level of predation risk (Sih 1987). *Aedes notoscriptus* appeared to be more visible and more attractive to predators by exhibiting thrashing behavior. In contrast the native mosquito species did not display any behavior that required a lot of movement and frequently displayed resting behavior in the presence of predators. The low risk behaviors of mosquito larvae least likely to result in predation are resting and staying near the water surface in response to *T. rutilus* (Juliano and Reminger 1992). These behaviors were also displayed by *Cx. pervigilans* when confronted with *A. wakefieldi*. This seems to be a successful strategy to reduce the chances of being attacked by predators and seems to fulfill the "threat sensitivity" hypothesis, which states that prey alter their avoidance response according to the magnitude of the threat. The conclusion that there is variation in the ability of different species to detect potential threats and adjust their behavior accordingly has wide support (e.g., Laurila et al. 1997, Rochette et al. 1997, Jackson et al. 2002). It seems logical that a native

species should be better able than an exotic species to detect and appropriately alter its behavior when confronted with a native predator due to predator-prey co-evolution.

The “escape from enemies” hypothesis is frequently used in reference to specialist predator-prey interactions (e.g., Helfmann 1989, Gyssels and Stoks 2005, Bailey et al. 2009). When an invasive species enters a food web containing generalist predators, lack of an ability to detect native predators and modify their behavior accordingly may frequently make these invaders more susceptible to attack. Also, in a newly invaded habitat range, exotic and non-native species often quickly gain a large number of enemies because they are essentially naïve and strongly affected by interactions with enemies (Case and Crawley 2000, Colautti et al. 2004). This increased susceptibility to generalist predator attack is effectively the opposite of the “escape from enemies” hypothesis.

Changes in prey behavior were observed in different water volumes. This result suggests that the mosquitoes may make themselves more or less apparent to predators in different environments. In nature the available habitats are diverse. Natural aquatic environments may contain different substrate, with different aquatic vegetation and flow velocities which can provide sub-habitats to prey species (Taniguchi et al. 2003, Taniguchi and Tokeshi 2004). Variable behavior may influence the susceptibility of mosquitoes to predators. In addition, in lower volume habitats the concentration of predator kairomones is presumably higher than that in larger volumes when the number of predators is at equal. Higher concentrations

could indicate a greater probability of encountering the predators within the small volume (Kesavaraju et al. 2006) and may indicate proximity of a predator by concentration of the cues (Kusch et al. 2004). Consequently, it is impossible to conclude that the exotic mosquito *Ae. notoscriptus* would always be more susceptible to native predators than the native *Cx. pervigilans* in all habitats, just as it would be impossible to make definitive conclusions about any predator-prey interaction in all potential habitats. Variable behavior or susceptibility to predators in different environments may help explain the establishment of *Ae. notoscriptus* in New Zealand despite its relatively higher susceptibility to this predator.

At this stage there is insufficient evidence to support the “escape from enemies” hypothesis as a major mechanism facilitating species invasions. Invasion success may depend on the ability of exotic species to respond adaptively to predators. In the absence of other adaptive responses, behavioral responses from prey may become one of the important factors in mediating the effect of predators on prey species.

CHAPTER FOUR:

Can adults of the New Zealand mosquito *Culex pervigilans* (Bergorth) detect the presence of a key predator in larval habitats?

In part published as: Zuharah WF and Lester PJ (2010) Can adult of New Zealand mosquito *Culex pervigilans* (Bergorth) detect the presence of a key predator in larval habitat?. Journal of Vector Ecology. 35(1): 100 - 105 (see Appendix C).

4.1 ABSTRACT

The influence of predators on mosquito populations may be direct through predation, or indirect through sub-lethal responses of adult mosquitoes in life history traits such as oviposition behavior. In New Zealand, the backswimmer, *Anisops wakefieldi*, is a common predator of mosquito larvae found in temporary and permanent water bodies. I predicted that the New Zealand native mosquito, *Culex pervigilans*, whose larvae are vulnerable to predation of *A. wakefieldi*, would likely avoid the containers with the presence of *A. wakefieldi* or its kairomones remnant. I established temporary water containers without predators, free-roaming predators, caged predators (which were unable to eat mosquitoes), or containers from which predators were removed immediately prior to the experiment (these containers would have remnant kairomones from the predators). Each treatment with *A. wakefieldi* had predator densities of one, three or nine *A. wakefieldi*. Contrary to my predictions, when choosing oviposition habitats, *Cx. pervigilans* appeared to ignore the presence of free-roaming *A. wakefieldi*, caged *A. wakefieldi* and water with *A. wakefieldi* kairomones. I thus observed no significant differences between the numbers of egg rafts laid by *Cx. pervigilans* in the different predator treatments nor were the number of egg rafts significantly affected by the density of predators. Rather than the presence of predators, environmental factors including temperature, humidity and pressure were significantly correlated with mosquito oviposition. These mosquitoes appeared to either ignore the presence of the predator, had no ability to detect the presence of predators, or perhaps the cues from *A.*

wakefieldi predators were not sufficiently strong enough to provide alarm to these mosquitoes. I argue that the mosquito has not evolved ability to detect the presence of these predators while ovipositing.

Keywords: Predation · Oviposition · Predator density · Kairomones · Environmental factors.

4.2 INTRODUCTION

The survival and growth of organisms without parental care is thought to be strongly dependent on the quality of habitat into which they are deposited. Adults of insects in which juveniles are incapable of migrating away from low quality habitat should be evolved to select oviposition sites that improve the likelihood of their offspring survival and growth (Kiflawi et al. 2003a, Blaustein et al. 2004, Bond et al. 2005). Thus a female mosquito should be able to detect and avoid ovipositing in sites which have a high risk of predation for their eggs or juvenile stages. Mosquitoes also compete with one-another and should selectively oviposit to minimize inter- or intra-specific competition. Blaustein et al. (2004) suggested that five factors have shaped the ability of mosquitoes to respond to the risk of predation during ovipositing: 1) mosquito larvae are highly vulnerable to predation, 2) they have only one or a few lifetime reproductive events, 3) their eggs are often laid together, 4) their predators have highly heterogeneous distributions, and finally, 5) the distribution of predators is often highly fixed from the time of prey oviposition event until prey progeny can either leave the patch or become large enough to be invulnerable to predation. Ideally, mosquitoes may have an ability to detect the presence of predators or competitors based on chemical or physical cues released (Stav et al. 1999).

Wisenden (2000) showed that chemical cues are released by the predator during detection, attack, capture and ingestion of prey. Prey may use these cues to minimize their exposure to predators. Several species of

mosquitoes have been shown to be able to detect the presence of predatory notonectid bugs and avoid ovipositing in pools containing these predators. In the presence of only one predatory backswimmer (*Notonecta maculata* Fabricious) in 10 litres of water, *Culiseta longirealata* (Macquart) oviposition was strongly reduced (Blaustein et al. 1995, Blaustein 1998, Kiflawi et al. 2003a, Kiflawi et al. 2003b). Eitam and Blaustein (2004) found that *Cs. longiareolata* and *Cx. laticintus* oviposited less in pools containing predators, but this avoidance of predators was unrelated to predator densities. Their work was also supported by Blaustein et al. (2005), who found that *Culex* mosquitoes strongly avoided ovipositing in pools containing the *Notonecta* predators. Mosquitoes continued to avoid ovipositing in pools with predators for two additional days after the predator was removed, suggesting a predator-released kairomones was the cue used by mosquitoes to detect the presence of predators.

Stav et al. (1999) suggested that the distribution and abundance of a mosquito species is dependent on their ability to selectively oviposit according to predator abundance. The density of predators may affect mosquito oviposition due to the ability of mosquitoes to detect predator densities and thus to assess the severity of predation risk. With increasing predators densities, concentration of kairomones are also increased and may result in reduced oviposition (Eitam and Blaustein 2004). Reduced oviposition by mosquitoes may be expected with increasing numbers of predators, due to the ability of these prey species to detect the presence of a

predator above a threshold density (Angelon and Petranka 2002) or concentration of kairomones (Eitam and Blaustein 2004).

My aim in this study is to examine the oviposition behaviour of the New Zealand mosquito *Cx. pervigilans* in response to the notonectid predator *A. wakefieldi* under the field conditions. *Anisops* spp. are known to be predators of mosquito larvae and are commonly found in permanent and temporary water bodies in New Zealand (Graham 1939, Laird 1990; 1995). *Culex pervigilans* is a vector species of the human and avian Whataroa virus (Maguire et al. 1967, Miles 1973), is suggested to be a vector of avian malaria caused by *Plasmodium relictum* (Holder et al. 1999, Massey et al. 2007), and has been highlighted as a species requiring further investigation for its potential to vector exotic arboviruses (Weinstein et al. 1997). I hypothesized that *Cx. pervigilans* are likely to avoid ovipositing in pools containing *A. wakefieldi* or its kairomones, and that the influence of the kairomones released by *A. wakefieldi* predator may last for several days after the predators are removed. I also hypothesized environmental factors could play a role in influence mosquito oviposition rates. Bentley and Day (1989) suggested temperature, rainfall, wind speed and relative humidity may affect the initiation of ovipositional flights by mosquitoes.

4.3 MATERIALS AND METHODS

4.3.1 Study sites

The study was undertaken in Otari-Wilton's Bush in Wellington, New Zealand (14°14'S, 174°45'E). Otari-Wilton's Bush is Wellington's largest area of original native forest. Experiments were undertaken on the edge of the forest in plant nursery, with no overhead canopy or shading. Temperatures over the period of experiments ranged from 14.8°C to 18.2°C and relative humidity was between 62.8% and 89.3%. Experiments were conducted during the southern hemisphere summer season for two consecutive summers (2007/08 and 2008/09). These experiments ran from 18th December 2007 to 11th February 2008, and from 7th December 2008 until 19th January 2009.

4.3.2 Predator colonies

The *A. wakefieldi* third and fourth instar (nymphal) used in this experiment were collected from permanent water troughs (with approximately volume of 800 liters of water) in Queen Elizabeth II Park near Waikanae, New Zealand (40°57'S, 174°58'E), approximately 70km from Wellington city. These predators were brought back to the laboratory for identification and deprived of food for 24 hours prior to their release into the experimental containers.

4.3.3 Study design

Eleven black plastic containers (25.5 × 25.5 × 23.5 cm) served as temporary pools for the experiment. Containers were filled with 10 l of water: 9.75 l of aged tap water (tap water that was left standing for more than 48 hours to reduce the chlorine content) mixed with 0.25 l of stream water from the nearest stream (Kaiwharawhara Stream). Water was left to age for 48 hours prior to initiating experiment. Stream water was taken from nearest river to introduce bacterial inoculates for food sources for mosquito larvae and was filtered with 0.2 mm mesh before being added to the containers. The water level was maintained at 10 l throughout the experiment by adding aged tap water or by natural rain to account for any loss due to evaporation. Nutrients were added once prior to experiment to the water in the form of “Kiwi” brand pelletised sheep manure which contained 3% nitrogen, 2% phosphorus and 4% potassium at a weight of 5 g/l of water, 24 hours prior to the experiment. Previous work has shown such conditions to be ideal for *Cx. pervigilans* (Leisnham et al. 2006). Containers were then covered with 1.0 cm wire mesh to prevent vertebrate animals from drinking or falling into the water.

These containers were set up so that the distance between containers was at least ~0.5m from its flanking neighbor. Treatments established were as follows: 1) controls without predators; 2) free-roaming *A. wakefieldi* (with one, three or nine *A. wakefieldi* per container); 3) caged *A. wakefieldi* (empty cage without predator, with one, three or nine *A. wakefieldi* in a cage, which were unable to consume mosquito larvae or directly seen by ovipositing

adults); and 4) *A. wakefieldi* kairomones remnant (with kairomones from one, three or nine *A. wakefieldi* which were present in the water for 24 h prior to the experiment, but removed at the start of each trial). All treatments were replicated for three times for each year. Cages used to contain predators in the caged *A. wakefieldi* treatment were constructed using 1.5 l clear plastic drinking bottles, each with one opening windows (10cm x 3cm) on each of two sides of the bottle and one end of the tops of the bottle was covered with 2 mm nylon mesh. The mesh was used to ensure that the predator could breathe and any predator-released kairomones from *A. wakefieldi* could diffuse into the main water-body. The cages were placed in a horizontal orientation within the water containers so that most of the cage was submerged. In the kairomones remnant treatment, *A. wakefieldi* were kept in the water for 48 hours immediately prior to the beginning of the experiment before their removal. Therefore, the water only contained kairomones without the presence of an actual predator.

All containers were set up 48 hours before the experiments began in order to introduce and familiarize mosquitoes to the new oviposition sites. All mosquito egg rafts were counted 48 hours after containers were introduced to the field. Each day for 12 days after the *A. wakefieldi* was added, *A. wakefieldi* was counted and any missing or dead *A. wakefieldi* were replaced. The eggs were daily collected into small containers with water and were brought back to laboratory for counting and identification at larval stages.

4.3.4 Statistical analysis

I used a repeated-measures general linear model (GLM) to test for significant effects of predator treatment (free-roaming, caged predators or no predators), predator density, and time on *Cx. pervigilans* oviposition rate in SPSS (2006). Data were tested for normality using a one-sample Kolmogorov-Smirnov test. All data were log (x+1) transformed prior to analysis to satisfy the assumptions of ANOVA. Sphericity could not be assumed in the study ($\epsilon = 0.704$, $p = 0.009$) and Huynh-Feldt epsilon value were used in the F-tests. The repeated measures within- subjects factor were period with 4 levels; day 1-3, day 4-6, day 7-9 and day 10-12. Predator treatment and density were treated as between-subjects factors.

The number of egg rafts observed during each period was correlated with five environmental factors: temperature, wind speed, rainfall, humidity and pressure, using a Pearson correlation coefficient. The environmental data were obtained from the Kelburn weather station approximately 3 km from my study site, by National Institute of Water and Atmospheric Research (NIWA), New Zealand. Any significant correlation data from Pearson's correlation test were then analyzed using t-test in order to determine the mean effects between years.

4.4 RESULTS

No significant differences in mosquito oviposition rates were detected between treatments with the presence of predators, either caged or uncaged, the presence of their kairomones remnant, and the control treatment with no predators ($F = 1.389$, $df = 3$, $P = 0.284$; Table 4.1 and Figure 4.1). Similarly, no statically significant effects of predator densities were observed on oviposition rates ($F = 0.224$, $df = 3$, $P = 0.879$). Thus, *Cx. pervigilans* mosquitoes appeared choose their oviposition habitat without taking into account of predator presence or density. There were no significant differences in oviposition between all four periods tested, nor was there any significant interaction between periods and treatments or a significant three way interaction ($P \geq 0.500$; refer to Table 4.1). Thus, mosquito oviposition rates were similar irrespective of how long the predators were in the water containers (or in the case of the kairomones remnant treatment, how long the predator had been removed). These results suggested that *Cx. pervigilans* mosquitoes were not able to detect, or ignored, the presence of *A. wakefieldi* or its kairomones remnant.

A total of 284 egg rafts were collected in 2007/2008, which was significantly more than 2008/09 when only 28 egg rafts were collected ($t = 3.801$, $df = 114$, $P < 0.001$). All of the egg rafts were identified as *Cx. pervigilans*. There were significant correlations between the number of *Cx. pervigilans* egg rafts and temperature, humidity, and pressure (Table 4.2). T-tests examining the mean effects between years indicated that fewer egg

rafts were oviposited by *Cx. pervigilans* mosquitoes when the temperature was significantly higher ($t = 67.43$, $df = 35$, $P < 0.001$) with significantly lower humidity and pressure ($t = 35.94$, $df = 35$, $P < 0.001$; $t = 798.08$, $df = 35$, $P < 0.001$ respectively) in 2008/09 summer season compared to 2007/08 summer season.

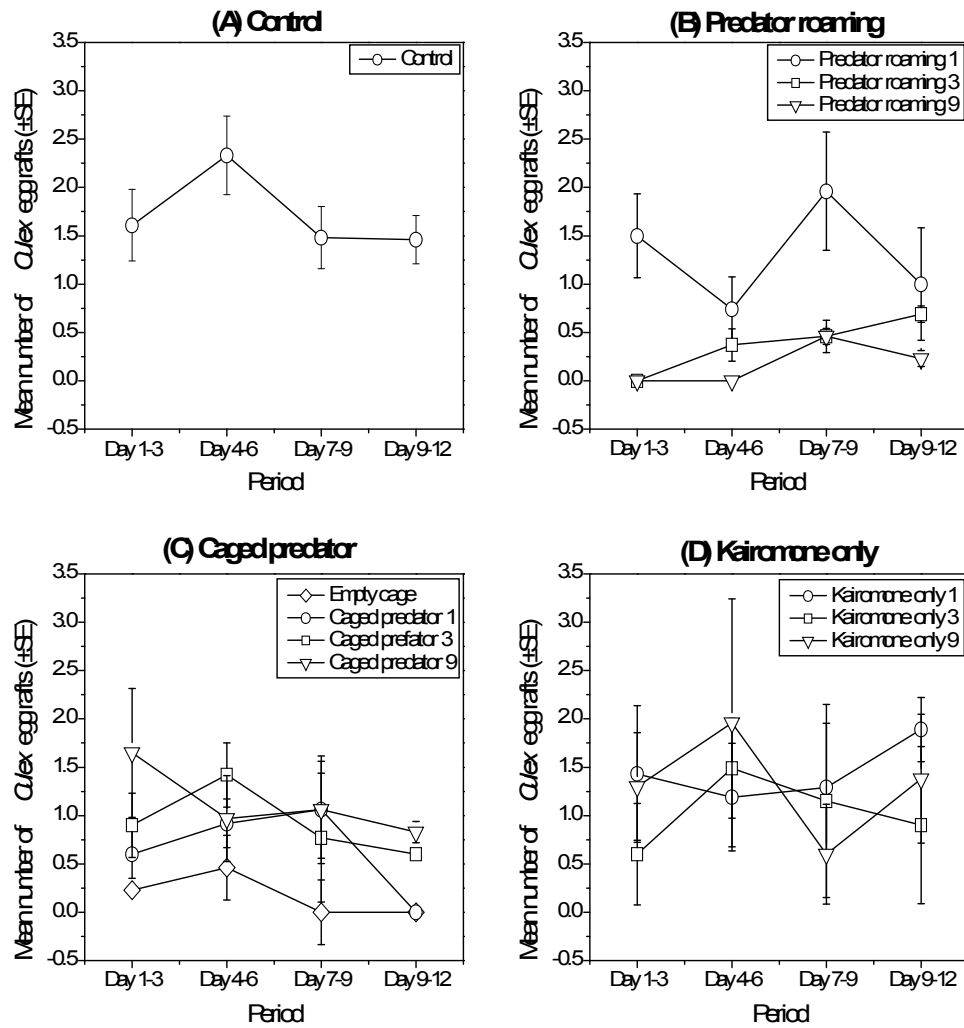


Figure 4.1: The effects of predator treatment and predator density (A. *wakefieldi*) on number of *Cx. peruvigilans* egg rafts (mean \pm SE) based on 4 periods time tested; (A) control (B) free-roaming predators, (C) caged predators (D) water with predator kairomones.

Table 4.1: Results of repeated measures ANOVA examining the cumulative number of *Cx. pervigilans* egg rafts in response different types of predator treatments and densities (*An. wakefieldi*). *df* = degree of freedom, MS = mean squared values. Significant values are in bold. Data were log transformed prior to analysis.

Source	<i>F</i>	<i>df</i>	MS	Significance
Treatments (T)	1.389	3	0.032	0.284
Densities (D)	0.224	3	0.005	0.879
Period (P)	1.030	3	0.004	0.381
Year (Y)	3.791	1	0.013	0.100
P x T	0.931	9	0.003	0.500
P x D	0.828	6	0.003	0.591
P x Y	1.689	3	0.006	0.173
P x T x D	0.693	12	0.002	0.600
P x Y x T	1.281	6	0.005	0.271
P x T x D x Y	0.395	12	0.001	0.963
Period x Subject within groups		126	0.004	
Period x Subject between groups		42	0.014	

Table 4.2: Pearson correlation analysis results between temperature, wind speed, rainfall, humidity, and pressure with *Cx. pervigilans* egg rafts after 12 days post-treatment based on two years study periods. Significant values are in bold. * indicated that the significant value is at $P < 0.05$ and ** are at 0.01. $df = 271$ for all factors.

Factor	Pearson's r	Significance
Temperature	0.159*	0.012
Wind speed	- 0.104	0.098
Rainfall	- 0.084	0.182
Humidity	0.187**	0.003
Pressure	-0.504**	<0.001

4.5 DISCUSSION

I predicted that the mosquito *Cx. pervigilans* would alter its oviposition behaviour in the presence of the predator *A. wakefieldi* or its kairomones. This prediction was based on several other studies that have found evidence for *Culex* spp. and *Culiseta* spp. avoiding oviposition in the presence of *Notonecta* sp. or its chemical traces (Blaustein et al. 1995, Blaustein 1998, Kiflawi et al. 2003a, Blaustein et al. 2004, Eitam and Blaustein 2004, Blaustein et al. 2005). Eitam et al. (2002) also found that in the presence of free swimming *A. sardea*, *Culiseta* mosquitoes were repelled from ovipositing in pools, resulting in a significant low number of eggs than in control pools. In contrast, I found no evidence that *Cx. pervigilans* modifies its oviposition behaviour in response to predators. I postulate that *Cx. pervigilans* either ignored the presence of the predator or that the cues from *A. wakefieldi* predators were not sufficiently strong enough to provide an alarm to these mosquitoes

The densities of predators that I used have elicited avoidance responses in *Culex* mosquitoes elsewhere. The densities of predators in my study were more than twice that used in the study by Eitam and Blaustein (2004), but I observed no significant response by mosquitoes to the predator or its kairomones. In regard to kairomones, other mosquitoes such as *Culiseta longireolata* can detect chemicals from *Notonecta* predators for periods of up to eight days after their removal from the pool (Blaustein et al. 2004) and at least two days for *Culex* species (Blaustein et al. 2005). It thus

seems reasonable to expect that the mosquitoes would have shown a response to this predator in my experimental design and I am relatively confident that no such response occurred. Nevertheless, I cannot rule out the possibility that the mosquito would have shown a response to the predator at higher predator densities.

Given the substantial effects of individual predators on *Cx. pervigilans* populations, I would have expected this mosquito to have evolved an ability to detect the densities of this predator that I used in the experiment. In laboratory experiments I have observed *A. wakefieldi* to kill large numbers of *Cx. pervigilans* (Zuharah and Lester, In Press B). A single adult predator can kill an average of 10-82 *Culex* larvae per day based on prey and predator size and density (Nabaneeta et al. 2007). There was thus clearly no reason why *Cx. pervigilans* should have ignored the presence of the *A. wakefieldi* predator. In the absence of any behavioural modification of oviposition, these lethal effects of *A. wakefieldi* seem likely to be the mechanism resulting from the near complete absence of mosquitoes co-occurring with this predator in my field studies (Zuharah and Lester, In Press A).

Given the lethal effects of *A. wakefieldi* on mosquito populations, why hasn't an ability to detect predators evolved in this mosquito? I can only speculate on explanations. It is possible that until recently, this species may have not naturally co-occurred together. Greig (2008) found that *Anisops* sp. in Canterbury, New Zealand, is likely to inhabit either in permanent or semi-permanent water bodies, but not in temporary pools. However, *Cx.*

pervigilans is able to breed in a wide variety of any water habitat. *Culex* larvae can easily found in fresh and polluted waters, permanent and temporary ground pools, natural and artificial containers and also slow moving streams (Belkin 1968, Dumbleton 1968, Holder et al. 1999). With the creation of water troughs by humans in farming industry, *A. wakefieldi* predators have perhaps only recently started to share the same habitat with *Cx. pervigilans*. Statistics New Zealand (2008) indicated that 76% of agricultural land was used for animal farming industries in year 2002 with many thousands of existing water troughs as potential habitat for *Cx. pervigilans*, *A. wakefieldi* and other aquatic insects. *Culex pervigilans* are now the most common species observed in water bodies such as animal trough where *A. wakefieldi* also occur (Laird 1990). Because of the immense selection pressure exerted by *A. wakefieldi* on *Cx. pervigilans* in this new habitat, the mosquito may develop an ability to detect this predator, but the evolution of such ability would be likely to take a long time to develop.

Given the lack response of *Cx. pervigilans* to *A. wakefieldi* predator, I predicted that variation within and between years in mosquito oviposition behaviour might be explained by environmental factors. Changes in temperature or precipitation may drive changes in mosquito density and populations (Canyon et al. 1998, Ritchie and Rochester 2001, Koenraadt et al. 2004, Hsu et al. 2008, Platonon et al. 2008). Similarly, my results also indicated that more egg rafts were oviposited by *Culex* mosquitoes when the temperatures were higher, humidity and pressure were lower as demonstrated in 2007/08 summer season. In my experiment, environmental

variables clearly had a far greater influence on oviposition than the presence of *A. wakefieldi* predators.

In summary, I found that *Cx. pervigilans* did not alter their oviposition behaviour in response to *A. wakefieldi* predators (or its kairomones), presence of different densities of predator and also environmental factors. This is the first published study to demonstrate that *Cx. pervigilans* do not alter their oviposition behaviour according to the presence of a voracious predator. The complexity of *Culex* sp. ability to detect predator presence still remains to be seen and should continue to be studied.

CHAPTER FIVE:

Lethal and sub-lethal exposure of prey to predators substantially influences the survival rate and life history traits of a mosquito

In part published as: Zuharah WF and Lester PJ. Lethal and sub-lethal exposure of prey to predators substantially influences the survival rate and life history traits of a mosquito.

(In review in *Population Ecology*, submitted on 10 June 2010)

5.1 ABSTRACT

The presence of predators can have dramatic consequences on prey communities, not only by the direct effects of consumption but also through sub-lethal effects. I investigated the survival rate and subsequent life history of the mosquito *Culex pervigilans* (Bergorth) under the influence of its major predator, the backswimmer *Anisops wakefieldi* (White). I established a field experiment with various treatments: 1) control without predators; 2) free-roaming *A. wakefieldi* (with one, three or nine *A. wakefieldi* per container); 3) caged *A. wakefieldi* (empty cage without predators, or with one, three or nine *A. wakefieldi* in each cage; and 4) *A. wakefieldi* kairomones remnant from predators (with kairomone concentrations of one, three or nine *A. wakefieldi* which were present in the water for 24 h prior to the experiment but removed at the start of each trial). *Culex pervigilans* eggs were then taken from these experiments and reared in two different laboratory conditions: (A) in clean water without any traces of predators, or (B) in water with the same treatments as in field. The survival rate of *Cx. pervigilans* was significantly reduced by the presence of predators or their kairomones. Even though the eggs were only exposed briefly to water containing either predators or kairomones in the field, and then were reared in clean water without any traces, sub-lethal effects still impacted the remaining survivors. These results were observed when adult mosquitoes were exposed to predators at the time of oviposition and when the predators or kairomones were present throughout the life history of the mosquito. The percentage of eggs that hatched, and the resulting mosquito population was influenced by the

presence of predators or their kairomones. No effect was observed on *Cx. pervigilans* sex ratio or changes in development times of each life stage. My results suggested that sub-lethal effects may be carried by surviving individuals primarily through the effects of stress, perhaps by epigenetic mechanisms. We may expect to observe similar plasticity in species or populations with high temporal or spatial variability in predation.

Keywords: *Culex pervigilans* · *Anisops wakefieldi* · Hatching rate · Sex ratio · Development time · Survival rate ·

5.2 INTRODUCTION

Predators influence prey populations not only through direct consumption but also by indirect effects, which include any type of interaction between the predator and prey that does not result in death. These sub-lethal effects may affect prey traits such as life history and growth rate (Havel 1987, Havel and Dodson 1987, Black and Dodson 1990, Laurila et al. 1998), morphology (Havel and Dodson 1984, Dodson 1988, Gilbert and Hartmen 1996, Johansson and Wahlström 2002), delayed or reduced fecundity (Havel and Dodson 1987, Walls and Ketola 1989, Black and Dodson 1990), and behavior (Lima 1998). Such sub-lethal effects typically induce a cost for prey populations (Lima 1998).

Avoiding predators may result in a reduction of food intake and ultimately a reduction in the reproductive success of prey (Gerritsen and Stickler 1977, Werner and Anholt 1996). For example, Bond et al. (2005) found that predatory fish reduced larval mosquito feeding opportunities. As a result the mosquitoes exhibited extended larval development times, adult mosquitoes were significantly smaller and had higher mortality compared to when predators were absent (Peckarsky et al. 1993, Bond et al. 2005, Mikolajewski et al. 2005). The sub-lethal influence of predators may even be sex-specific. Braune and Rolff (2001) suggest that mass and size are relatively important for female prey as predators likely affect their survival and fecundity. Male mosquitoes may emerge earlier as a trade-off in presence of predator to maximize their fitness (Nylin and Gothard 1998).

Chemical recognition of predators by prey may play an important role for many aquatic insects, especially for those that live in limited visibility habitats or where predators are cryptic (Kats and Dill 1998). Prey may be able to detect the presence of a predator by their chemical cues (or 'kairomones'); these are usually released directly by a potential predator or by injured conspecific prey (Beketov and Liess 2007). Upon sensing kairomones released by potential predators, prey may display diverse changes in behavior, morphology, or even life history (Kats and Dill 1998). However, it is unlikely an individual prey species can detect kairomones from all potential predators. Understanding if prey can detect predator kairomones and how this may modify their life history is important for understanding the population dynamics of prey populations.

The mosquito *Cx. pervigilans* (Bergorth) is New Zealand's most common native mosquito species observed in artificial containers (Laird 1990; 1995). This mosquito is a vector of the human and avian Whataroa virus (Maguire et al. 1967, Miles 1973) and is suggested to be a vector of avian malaria (Holder et al. 1999, Massey et al. 2007). A common predator is the backswimmers, *A. wakefieldi* (White). These predators are known to consume mosquito larvae and are commonly found in permanent and temporary water bodies in New Zealand (Graham 1939, Laird 1990; 1995). My aim in this study was to examine the lethal and sub-lethal effects of these predators and their kairomones on the mosquito. I examined the lethal and sub-lethal effects of this predator on egg hatching rates, development time of each life stage, sex ratio, and survival rate of *Cx. pervigilans* first progeny

after exposure to *A. wakefieldi*. These scenarios differed in the length of time the developing mosquitoes were exposed to predators or their kairomones; (1) only the eggs were exposed to predators or their kairomones in the field but then reared in clean water without any traces of predator. (2) eggs were hatched and the larvae maintained in treatments that had free-roaming predators or predators in cages, from the treatment with predator kairomones remnant, and from the control treatment where no predators or their traces were present.

5.3 MATERIALS AND METHODS

5.3.1 Insect colonies

The predators, *A. wakefieldi*, used in this experiment were collected from water troughs in Queen Elizabeth II Park near Waikanae, New Zealand (40°57'S, 174°58'E), approximately 70 km from Wellington city. These predators were brought back to laboratory and fed daily with mixed instar mosquito larvae.

Egg rafts of *Cx. pervigilans* were obtained opportunistically from another experiment on mosquito oviposition behavior (Zuharah and Lester 2010). Briefly, this experiment utilized black circular 10 l containers 25.5 × 25.5 × 23.5 cm, at Otari-Wilton's Bush in Wellington, New Zealand (14°14'S, 174°45'E). Nutrients were added to the water in the form of "Kiwi" brand pelletised sheep manure which contained 3% nitrogen, 2% phosphorus and 4% potassium at a rate of 5 g/l of water, 1 day prior to the experiment.

Previous work has shown such conditions to be ideal for *Cx. pervigilans* (Leisnham et al. 2006). Containers were then covered with 1 cm wire mesh to prevent vertebrate animals from drinking or falling into the water. The treatments from which eggs were collected were as follows: 1) control without predators; 2) free-roaming *A. wakefieldi* (with one, three or nine *A. wakefieldi* per container); 3) caged *A. wakefieldi* (empty cage without predators, or with one, three or nine *A. wakefieldi* in each cage, these predators were either unable to consume mosquito larvae or been directly seen by ovipositing mosquito adults); and 4) *A. wakefieldi* kairomones remnant from predators (with kairomone concentrations of one, three or nine *A. wakefieldi* which were present in the water for 24 h prior to the experiment but removed at the start of each trial). Cages used to contain predators in the caged *Anisops* treatment were constructed using 1.5 l clear plastic drinking bottles, each with one opening windows (10cm x 3cm) on each of two sides of the bottle and one end of the tops of the bottle was covered with 2 mm nylon mesh. Eggs laid by wild female adult mosquitoes were collected daily at 0900, brought back to laboratory and immediately counted under the microscope. Experiment with treatments above was replicated for three times. Temperatures over the period of experiments ranged from 14.8°C to 18.2°C and relative humidity was between 62.8% and 89.3%. These containers were set up in the southern hemisphere summer season from December to January 2007/08 and 2008/09.

5.3.2 Life-history influence of predators at the time of oviposition

In this trial I tested for any life-history effects of the presence of predators, when these predators were present only at the time of oviposition by adult mosquitoes. After oviposition, the eggs were reared away from predators or their kairomones. I collected eggs that had been oviposited into the above treatments that had predators' present, free-roaming, or in cages, from the treatment with predator kairomones, and from the control treatment where no predators or kairomones were present. These eggs were then transferred into clean water (aged tap water) and monitored daily from egg hatch until adult mosquito death. I used between 2 -32 replicates in which I considered single egg raft as one replicate. These replicate varied according to the availability of egg rafts collected in the field. This experiment and the below experiment investigating the life-history influence of predators throughout the mosquito life history were conducted in 1 l containers (20cm × 13cm × 5cm), each with 500ml of water. Each of egg rafts from various treatments were placed in separate containers and reared under laboratory controlled conditions at $25 \pm 1^{\circ}\text{C}$ in photoperiod LD 14: 10 hours. Hatching larvae were fed with a mixture of cat biscuit, beef liver, yeast and milk powder in the ratio of 2:1:1:1 by weight, prepared as a fine powder and added to the water at a rate of 10 mg/ml, as proposed by WHO (1996). Water was changed every 4 days to avoid a film of yeast forming on the water surface causing larval mortality (Bar-Zeev 1957). Adult mosquitoes were then placed in cage size $30.5 \times 30.5 \times 22.8\text{cm}$ and fed with 10% sucrose.

I monitored the percentage of eggs hatching and the survival of mosquitoes until adult death. Data for egg hatching rates are presented as mean percentages (\pm SE). I tested the hatching rate of eggs between treatments with two separate water conditions using one-way ANOVA followed by a post hoc comparison (Tukey HSD). Prior to statistical analysis I examined this and all subsequent data for heteroscedasticity using Levene's test, and for fit to a normal distribution using the one-sample Kolmogorov-Smirnov test in SPSS (2006).

Larvae were counted, sorted according to larval stages, and transferred each day to new containers. Pupae were removed daily from the containers and placed in 250ml paper cups covered with 0.2 mm mesh and were left until emergence of adults. The sex of the emerged adults was then determined. I used chi-square test to detect deviations from expected 1:1 ratio between male: female in the adult mosquito sex. All treatments were compared with control where the ratio was 1:1 (there were insufficient data in some treatments, such as in treatments with predators, for other tests such as contingency tables).

Mosquito development and mortality during larval, pupal and adult stages was recorded daily. To analyze differences in stage-specific developmental time I used a MANOVA with the dependent variables being life stage (4 larval instars, pupae, and male or female adults), treatments were the independent variables. Post-hoc comparisons between treatments

were performed using Tukey HSD tests in order to calculate the significant effects of development times for each stage.

The survival rate of *Cx. pervigilans* from egg hatch until adult death was analyzed using Kaplan-Meier survival analysis with 95% confidence intervals in SPSS (2006). Survival analysis was used to estimate population survival curve from the treatments tested. Types of treatment served as factor whereas status was defined as 0 for alive and 1 for dead. All treatments were compared with the survival rate in the control treatment (without any predators). I then ran pair-wise comparisons using log-rank tests in order to determine the survival differences between types of treatment tested.

5.3.3 Life-history influence of predators throughout mosquito life history

In the previous experiment I tested for any influence of predators, when these predators were present only at the time of oviposition. In this experiment I examined the direct influence of *A. wakefieldi* on mosquitoes from oviposition until adult death, with treatments in which the predators were maintained with the mosquito larvae. In these treatments I collected eggs from the various treatments in the field experiment as detailed above. After the egg rafts were brought back to the laboratory I allocated them to containers with *A. wakefieldi*, or water with its traces with either: 1) one, three or nine free-roaming *A. wakefieldi*; 2) one, three or nine *A. wakefieldi* in cages; or 3) kairomone treatments. These kairomone treatments consisted of

containers in which one, three or nine *A. wakefieldi* were present in the water for 24 h immediately prior to experiment and were then removed before the introduction of eggs. The kairomones were not replaced during the remainder of the experiment as such a replacement may have significantly disturbed the mosquitoes and their food supply. These eggs, developing larvae, and adults were monitored daily until mosquito death. I used between 2 - 15 replicates in these experiments which I considered single egg raft as one replicate. These replicates were varied according to the availability of eggs collected in the field.

The egg hatching rate and sex ratio of adults were analyzed using one-way ANOVA and sex ratio were analyzed using chi-square test with same procedure as above. I used a MANOVA with the same variables as in the oviposition experiment for examination of the effects of each life-stage. The survival rate of *Cx. pervigilans* from egg hatch until adult death was analyzed using Kaplan-Meier survival analysis as in the oviposition experiment with 95% confidence intervals in SPSS (2006).

5.4 RESULTS

5.4.1 Life-history influence of predators at the time of oviposition

In this trial I tested for any life-history effects of the presence of predators, when these predators were present only at the time of oviposition by adult mosquitoes. After oviposition, the eggs were reared away from predators or their kairomones. I examined egg hatching, mosquito development and

survival rates after eggs were collected from a field experiment with nine different treatments (with free roaming predators, predators in cages, etc). These eggs were reared to adult stage in clean water without any predators or kairomones.

The mean percentage of eggs that hatched ranged between 70.8 - 92.18%. One-way ANOVA revealed no significant difference in the percentage of eggs that hatched between treatments ($F= 1.06$, $df = 9$, $P = 0.41$; Figure 5.1A). In the treatment with three free-roaming predators the ratio of emerged females was lower than male adult mosquitoes at a ratio of 1 : 0.42, though this was significant ($\chi^2 = 28.051$, $df = 1$, $P = 0.000$). In kairomones treatment with three predators the sex ratio was also significantly different from control (1 : 1) with ratio of 1 male to 0.78 females ($\chi^2 = 8.718$, $df = 1$, $P = 0.003$; Table 5.1).

I monitored the developmental period of eggs until they emerged to adult stage, including the duration of the four larval instars, pupae, and male or female adults. The MANOVA test indicated that the development period of all 4 stages of larvae and female adults were not significantly different between treatments ($P > 0.05$, Figure 5.2). However, the post-hoc test revealed that eggs collected from the field treatment with one free-roaming predator showed significantly longer development times at the pupal stage ($P = 0.046$) and time to male adult emergence ($P = 0.011$) than control conditions with no predators present at the time of oviposition.

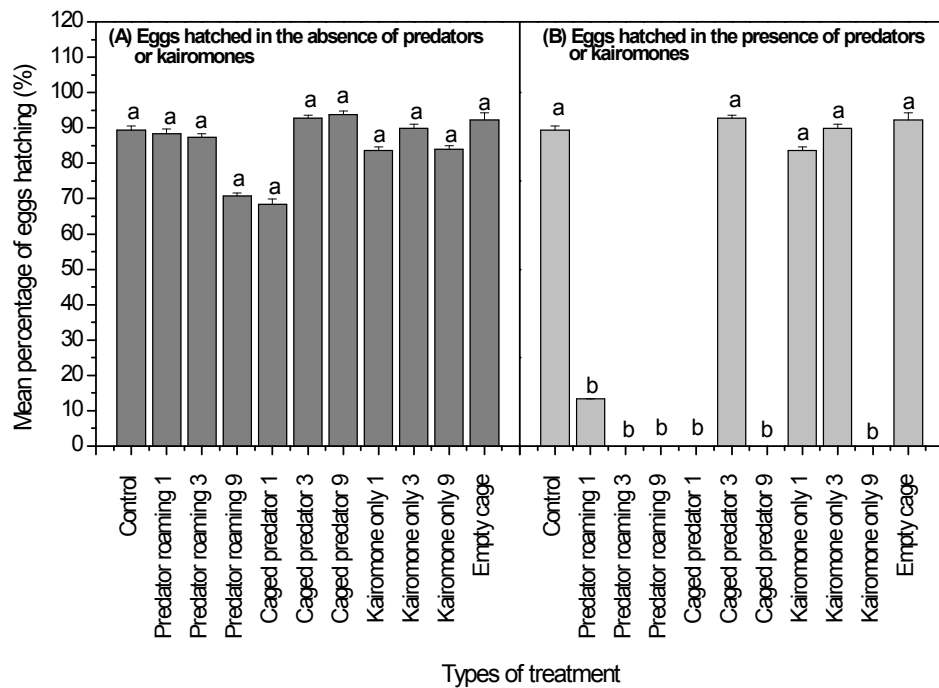


Figure 5.1: Egg hatching rates in the absence or presence of the predator *A. wakefieldi*. (A) shows the hatching rates of eggs collected from field experiments with varying predator treatments (free-roaming predators, caged predators, etc), but then with these eggs removed and then reared in water without predators or kairomones. (B) shows hatching rates of eggs collected from field experiment with the same treatments and then reared in the laboratory with those same predator treatments. The control treatment and empty cage treatments are repeated on both graphs. Treatments with the same letter represent no significant difference, at $P < 0.05$, based on Tukey HSD tests after analysis using one-way ANOVA.

Table 5. 1: Results from Chi-square to detect deviation from an expected sex ratio at 1 : 1 (control) for mosquito adult where eggs were collected from field experiments with varying predator treatments. Eggs were then hatched in two water conditions; (A) hatched in water without predators or kairomones. (B) hatched in water with the presence of predators or kairomones. All treatments were compared with control. *No data were gained from treatments with traces of free-roaming predators, predators in cages with three and nine predators, and kairomones from nine predators as none of the larvae reached adult stage. Sex ratio is between male: female. Significant values are in bold at $P < 0.05$, $df = 1$.

Water conditions	Subjects	Control	1 Free-Roaming Predator	3 Free-Roaming Predators	9 Free-Roaming Predators	1 Caged Predator	3 Caged Predator	9 Caged Predator	Kairomones (from 1 predator)	Kairomones (from 3 predators)	Kairomones (from 9 predators)	Empty cage
Without predator or its kairomones remnant	Sex ratio	1 : 1	1 : 1	1 : 1	1 : 1	1 : 0.5	1 : 0.93	1 : 1.12	1 : 0.78	1 : 0.66	1 : 0.68	1 : 0.78
	χ^2	-	0.002	0.42	0.000	1.930	0.11	1.095	3.616	8.718	0.780	0.460
With the presence of predators or its kairomones remnant	Sig. value	-	0.965	0.000	0.986	0.165	0.73	0.295	0.057	0.003	0.182	0.498
							4	6				
With the presence of predators or its kairomones remnant	Sex ratio	1 : 1	*	*	*	*	1 : 3	*	1 : 0	1 : 1	*	1 : 1
	χ^2	-					16.4		0.992	1.75		1.29
	Sig. value	-					28		0.319	2.462		0.460
							0.00			0.117		0.498

Overall, survival probabilities varied significantly between treatments, as revealed by log-rank test ($P < 0.05$, Table 5.2). Surprisingly, significant differences were observed in mosquito survival when the eggs were deposited in treatment containers in the field and hatched in water without the traces of predators or its kairomones (Table 5.2 and Figure 5.4). The time to death was significantly shorter in all nine treatments which were less than 48 days as performed in the control treatment (Figure 5.4). For example, for eggs collected from the one free-roaming predator treatment, the overall survival time of mosquitoes from eggs until adult death was shorter and died faster than control mosquitoes. It appears that survival rates and survival times were reduced even when the eggs were only exposed briefly to predators or kairomones, and spent the remainder of their life stages without contact with predators or kairomones.

(A) Progeny reared in the absence of predators or kairomones

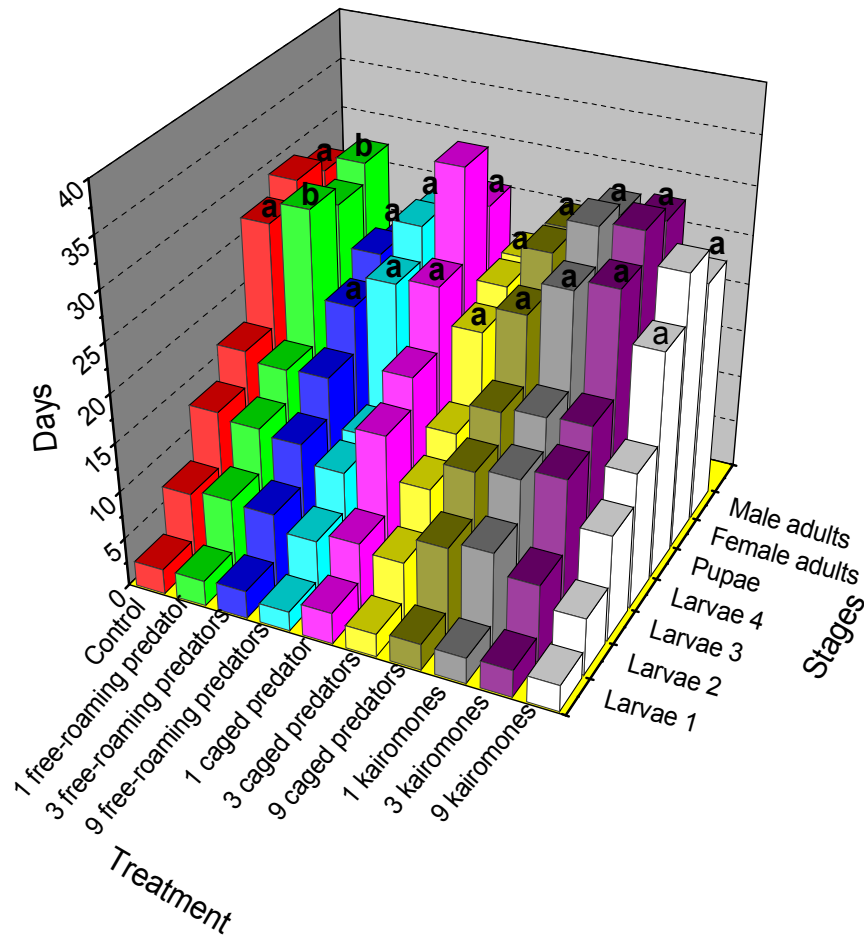


Figure 5.2: The development time needed by eggs that were exposed to the predators or kairomones in the field. Progeny was then reared in the absence of predators and sorted into stage-specific treatments (4 larval instars, pupae, and male or female adults). Treatments with the same letter represent no significant difference, at $P < 0.05$, based on Tukey HSD tests, after analysis using MANOVA.

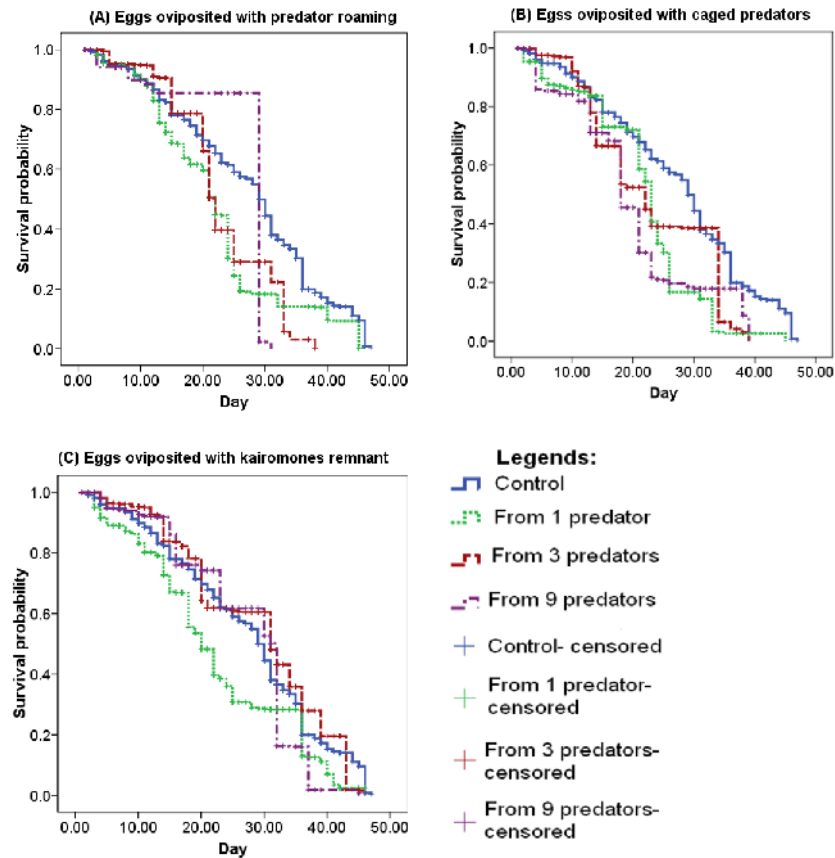


Figure 5.3: The influence of the presence of predators at the time of oviposition by adult mosquitoes on the survival rate of developing mosquitoes. Eggs were collected from treatments that (A) had predators' present free-roaming or (B) in cages, (C) from the treatment with kairomones, and from the control treatment where no predators or their traces were present (survival in the control treatment is represented by the same line on all three graphs). These eggs were then transferred into clean water and monitored from egg hatch until adult mosquito death.

Table 5.2: Pairwise comparison using log-rank test in order to test the differences between survival rates of treatments in at oviposition. Eggs were collected from field experiments with varying predator treatments. (free-roaming predators, caged predators, etc). These eggs were then and hatched in water without predators or kairomones. Significant values are in bold at $P < 0.05$. Values in square brackets are Chi-Square values from the log rank test.

Treatment	Control	1 Free-Roaming Predator	3 Free-Roaming Predators	9 Free-Roaming Predators	1 Caged Predator	3 Caged Predators	9 Caged Predators	Kairomones (from 1 predator)	Kairomones (from 3 predators)	Kairomones (from 9 predators)
Control	-	[417.3]	[65.69]	[19.53]	[528.88]	[90.90]	[561.64]	[474.75]	[40.57]	[0.028]
1 Free-Roaming Predator	<0.001	-	[21.02]	[43.46]						
3 Free-Roaming Predators	<0.001	<0.001	-	[15.33]						
9 Free-Roaming Predators	<0.001	<0.001	0.02	-						
1 Caged Predator	<0.001				-	[83.76]	[46.37]			
3 Caged Predators	<0.001				<0.001	-	[83.23]			
9 Caged Predators	<0.001				<0.001	<0.001	-			
Kairomones (from 1 predator)	<0.001							-	[557.49]	[257.96]
Kairomones (from 3 predators)	<0.001							<0.001	-	[51.71]
Kairomones (from 9 predators)	<0.001							<0.001	<0.001	-

5.4.2 Life-history influence of predators throughout mosquito life history

In this experiment I examined the direct influence of *A. wakefieldi* on mosquitoes from oviposition until adult death, with treatments in which the predators were maintained with the mosquito larvae. I examined egg hatching, mosquito development and survival rates after eggs were collected from a field experiment with nine different treatments (with free roaming predators, predators in cages, etc), and reared to adult stage in the laboratory in water subject to the same predator treatments.

The mean percentage of eggs that hatched ranged between 0 - 92.74%. The one-way ANOVA results showed a significant difference in egg hatching rates between treatments ($F = 139.09$, $df = 10$, $P < 0.01$). In the treatments with three and nine free-roaming *A. wakefieldi*, no eggs hatched, the predators attacked and killed all eggs in these treatments. Additionally, no eggs hatched in the treatments with nine caged predators, or the treatment with kairomones of nine predators (with no predators present) (Figure 5.1B). Only a small percentage of eggs lived to hatch in the treatment with one predator. The post-hoc analysis revealed egg hatching rates in the other treatments tested were not significantly different from the control treatment with no predators or kairomones ($P \geq 0.05$). When larvae were reared in water with the presence of predators, predators in cages or kairomones, few or no larvae survived until eclosion. Several treatments had low numbers of adults, resulting in difficulties analyzing aspects such as

mosquito sex ratio. The emerging adults in three caged predator treatments were at ratio of 1 male: 3 females. This ratio was statistically significant when compared to control treatment at ratio of 1:1 ($\chi^2 = 16.428$, $df = 1$, $P = 0.000$; Table 5.1).

The availability of data for developmental times for each life-stage of *Cx. pervigilans* were similarly affected by the predator treatments, with no information available from the treatments with three and nine free-roaming predators, one and nine predators in cages and kairomones from nine predators. Only 33 larvae hatched from 248 eggs in treatments with single free-roaming predators, but these larvae failed to grow into second instars as they were all consumed by the predator. The post-hoc test from the MANOVA revealed that all stage-specific growth rates for mosquitoes in the three caged predator treatments were significantly shorter from the control with no predators present ($P > 0.05$), and 4th instar larvae failed to grow to pupae stage perhaps due to the effect of kairomones (Figure 5.3). The development time to reach each life stage was shorter, but not significantly so, in the treatments with kairomones with one or three predators in comparison with the control with no predators ($P < 0.05$). The presence of an empty cage in the containers had no effect on development time of the mosquitoes at any development stage ($P > 0.05$).

The survival analysis indicated that when the eggs were reared in water with the presence of predators or its kairomones remnant, all treatments tested were significantly different ($P < 0.001$, Table 5.3). All of treatments tested showed that the time to adult death was faster than in the

control treatment (Figure 5.5). No survival curves were plotted for the free roaming predator treatments with three and nine predators, caged predator treatments with one and nine predators, and kairomone only treatments from nine predators, because all *C. pervigilans* eggs failed to hatch in these treatments (Figure 5.5). In the treatment with one free roaming predator, all of the first instar larvae were eaten by the predators on the first day of treatment after being placed in water with predators (Figure 5. 5A). The presence of an empty cage showed no significant effect on *Cx. pervigilans* mosquito survival rate when compared with the control treatment ($\alpha = 0.01$, $P = 0.92$, Table 5.3; Figure 5.5B).

(B) Progeny reared in the presence of predators and kairomones

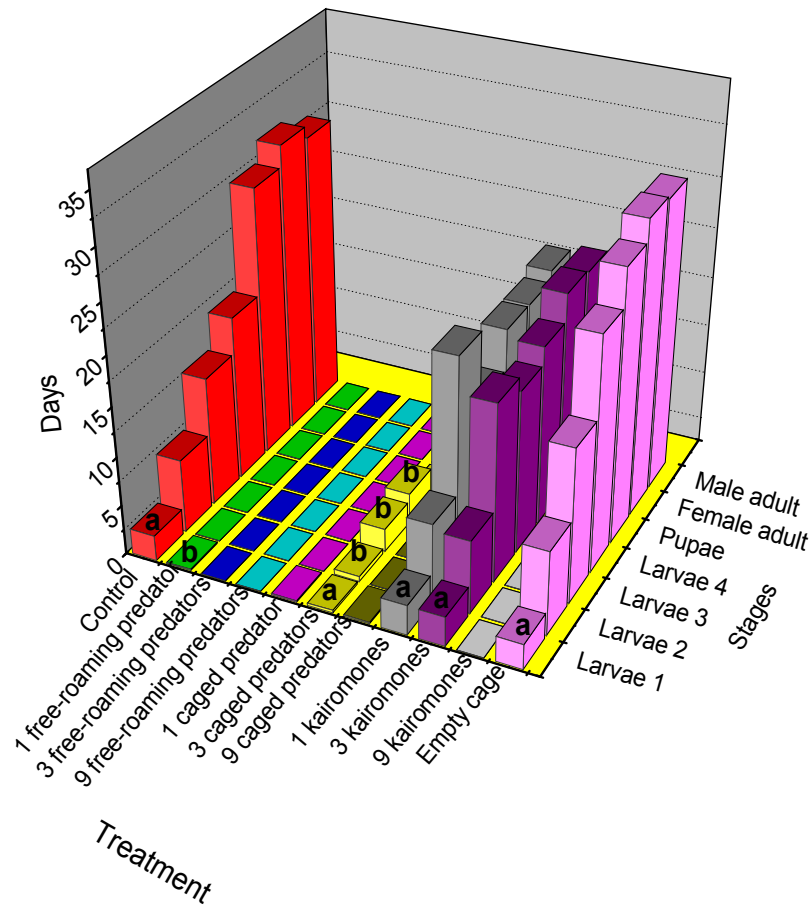


Figure 5.4: The influence of direct exposure to the presence of predators or kairomones on development time of stage-specific progeny (4 larval instars, pupae, and male or female adults) in ten different treatments (control, free-roaming predator, etc.). Treatments with the same letter represent no significant difference, at $P < 0.05$, based on Tukey HSD tests, after analysis with MANOVA. Some points are omitted due to insufficient data.

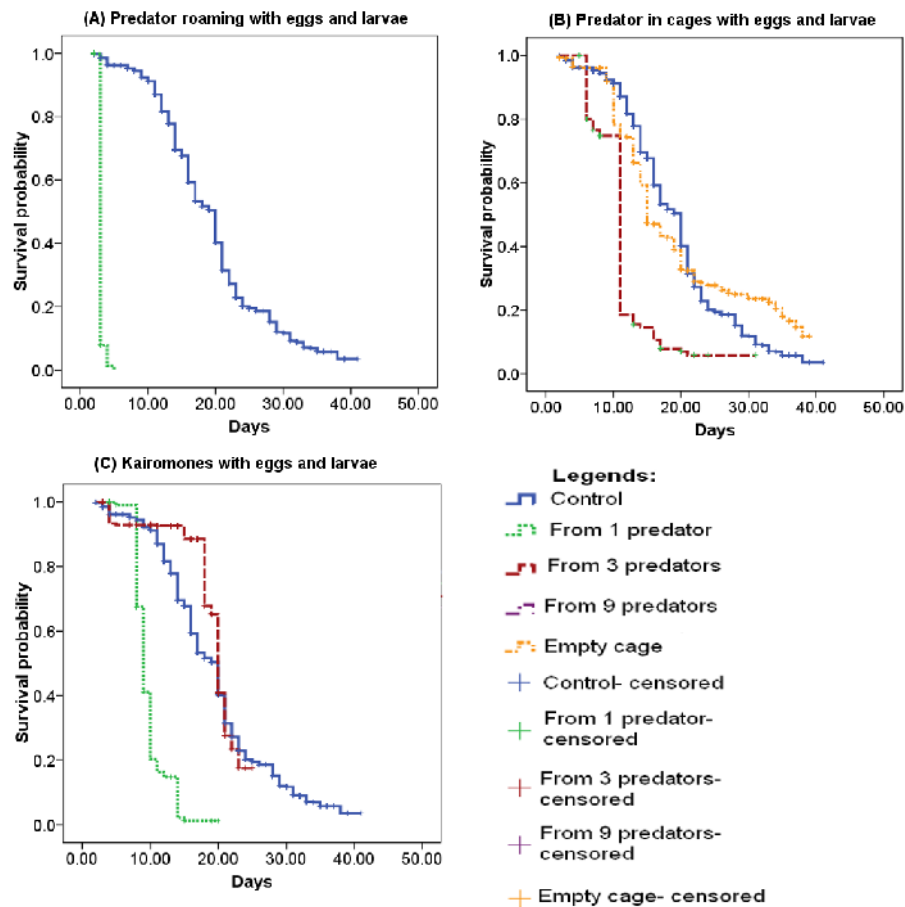


Figure 5.5: Survival rate of developing *Cx. pervigilans* mosquito progeny until adult death after eggs were hatched and larvae were reared in the water with the presence of predators or kairomones. Graphs represents survival rate that had (A) predators' present free-roaming, (B) caged predator (C) kairomones remnant from predators, and from the control treatments with the absence of predators or kairomones. (Survival in the control treatment is represented by the same line on all three graphs).

Table 5.3: Log-rank test results to test the differences of survival rates after eggs were collected from field experiments with varying predator treatments (free-roaming predators, caged predators, etc). These eggs were then reared in the laboratory with the same predator treatments as in the field. Significant values are in bold at $P < 0.05$. Values in square brackets are Chi-Square values from the log rank test.

Treatment	Control	1 Free - Roaming Predator	3 Free - Roaming Predators	9 Free - Roaming Predators	1 Caged Predator	3 Caged Predators	9 Caged Predators	Empty Cage	Kairomones (from 1 predator)	Kairomones (from 3 predators)	Kairomones (from 9 predators)
Control	-	[3106.24]	[5227.48]	[5105.29]	[5042.93]	[538.22]	[5231.97]	[0.01]	[958.41]	[18.49]	[5022.36]
1 Free- Roaming Predator	<0.001	-	[483.00]	[435.00]	-	-	-	-	-	-	-
3 Free- Roaming Predators	<0.001	<0.001	-	-	-	-	-	-	-	-	-
9 Free- Roaming Predators	<0.001	<0.001	-	-	-	-	-	-	-	-	-
1 Caged Predator	<0.001	-	-	-	-	[888.00]	-	[1712.65]	-	-	-
3 Caged Predators	<0.001	-	-	-	<0.001	-	[958.00]	[200.15]	-	-	-
9 Caged Predators	<0.001	-	-	-	-	<0.001	-	[1807.92]	-	-	-
Empty Cage	0.92	-	-	-	<0.001	<0.001	<0.001	-	-	-	-
Kairomones (from 1 predator)	<0.001	-	-	-	-	-	-	-	-	[493.03]	[720.00]
Kairomones (from 3 predators)	<0.001	-	-	-	-	-	-	-	<0.001	-	[737.00]
Kairomones (from 9 predators)	<0.001	-	-	-	-	-	-	-	<0.001	<0.001	-

5.5 DISCUSSION

The predators that I examined had a lethal effect, and dramatic sub-lethal effect on the survival rate of the mosquitoes, even when only the egg stage was briefly exposed to predators or kairomones. Even though the eggs were exposed only to water containing predators or kairomones in the field and were then reared in clean water without any traces afterward, sub-lethal effects still continued in the remaining survivors. My study demonstrated a significant reduction in the survival rates and survival time as a result of exposure to predators or their chemical cues. These results were observed when adult mosquitoes were exposed to predators at the time of oviposition and when the predators or kairomones were present throughout the life history of the mosquito.

Other studies have demonstrated major changes in life-history after only a short exposure to predators. For example, within 3 - 5 weeks of exposure to chemical cues released from predatory fish *Daphnia lumholtzi* formed rigid head spines as a defense system against these predators (Laforsch and Beccara 2006, Engel and Tollrian 2009). Inducible defense tends to be a trade-off with life history as an adaptation to heterogeneity in predation risk (Tollrian 1995). Chiver et al. (2001) also noted a change in the life-history of frogs as a result of exposure to predator stimuli. Chemical cues from leech predators demonstrated that injured Pacific tree-frog eggs tended to hatch earlier, resulting in smaller and less developed individuals (Chiver et al. 2001). Sih and Moore (1993) found that salamander eggs delayed their hatching response when exposed to chemical cues from predators. The sub-

lethal effects of predation can be seen in changes on metamorphosis time and size for a variety of other animals (See Werner 1991, Lounibos et al. 1993, Perkarsky et al. 1993, Ball and Baker 1996, Hetchel and Juliano 1997).

The sub-lethal effects of predators on *Cx. pervigilans* were carried by the progeny throughout their whole life history and appeared to be triggered by the predator's chemical cues (kairomones) without any direct contact with the actual predators. It is likely that an egg embryo that is exposed to stressful stimuli from predation during development may cause this change in post-embryonic physiology or behavior. Early warning of predators through chemical cues, without direct contact with predators, can benefit the prey by detecting an early risk of predation (Lima and Dill 1990). Herper (1996) suggested that the embryo's learning traits have been primarily associated with maternal and/or auditory recognition. The anxiety-like behavior in mothers which receive stressful stimuli is passed down to the embryo (Kats and Dill 1998) and long lasting disturbances may persist throughout adulthood (Dugovic et al. 1999). Exposure to predator stimuli experienced by *Ambystoma annulatum* (ringed salamander) embryos later appeared to influence larvae by reducing activity and provoking greater shelter-seeking behavior as an anti-predator strategy (Manthis et al. 2003; 2008). But without the reinforcement of risk, the embryo learned to recognize stimuli of predator as non-threatening agent (Ferrari and Chiver 2008).

Adult females of some mosquito species appear able to detect and actively avoid ovipositing in water containing predators or kairomones

(Blaustein 1988, Blaustein et al. 1995, Kiflawi et al. 2003a; 2003b). This avoidance does suggest mosquitoes can interpret stressful stimuli that may be passed down to their offspring, as suggested by Kats and Dill (1990). However, my previous work has found no evidence that *Cx. pervigilans* can detect *A. wakefieldi* when making a choice where to lay their eggs (Zuharah and Lester 2010). While it is still possible that those adult female mosquitoes in some way indicate stress to their offspring it seems more likely that the developing eggs or larvae directly interpreted the presence of a predator through chemical cues. This detection appears to result in stress for the larvae and in shorter life histories. Trade-offs in life history characteristics often results in a change in survival and fitness (Calow 1973, Cowl and Covich 1990). Such threat-sensitive learning may allow *Cx. pervigilans* to optimize the trade-off between anti-predator behaviors by enabling individuals to reach earlier maturity. However, I did not observe significantly shorter larval periods for the mosquitoes in this study except when the larval were exposed in treatment with three caged predators. These results indicate that while the mosquitoes detected predators, this detection may have resulted primarily in stress rather than a change in life history. The stress may have come about through anti-predatory behavior such as reduced feeding activity (Peckarsky 1996, Moses and Sih 1998). This potential mechanism would be consistent with the significantly reduced survivorship of mosquitoes in kairomone only treatments. In subsequent experiments I have observed that these mosquitoes do change their behavior in the presence of predators, appearing to feed less and exhibiting

more resting behavior associated with escape from predators (Zuharah and Lester, In Press B).

The change in survival rate of mosquitoes as a consequence of exposure to predators only at the egg stage could be consistent with epigenetic effects. Epigenetic effects are observed as changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence, and is thought to be triggered by environmental effects during development (Jaenisch and Bird 2003). DNA methylation is a central mechanism of epigenetic regulation, which has been studied in mosquito species (Marhold et al. 2004). Whether or not epigenetic effects are responsible for my results remains unknown, but are clearly one potential mechanism worthy of further investigation.

The sex ratio of mosquitoes is usually approximately equal between males and females in natural environment (Sweeney and Barr 1977). In my study the sex ratio between male and female of *Cx. pervigilans* in some treatment were statistically different; even when *Cx. pervigilans* was only exposed to *A. wakefieldi* predation at oviposition stage. However, Hagman and Shine (2006) found that the sex ratio of emerging mosquitoes was not affected by invasive cane tadpoles. Female mosquitoes in other studies prolonged their developmental time (Banks and Thompson 1987, Stav et al. 2007). More male mosquitoes were emerged compared to female mosquitoes in my study. The results suggested that the stress induced in

mosquitoes in this study is likely to influence female mosquito more than male mosquito.

I suspect that we are only beginning to understand the complex ways in which predators can have a dramatic sub-lethal effect on prey populations. Empirical studies examining predator-induced changes in hatching characteristics and the subsequent effects on juveniles and adults are rare. This study may have implications for the dynamics of other predator-prey systems. We may expect to observe plasticity in species or populations with high temporal or spatial variability in predation.

CHAPTER SIX:

General Discussion

This study has made a considerable contribution towards understand the role played by an aquatic predator in shaping endemic and exotic mosquito populations dynamics in New Zealand. In particular, this study has made four specific contributions: (1) Chapter two described the association between predator and mosquito occurrence and abundance in water troughs. Using logistic regression, I investigated factors such as environmental variables and water volume that may influence the predator-prey system; (2) Chapter three described the ability of a common predator to limit the abundance of endemic and exotic mosquitoes based on two hypotheses: “escape from natural enemies” and “threat sensitivity”; (3) Chapter four examined the sub-lethal effects of predators on adult mosquitoes in selecting oviposition habitats; (4) Chapter five expanded the study of sub-lethal effects on mosquito progeny after eggs were taken from the field experiment in Chapter four, and reared either with or without predators. This chapter suggested that the presence of *A. wakefieldi* has a substantial impact on survival rates of *Cx. pervigilans* progeny, even when the eggs were only briefly exposed to water containing predators or kairomones and then reared in clean water without any traces of the predators.

In this chapter, I will discuss the links between these empirical studies by examining the lethal and sub-lethal effects exhibited by mosquitoes in response to predators, as examined in the previous chapters.

6.1 Do predators influence mosquito populations?

Predators are known to have substantial impacts on the community structure, distribution and diversity of their prey. Predators may drive prey populations to extinction, reduce the prey population, or even co-exist with a wide range of prey species. However, few studies have been undertaken relating to mosquito population dynamics in response to the presence of a predator.

The “escape from natural enemies” hypothesis is one of the better known explanations as to the successful establishment of non-native species, and reasons for their enhanced growth and higher rate of reproduction in a new habitat range (Elton 1958, Blossey and Notzold 1995, Maron and Vilá 2001). My research has found evidence that predators do influence mosquito populations, but my results did not support the “escape from natural enemies” hypothesis specifically. I found that the native predator preferentially consumed the exotic species, *Ae. notoscriptus*, over the endemic species *Cx. pervigilans*. This behavior may have limited the invasion and subsequently reduced numbers of this exotic mosquito species in New Zealand. I suggested that when this exotic mosquito coexists with the native predator and enters the food web, this prey is more susceptible to attack because of non-adaptive changes in its behavior. This exotic mosquito species is essentially naïve to New Zealand predators. The predator appeared to preferentially consume the exotic mosquito species, but they did also respond to the endemic mosquito species. When only the endemic

species is present the predator clearly showed that it can control and reduce the number of endemic mosquitoes. The occurrence and abundance of mosquitoes in field populations may be associated with the abundance of predators. With the presence of only nine *A. wakefieldi* predators and 70 *Cx. pervigilans* prey in a water trough, all prey were consumed within two days. Similar ratios of predators to prey are commonly observed in field populations.

I also found evidence that the presence of predators can have dramatic consequences for prey communities, not only by the direct effects of consumption but also through sub-lethal effects. I initially hypothesized that the extent to which mosquito populations are influenced by predation may depend on other mechanisms such as an oviposition response in adult mosquitoes, behavioral responses of prey when facing a predator, and sub-lethal effect of predators on prey progeny.

6.2 What are the major mechanisms by which predators influence mosquito populations?

In this thesis, I investigated several different mechanisms of lethal and sub-lethal effects of predation that may influence mosquito populations in New Zealand. My results suggest that direct consumption by *A. wakefieldi* is the primary effect of this predator on mosquito populations. The functional response describes the relationship between prey density and a predator's consumption rate. It is a key factor for understanding the population

dynamics of predator-prey systems. However, the abundance of mosquitoes in the environment is also affected by the abundance of predators, environmental factors, and water volume (Chapters 2 & 3).

In aquatic environments predators may coexist in the same habitat with more than one prey species. In the presence of more than one prey species, predators preferentially consumed the exotic mosquito, *Ae. notoscriptus*, exhibiting a higher attack rate towards this species (Chapter 2). Observations show that one *A. wakefieldi* may consume an average of 31 *Ae. notoscriptus* larvae, but only 18 *Cx. pervigilans* larvae per day. The differential consumption of various mosquito species by predators has been shown to allow coexistence of different mosquito species (Griswold and Lounibos 2005).

The ability of mosquito adults to detect the presence of predators while searching for oviposition sites also affects the abundance of mosquito species in the environment (Spencer et al. 2002). Mosquitoes may have the ability to detect the presence of predators or competitors based on chemical or physical cues when choosing oviposition sites (Stav et al. 1999). In my study, I found no evidence that *Cx. pervigilans* modifies its oviposition behaviour in response to predators (Chapter 4). There are two possible reasons for this result; either *Cx. pervigilans* ignored the presence of the predator because the predator does not pose a threat to mosquitoes, or the cues from *Anisops* predators do not provoke alarm, or were not sufficiently strong enough to provoke alarm in these mosquitoes. However, other mosquito species such as *Culiseta longireolata* can detect chemicals from *Notonecta* predators in lower numbers than those were examined (Blaustein

et al. 2004). It seems likely that the adult mosquitoes were unable to detect the presence of predators. This inability to detect predators by *Cx. pervigilans* adults while choosing oviposition sites exposes their progeny to significant predation risk and is reflected in the low survival rate of larvae, as observed in chapter five of this thesis.

While adults appeared unable to detect predators, their offspring exhibited substantial sub-lethal effects in response to the presence of predators. The survival rate of *Cx. pervigilans* was significantly reduced by the presence of predators and kairomones, even when the eggs were exposed only briefly to water containing either predators or kairomones in the field, and were then reared in clean water without any traces (Chapter 5). It is possible that adult female *Cx. pervigilans* in some way indicate stress to their offspring after exposure to predators. However, it seems more likely that the developing eggs or larvae directly interpreted the presence of a predator through chemical cues. Trade-offs in life history characteristics often results in a change in survival and fitness (Calow 1973, Crowl and Covich 1990). In experiments in Chapter three, I observed that these *Cx. pervigilans* mosquitoes do change their behavior in the presence of predators; they appeared to feed less and exhibited more of the resting behaviour associated with escape from predators. However, this behaviour did influence the mosquito's life history by influencing the development time between life stages (Chapter 5).

Predators such as *A. wakefieldi* are voracious predators. They clearly do attack and kill exotic mosquito species. They probably have a role in limiting the invasion of new mosquito species in New Zealand.

6.3 The variable role of sub-lethal effects of predators on mosquito populations.

Sub-lethal effects of predators can have impacts on prey in many different ways; such as influencing feeding, growth rates, competitive ability, oviposition behaviour, movement rates, etc. In response to predation risk, prey will frequently adjust their behavior accordingly (Sih 1987). In the presence of cutthroat trout fish odours, mayflies (*Baetis bicaudatus*) showed sub-lethal effects by reducing drifting behavior (McIntosh et al. 1999, McIntosh et al. 2000, McIntosh and Peckarsky 2004). This behavior reduced their entry rate to the water column which reduced the possibility of being taken by the predator. Alteration in the feeding rate and diet composition in damselfly larvae is another example of sub-lethal effects of *Notonecta* and fish predators (Koperski 1997). This behavior is a defense against predators that may be able to detect the presence of prey by water movement. All of these sub-lethal effects of predators can have a major role in influencing mosquito populations.

In contrast to other mosquitoes, I did not observe *A. wakefieldi* to have a sub-lethal effect on adult *Cx. pervigilans*, wherein oviposition behavior is changed. But clearly, substantial sub-lethal effects occurred for eggs and

larval stages. If *Cx. pervigilans* larvae escaped the lethal effects of the predator, the remaining prey still continued to display sub-lethal responses. *Culex pervigilans* clearly responded to predators by exhibiting more resting behavior and decreasing their movement. This behavior is known as the safest way to avoid possible threat from predators. However, this behavior may result in reduced food intake by prey. The observed increase in larval mortality, delay in developmental times, and a reduction in body size are likely to be caused by a decrease in foraging activity which results in food limitation (Beketov and Liess 2007). In my study, the decrease in food foraging activity decreases the survival rate of progeny. The threat of predation is often sufficient to modify prey populations. In this study I suggested that food scarcity and stress stimuli passing through the embryo during development both cause a decrease in survival. Lima and Dill (1990) suggested that even without direct contact from predators, prey can still detect an early risk of predation through chemical traces left by predators.

6.4 Future research

I suspect that we only beginning to understand the complex ways in which predators can influence mosquito population dynamics. This study may have implications for the dynamics of many predator-prey systems. However, I have several suggestions to improve or enhance similar studies in the future. In chapter two, I examined the influences of a predator on mosquito populations. The result clearly showed that *A. wakefieldi* are able

to consume and control mosquito populations in water troughs. However, the number of predators and mosquito larvae were greater in 2007/08 than in 2008/09. What is the factor that may cause the changing in abundance between years? I suspect that changes in environmental factors such as temperature are the main drivers that may limit populations, even though I observed no such correlation during my sampling periods. Overwintering mortality may be quite variable in different years.

The interaction between mosquito populations and predators in water troughs needs to be tested more widely in other locations, and should extend to warmer areas such as the northern North Island. The warmer climate, large human population, and larger seaport and airport are potential factors in increasing the numbers of mosquito invasions that could potentially cause mosquito-borne disease outbreaks in New Zealand (Laird 1990, Laird 1995, Weinstein et al. 1997).

In Chapter three, more exotic species than endemic species were consumed by *A. wakefieldi*. Both were Type II functional responses. In nature, predator-prey populations do not consist of only one predator and one prey species. The observed functional response may change in the presence of alternative prey. Several studies have shown that the Type II functional response is not stable and does not allow long-term population persistence, while a type III response is more demographically stabilizing (Murdoch and Oaten 1975, Hassell and Comins 1978, Pech et al. 1992). It is important that future work on functional responses involve mixed prey species and several predator species. This more natural study system would

enable us to have a better understanding of the persistence of predator-prey systems in the natural environment.

In the situations where predators do not result in the death of prey by lethal effects, sub-lethal effects may positively or negatively affect behavior, growth rate, or life history traits of prey species. My study has confirmed that the presence of *A. wakefieldi* has sub-lethal effects on mosquito larvae (Chapter 5). This behavior was typified by an alteration in feeding behavior and resulted in a decreasing number of prey surviving to the next generation even when eggs were exposed only briefly to the predator's kairomones and then reared in clean water without any traces of the predator. However, it is possible that the sub-lethal effects may still continue in the second and third generation of mosquitoes, which is worthy of examination in the future. However, the sub-lethal effect on adult longevity is particularly important. By knowing the lifespan of adults we can easily control mosquito populations.

A better understanding of various mosquito species' response to predators and environmental factors will allow us to make more accurate predictions of the population level outcomes of mosquito invasions.

APPENDICES

APPENDIX A:

**Abstract from The 25th Annual Meeting of The Society
of Population Ecology**

個体群生態学会
第 25 回年次大会
プログラム・講演要旨集

Program and Abstracts
The 25th Annual Meeting
The Society of Population Ecology

2009 年 10 月 17 日 (土)・18 日 (日)

同志社大学寒梅館 (京都市上京区)

October 17-18, 2009

Kanbai-kan, Doshisha University (Kamigyo-ku, Kyoto)

個体群生態学会
第 25 回年次大会実行委員会

Can *Anisops* predators (Backswimmer) reducing the population abundance of New Zealand mosquito?

○Wan Fatma Zuharah (School of Biological Sciences, Victoria University of Wellington) · Phil Lester

The influence of these predators on mosquito populations may be direct, through predation, or indirectly through sub-lethal responses of adult mosquitoes in life history traits like oviposition behavior. *Anisops wakefieldi* are a common predator of mosquito larvae and can be found in temporary and permanent water bodies in New Zealand. We established temporary containers with four types of treatments in order to study the effects of *Anisops* predator on mosquito population: 1) Control, 2) Free roaming *Anisops* (with one, three and nine *Anisops*), 3) Caged *Anisops* (with one, three and nine *Anisops* in caged and empty cage), 4) *Anisops* kairomone (with kairomone concentration of one, three and nine *Anisops* in present in the water for 24 h prior to the experiment, but removed at the start of each trial). We predicted that in the presence of *Anisops wakefieldi* or its kairomone, fewer mosquito eggs would be oviposited. Contrary to our predictions, *Cx. pervigilans* appeared to ignore the presence of free roaming *Anisops*, caged *Anisops*, and water with *Anisops* kairomone when choosing their oviposition sites. As a result, no significant differences were detected between treatments ($F=1.389$, $df=10$, $p=0.284$) and the presence of *Anisops* did not affect the mosquitoes' oviposition behaviour. If there were no effects of the predator's presence on oviposition behavior by mosquito, the variation in the abundance of mosquitoes in pools may be primarily driven by the lethal effects of this predator. We also conducted a field survey using water troughs at farming areas in Queens Elizabeth II Park, Waikanae, New Zealand. We attempted to tease apart the relationship between *Anisops* predator, mosquito population abundance, water depth and environmental variables; temperature, wind speed, rainfall, humidity, and pressure. We have predicted that the presence of *Anisops* predator may be significantly correlated with the presence of mosquito. The abundance of *Anisops* predators in troughs was not correlated to the abundance of mosquito ($r=-0.052$, $p=0.454$) or other environmental variables. But, their predator presence had a positive correlation with the water depth ($r=0.176$, $p<0.05$). The presence of mosquito larvae in water troughs was correlated to water depth ($r=-0.159$, $p=0.022$) and wind speed ($r=0.142$, $p=0.041$) but not other environmental variables. We conclude that the existence of *Anisops* predator has little role in controlling the population abundance of mosquitoes in New Zealand.

APPENDIX B:

**Abstract from The 58th Annual Conference of the
Entomological Society of New Zealand**



***The 58th Annual Conference of
the Entomological Society of
New Zealand***

"Islands and Hotspots"

April 5 - 8th 2009

**Tamaki Campus,
University of Auckland**

✓ Can adults of the mosquito *Culex pervigilans* detect the presence of a key predator in larval habitats?

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Anisops wakefieldi are a common predator of mosquitoes and can be found in temporary and permanent water bodies. The influence of these predators on mosquito populations may be direct, through predation, or indirectly through sublethal responses of adult mosquitoes in life history traits like oviposition behaviour. We established temporary containers with four types of treatments: 1) Control, 2) Free roaming *Anisops* (with one, three and nine *Anisops*), 3) Caged *Anisops* (with one, three and nine *Anisops* in caged), 4) *Anisops kairomone* (with kairomone concentration of one, three and nine *Anisops* in present in the water for 24 h prior to the experiment, but removed at the start of each trial). We predicted that the New Zealand native mosquito, *Culex pervigilans*, whose larvae are highly vulnerable to predation of *Anisops wakefieldi*, would likely to avoid the containers with the presence of *Anisops* or its kairomone. Contrary to our predictions, *C. pervigilans* appeared to ignore the presence of free roaming *Anisops*, caged *Anisops* and water with *Anisops kairomone*. As a result, no significant differences were detected between treatments. We also predicted that *C. pervigilans* would be able to discriminate *Anisops* densities when choosing oviposition sites. The experimental evidence indicates that the number of egg rafts was not significantly affected by the density of predators. It appears that *C. pervigilans* are unable to determine the presence or absence of *Anisops* predators while ovipositing. Variation in the abundance of mosquitoes in pools appears to be primarily driven by the lethal effects of this predator on *C. pervigilans*.

APPENDIX C:

Zuharah WF and Lester PJ (2010) Can adult of New Zealand mosquito *Culex pervigilans* (Bergorth) detect the presence of a key predator in larval habitat?.

Journal of Vector Ecology. 35(1) (In Press)

Can adults of the New Zealand mosquito *Culex pervigilans* (Bergorth) detect the presence of a key predator in larval habitats?

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ABSTRACT: The influence of predators on mosquito populations may be direct through predation or indirect through sub-lethal responses of adult mosquitoes in life history traits such as oviposition behavior. In New Zealand, the backswimmer, *Anisops wakefieldi*, is a common predator of mosquito larvae found in temporary and permanent water bodies. We predicted that the New Zealand native mosquito, *Culex pervigilans*, whose larvae are vulnerable to predation of *Anisops*, would likely avoid the containers with the presence of *Anisops* or its kairomone. We established temporary water containers without predators, free-roaming predators, caged predators (which were unable to eat mosquitoes), or containers from which predators were removed immediately prior to the experiment (these containers would have remnant kairomones from the predators). Each treatment with *Anisops* had predator densities of one, three, or nine *Anisops*. Contrary to our predictions, when choosing oviposition habitats, *Cx. pervigilans* appeared to ignore the presence of free-roaming *Anisops*, caged *Anisops*, and water with *Anisops* kairomone. We thus observed no significant differences between the numbers of egg rafts laid by *Cx. pervigilans* in the different predator treatments nor were the number of egg rafts significantly affected by the density of predators. Rather than the presence of predators, environmental factors including temperature, humidity, and pressure were significantly correlated with mosquito oviposition. These mosquitoes appeared to either ignore the presence of the predator, had no ability to detect the presence of predators, or perhaps the cues from *Anisops* predators were not sufficiently strong enough to alarm these mosquitoes. We argue that the mosquito has not evolved the ability to detect the presence of these predators while ovipositing. *Journal of Vector Ecology* 35 (1): 100-105. 2010.

Keyword Index: Predation, oviposition, predator density, kairomone, environmental factors.

INTRODUCTION

The survival and growth of organisms that lack parental care is thought to be strongly dependent on the quality of habitats into which they are deposited. Adults of insects with juveniles that are incapable of migrating away from low quality habitat should evolve to select oviposition sites that improve the likelihood of the survival and growth of their offspring (Kiflawi et al. 2003a, Blaustein et al. 2004, Bond et al. 2005). Thus, a female mosquito should be able to detect and avoid ovipositing in sites which have a high risk of predation for their eggs or juvenile stages. Mosquitoes also compete with one another and should selectively oviposit to minimize inter- or intra-specific competition. Blaustein et al. (2004) suggested that five factors have shaped the ability of mosquitoes to respond to the risk of predation during oviposition: 1) mosquito larvae are highly vulnerable to predation, 2) they have only one or a few lifetime reproductive events, 3) their eggs are often laid together, 4) their predators have highly heterogeneous distributions, and finally, 5) the distribution of predators is often highly fixed from the time of the prey oviposition event until prey progeny can either leave the patch or become large enough to better avoid predation. Ideally, mosquitoes may have an ability to detect the presence of predators or competitors based on chemical or physical cues released (Stav et al.

1999).

Wisenden (2000) showed that chemical cues are released by the predator during detection, attack, capture, and ingestion of prey. Prey may use these cues to minimize their exposure to predators. Several species of mosquitoes have been shown to be able to detect the presence of predatory notonectid bugs and avoid ovipositing in pools containing these predators. In the presence of only one predatory backswimmer (*Notonecta maculata* Fabricious) in 10 liters of water, *Culiseta longirealata* (Macquart) oviposition was strongly reduced (Blaustein 1998, Blaustein et al. 1995, Kiflawi et al. 2003a, Kiflawi et al. 2003b). Eitam and Blaustein (2004) found that *Cs. longiareolata* and *Cx. laticinctus* oviposited less in pools containing predators, but this avoidance of predators was unrelated to predator densities. Their work was also supported by Blaustein et al. (2005), who found that *Culex* mosquitoes strongly avoided ovipositing in pools containing the *Notonecta* predators. Mosquitoes continued to avoid ovipositing in pools with predators for two additional days after the predator was removed, suggesting a predator-released kairomone was the cue used by mosquitoes to detect the presence of predators.

Stav et al. (1999) suggested that the distribution and abundance of a mosquito species is dependent on their ability to selectively oviposit according to predator abundance. Mosquito oviposition may be affected by predator densities

because gravid females may detect their densities and assess the severity of predation risk. With increasing predator densities, concentrations of kairomone are also increased and may result in reduced oviposition (Eitam and Blaustein 2004). Reduced oviposition by mosquitoes may be expected with increasing numbers of predators, due to the ability of these prey species to detect the presence of a predator above a threshold density (Angelon and Petranka 2002) or concentration of kairomones (Eitam and Blaustein 2004).

Our aim in this study was to examine the oviposition behavior of the New Zealand mosquito *Cx. pervigilans* in response to the notonectid predator *Anisops wakefieldi* under field conditions. *Anisops* spp. are known to be predators of mosquito larvae and are commonly found in permanent and temporary water bodies in New Zealand (Graham 1939, Laird 1990, Laird 1995). *Culex pervigilans* is a vector species of the human and avian Whataroa virus (Maguire et al. 1967, Miles 1973), is suggested to be a vector of avian malaria (Holder et al. 1999), and has been highlighted as a species requiring further investigation for its potential to vector exotic arboviruses (Weinstein et al. 1997). We hypothesized that *Cx. pervigilans* are likely to avoid ovipositing in pools containing *Anisops* or its kairomone traces and that the influence of the kairomones released by *Anisops* predator may last for several days after the predators are removed. We also hypothesized environmental factors could play a role in influencing mosquito oviposition rates. Bentley and Day (1989) suggested that temperature, rainfall, wind speed, and relative humidity may affect the initiation of ovipositional flights by mosquitoes.

MATERIALS AND METHODS

Study sites

The study was undertaken in Otari-Wilton's Bush in Wellington, New Zealand (14°14'S, 174°45'E). Otari-Wilton's Bush is Wellington's largest area of original native forest. Experiments were undertaken on the edge of the forest in a plant nursery, with no overhead canopy or shading. Temperatures over the period of experiments ranged from 14.8° C to 18.2° C and relative humidity was between 62.8% and 89.3%. Experiments were conducted during the southern hemisphere summer season for two consecutive summers (2007/08 and 2008/09). These experiments ran from 18 December 2007 to 11 February 2008, and from 7 December 2008 until 19 January 2009.

Predator colonies

The *A. wakefieldi* used in this experiment were collected from permanent water troughs (with an approximate volume of 800 liters of water) in Queen Elizabeth II Park near Waikanae, New Zealand (40°57'S, 174°58'E), approximately 70 km from Wellington city. These predators were brought back to the laboratory for identification and deprived of food for 24 h prior to their release into the experimental containers.

Study design

Twelve black plastic containers (25.5 × 25.5 × 23.5 cm) served as temporary pools for the experiment. Containers were filled with 10 liters of water: 9.75 liters of aged tap water (tap water that was left standing for more than 48 h to reduce the chlorine content) mixed with 0.25 liters of stream water from the nearest stream, Kaiwharawhara Stream. Stream water was used to introduce bacterial inoculates as food sources for mosquito larvae and was filtered with 0.2 mm mesh before being added to the containers. The water level was maintained at 10 liters throughout the experiment by adding aged tap water or by natural rain to account for any loss due to evaporation. Nutrients were added to the water once prior to the experiment in the form of "Kiwi" brand pelletized sheep manure which contained 3% nitrogen, 2% phosphorus, and 4% potassium at a weight of 5 g/liter of water, 24 h prior to the experiment. Previous work has shown such conditions to be ideal for *Cx. pervigilans* (Leisnham et al. 2006). Containers were then covered with 1.0 cm wire mesh to prevent vertebrate animals from drinking or falling into the water.

These containers were set up so that the distance between containers was at least 0.5 m from its flanking neighbor. Treatments were established as follows: 1) controls without predators; 2) free-roaming *Anisops* (with one, three, or nine *Anisops* per container); 3) caged *Anisops* (empty cage without predator, with one, three, or nine *Anisops* in a cage that were unable to consume mosquito larvae or be directly seen by ovipositing adults); and 4) *Anisops* kairomone only (with kairomones from one, three, or nine *Anisops* which were present in the water for 24 h prior to the experiment but removed at the start of each trial). Cages used to contain predators in the caged *Anisops* treatment were constructed using 1.5 liter clear plastic drinking bottles, each with one opening window (10 cm x 3 cm) on each of two sides of the bottle and 2 mm nylon mesh covering one end of the bottle tops. The mesh was used to ensure that the predator could breathe and any predator-released kairomones from *Anisops* could diffuse into the main body of water. The cages were placed in a horizontal orientation within the water containers so that most of the cage was submerged. In the kairomone-only treatment, *Anisops* were kept in the water for 48 h immediately prior to the beginning of the experiment before their removal. Therefore, the water only contained kairomones without the presence of an actual predator.

All containers were set up 48 h before the experiments began in order to introduce and familiarize mosquitoes to the new oviposition sites. All mosquito egg rafts were counted 48 h after containers were introduced to the field. Each day for 12 days after the *Anisops* were added, they were counted and any missing or dead *Anisops* replaced. Each day, eggs were collected into small containers with water and were brought back to laboratory for counting and identification at larval stages.

Statistical analysis

We used a repeated-measures general linear model

(GLM) to test for significant effects of predator treatment (free-roaming, caged predators, and no predators), predator density, and time on *Cx. pervigilans* oviposition rate in SPSS (2006). Data were tested for normality using a one-sample Kolmogorov-Smirnov test. All data were log (x+1) transformed prior to analysis to satisfy the assumptions of ANOVA. Sphericity could not be assumed in the study ($\epsilon = 0.704$, $p = 0.009$) and the Huynh-Feldt epsilon values were used in the F-tests. The repeated measures within-subjects factor were period with four levels; day 1-3, day 4-6, day 7-9, and day 10-12. Predator treatment and density were treated as between-subjects factors.

The number of egg rafts observed during each period was correlated with five environmental factors: temperature, wind speed, rainfall, humidity, and pressure, using a Pearson correlation coefficient. The environmental data were obtained from the Kelburn weather station approximately three km from our study site, by the National Institute of Water and Atmospheric Research (NIWA), New Zealand. Any significant correlation data from Pearson's correlation test were analyzed with a t-test in order to determine the mean effects between years.

RESULTS

No significant differences in mosquito oviposition rates were detected among treatments with the presence of predators, either cages or uncaged, the presence of their kairomone only, and the control treatment with no predators ($F = 1.389$, $df = 3$, $p = 0.284$; Table 1 and Figure 1). Similarly, no statistically significant effects of predator densities were observed on oviposition rates ($F = 0.224$, $df = 3$, $p = 0.879$). Thus, *Cx. pervigilans* mosquitoes appeared to choose their oviposition habitat without taking into account predator presence or density. There were no significant differences

in oviposition among all four periods tested, nor was there any significant interaction among periods and treatments or a significant three-way interaction ($p \geq 0.500$, Table 1). Thus, mosquito oviposition rates were similar irrespective of how long the predators were in the water containers (or in the case of the kairomone-only treatment, how long the predator had been removed). These results suggested that *Cx. pervigilans* mosquitoes were not able to detect, or ignored, the presence of *Anisops* or its kairomones.

A total of 284 egg rafts was collected in 2007/2008, which was significantly more than 2008/2009 when only 28 egg rafts were collected ($t = 3.801$, $df = 114$, $p < 0.001$). All of the egg rafts were identified as *Cx. pervigilans*. There were significant correlations between the number of *Cx. pervigilans* egg rafts and temperature, humidity, and pressure (Table 2). T-tests examining the mean effects between years indicated that fewer egg rafts were oviposited by *Cx. pervigilans* mosquitoes when the temperature was significantly lower ($df = 35$, $t = 67.43$, $p < 0.001$), with significantly higher humidity and pressure ($df = 35$, $t = 35.94$, $p < 0.001$; $df = 35$, $t = 798.08$, $p < 0.001$, respectively) in the 2008/2009 summer season compared to the 2007/2008 summer season.

DISCUSSION

We had predicted that the mosquito, *Cx. pervigilans*, would alter its oviposition behavior in the presence of the predator *Anisops* or its kairomone. This prediction was based on several other studies that have found evidence for *Culex* spp. and *Culiseta* spp. avoiding oviposition in the presence of *Notonecta* sp. or its chemical traces (Blaustein et al. 1995, Blaustein 1998, Blaustein et al. 2004, Eitam and Blaustein 2004, Kiflawi et al. 2004a, Blaustein et al. 2005). Eitam et al. (2002) also found that in the presence of free

Table 1. Results of repeated measures ANOVA examining the cumulative number of *Cx. pervigilans* egg rafts in response to different types of predator treatments and densities (*A. wakefieldi*). df = degree of freedom, MS = mean squared values. Significant values are in bold. Data were log transformed prior to analysis.

Source	F	Df	MS	Significance
Treatments (T)	1.389	3	0.032	0.284
Densities (D)	0.224	3	0.005	0.879
Period (P)	1.030	3	0.004	0.381
Year (Y)	3.791	1	0.013	0.100
P x T	0.931	9	0.003	0.500
P x D	0.828	6	0.003	0.591
P x Y	1.689	3	0.006	0.173
P x T x D	0.693	12	0.002	0.600
P x Y x T	1.281	6	0.005	0.271
P x T x D x Y	0.395	12	0.001	0.963
Period x Subject within groups		126	0.004	
Period x Subject between groups		42	0.014	

Figure 1. The effects of predator treatment and predator density (*A. wakefieldi*) on number of *Cx. pervigilans* egg rafts (mean \pm SE) based on four time periods tested; (A) control (B) free-roaming predators, (C) caged predators (D) water with predator kairomones.

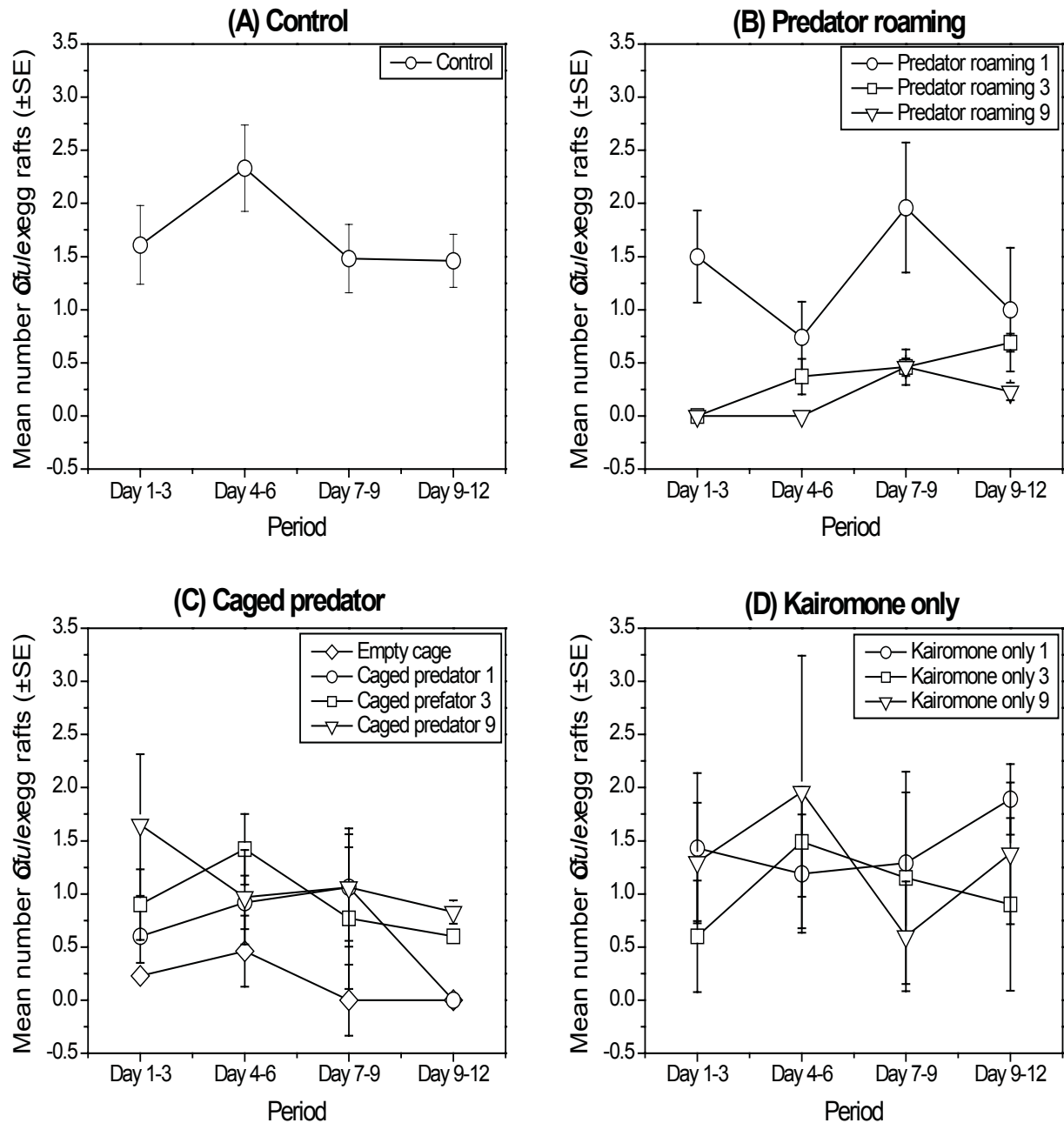


Table 2. Pearson correlation analysis results between temperature, wind speed, rainfall, humidity, and pressure with *Cx. pervigilans* egg rafts after 12 days post-treatment based on two-year study periods. Significant values are in bold. * indicated that the significant value is at 0.05 and ** is at 0.01. df = 271 for all factors.

Factor	Pearson r	Significance
Temperature	0.159*	0.012
Wind speed	- 0.104	0.098
Rainfall	- 0.084	0.182
Humidity	0.187**	0.003
Pressure	-0.504**	<0.001

swimming *A. sardea*, *Culiseta* mosquitoes were repelled from ovipositing in pools, resulting in a significantly lower number of eggs than in control pools. However, we found no evidence that *Cx. pervigilans* modifies its oviposition behavior in response to predators. We postulate that *Cx. pervigilans* either ignored the presence of the predator or that the cues from *Anisops* predators were not sufficiently strong enough to provide an alarm to these mosquitoes.

The densities of predators that we used have elicited avoidance responses in *Culex* mosquitoes elsewhere. The densities of predators in our study were more than twice that used in the study by Eitam and Blaustein (2004), but we observed no significant response by mosquitoes to the predator or its kairomone. In regard to kairomones, other mosquitoes such as *Culiseta longireolata* can detect chemicals from *Notonecta* predators for periods of up to eight days after their removal from the pool (Blaustein et al. 2004) and at least two days for *Culex* species (Blaustein et al. 2005). It thus seems reasonable to expect that the mosquitoes would have shown a response to this predator in our experimental design but we failed to detect one. Nevertheless, we cannot rule out the possibility that the mosquito would have shown a response to the predator at higher predator densities.

Given the substantial effects of individual predators on *Cx. pervigilans* populations, we would have expected this mosquito to have evolved an ability to detect the densities of the predator we used in the experiment. In laboratory experiments, we have observed that *Anisops* kill large numbers of *Cx. pervigilans* (Fatma Zuharah, unpublished data). A single adult predator can kill an average of 10-82 *Culex* larvae per day based on prey and predator size and density (Nabaneeta et al. 2007). There was thus clearly no reason why *Cx. pervigilans* should have ignored the presence of the *Anisops* predator. In the absence of any behavioral modification of oviposition, these lethal effects of *Anisops* seem likely to be the mechanism resulting from the nearly complete absence of mosquitoes co-occurring with this predator in our field studies (Fatma Zuharah, unpublished data).

We can only speculate, given the lethal effects of *Anisops*

on mosquito populations, why an ability to detect predators has not evolved in this mosquito. It is possible that until recently, these species may not have naturally occurred together. Greig¹ found that *Anisops* sp. in Canterbury, New Zealand, is likely to inhabit either permanent or semi-permanent water bodies but not temporary pools. However, *Cx. pervigilans* is able to breed in a wide variety of water habitats. *Culex* larvae can easily be found in fresh and polluted waters, permanent and temporary ground pools, natural and artificial containers, and also slow moving streams (Belkin 1968, Dumbleton 1968, Holder et al. 1999). With the creation of water troughs by humans in the farming industry, *Anisops* predators have perhaps only recently started to share the same habitat with *Cx. pervigilans*. Statistics New Zealand (2008) indicated that 76% of agricultural land was used for animal farming industries in 2002, with many thousands of existing water troughs as potential habitats for *Cx. pervigilans*, *Anisops*, and other aquatic insects. *Culex pervigilans* are now the most common species observed in water bodies such as animal troughs where *Anisops* also occur (Laird 1990). Because of the immense selection pressure exerted by *Anisops* on *Cx. pervigilans* in this new habitat, the mosquito may develop an ability to detect this predator, but the evolution of such an ability would likely take a long time to develop.

Given the lack of response of *Cx. pervigilans* to *Anisops* predators, we predicted that variation within and between years in mosquito oviposition behavior might be explained by environmental factors. Changes in temperature or precipitation may drive changes in mosquito density and populations (Canyon et al. 1998, Ritchie and Rochester 2001, Koenraad et al. 2004, Hsu et al. 2008, Platonon et al. 2008). Similarly, our results also indicated that more egg rafts were oviposited by *Culex* mosquitoes when the temperatures were higher and the humidity and pressure were lower, as demonstrated in the 2007/2008 summer season. In our experiment, environmental variables clearly had a far greater influence on oviposition than did the presence of *Anisops* predators.

In summary, we found that *Cx. pervigilans* did not alter their oviposition behavior in response to *Anisops* predators (or its kairomone traces), the presence of different densities of predators, or environmental factors. This is the first published study to demonstrate that *Cx. pervigilans* do not alter their oviposition behavior according to the presence of a voracious predator. The complexity of possible abilities to detect predator presence still remains to be examined.

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¹Greig, S.H. 2008. Community assembly and food web interaction across ponds permanence gradients. Ph.D. thesis, University of Canterbury. 142 pp.

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APPENDIX D:

Zuharah WF and Lester PJ (2010) The influence of aquatic predators on mosquito abundance in animal drinking troughs in New Zealand. Journal of Vector Ecology. 35(2): In Press

The influence of aquatic predators on mosquito abundance in animal drinking troughs in New Zealand

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ABSTRACT: The occurrence and abundance of mosquito populations may be associated with the abundance of predators. We examined the relationship between aquatic predators and populations of mosquitoes in animal water troughs in Waikanae, New Zealand. We also investigated the effects of water volume and environmental factors (temperature, rainfall, wind speed, humidity, and pressure) in order to further understand factors influencing mosquito and predator populations. Logistic regression indicated that the presence or absence of mosquitoes was primarily affected by three factors: predator abundance, week of observation, and water volume. Pearson's correlation indicated that the presence of predators had a positive correlation with water volume ($r^2 = 0.176$, $p < 0.05$). Otherwise, the presence of mosquito larvae in water troughs was negatively correlated with water volume ($r^2 = -0.159$, $p = 0.022$) and wind speed ($r^2 = 0.142$, $p = 0.041$). We established a translocation experiment in which predators or mosquitoes were moved between troughs in order to examine the prey survival rate after exposure to *Anisops wakefieldi* predators. The survival rate of mosquitoes was not significantly different, between 0-0.1%, irrespective of the number of predators translocated (1-9) or the initial mosquito density (20-70 larvae). Our results suggested that *A. wakefieldi* predators may have the potential to be a promising biological control tool for the control of mosquito populations by altering mosquito population dynamics. **Journal of Vector Ecology 35 (2): xxx-xxx. 2010.**

Keyword Index: Environmental factors, mosquito population, predator, translocation, water trough, water volume.

INTRODUCTION

Artificial containers such as bottles, cans, tires, pots, and animal drinking troughs are major habitats for mosquito populations. However, aquatic predators also occur in many of these habitats, including animal drinking troughs, and have been suggested to influence the abundance of mosquitoes (Laird 1990). Mosquito species may share the same habitat with predators, with some predators appearing to have little influence on mosquito populations (Lundkvist et al. 2003). Different mosquito species may be affected differently by predators (Griswold and Lounibos 2006), with the differential consumption of various mosquito species by predators resulting in the coexistence of some mosquito species (Griswold and Lounibos 2005).

A variety of aquatic insects in the orders Coleoptera, Hemiptera, Odonata, and Diptera is known to attack and eat mosquito larvae. In general, most predators are polyphagous and consume a broad range of prey species (Shaalan et al. 2007). In New Zealand, the common mosquito predators seen in natural and anthropogenic water bodies are the notonectid backswimmer *Anisops* spp. (Hemiptera), diving beetles in the genus *Rhantus* (Coleoptera), and damselfly larvae (Odonata) (Graham 1939). Several studies have focused on notonectid predators because of their effectiveness in controlling mosquito populations (Scott and Murdoch 1983, Murdoch et al. 1984, Koivisto et al. 1997). Notonectid predators clearly have the potential to alter mosquito communities via direct or indirect effects (Gilbert and Burns 1999). The indirect effects of

the presence of notonectid predators in water bodies can include a reduction in oviposition by adult mosquitoes (Chesson 1984, Blaustein et al. 1995), while direct effects occur primarily through predation.

New Zealand currently has only 16 mosquito species, with 12 indigenous species and four exotic species (Derraik 2004a, 2004b, Laird 1990, 1995). No outbreaks of mosquito-borne disease have been recorded. However, out of 12 indigenous mosquito species, *Culex pervigilans* and *Culiseta tonnoiri* have been identified as vectors of the avian arbovirus (Whataroa Virus) (Maguire et al. 1967). *Culex pervigilans* is New Zealand's most abundant and widespread species and has the potential to be a vector of human pathogens. *Culex pervigilans* is often found in artificial containers including animal drinking troughs (Graham 1929, 1939, Belkin 1968, Laird 1990, 1995). There are other species which are of much greater concern such as *Aedes notoscriptus* and *Coquilletidia iracunda*, with the former also being found in drinking water troughs in New Zealand (Laird 1995).

Environmental factors may affect the abundance of predator and mosquito populations. A backtrack simulation study of wind speeds sufficient for mosquito transport from Papua New Guinea to Australia was performed by Ritchie and Rochester (2001). Winds at 36 and 72 km/h were capable of carrying mosquitoes from New Guinea to the Northern Peninsula of Australia. Slow velocity winds are also important as they allow female mosquitoes to place their eggs calmly without disturbance in water containers (Service 2000). Temperatures can also strongly influence predation rates. For example, Anderson et al. (2001) showed

that tadpoles raised in warmer water experienced a higher probability of capture by notonectids predators than those reared in cooler conditions.

Our aim in this study was to examine the relationship and interactions between predators and mosquito populations in animal drinking troughs. We translocated predators between troughs in order to better understand their influence on mosquito populations. We also examined the effects of water volume and environmental factors on predator and mosquito populations.

MATERIALS AND METHODS

Study site

Our study was undertaken at Queen Elizabeth II Park, Waikanae, New Zealand (41°06'31.43 S, 175°13'12.91 E). This area contains a variety of habitats, including coastal and inland sand dunes as well as pastoral areas for animal farming. A total of 13 water troughs within an area of 4.8 km² were sampled. Troughs ranging in volume from 0.32–0.84 m³ were sampled at weekly intervals. Sampling continued for two southern hemisphere summers from December to February of 2007/2008 and 2008/2009.

The relationship between predator and mosquito populations

During each sampling occasion, predators such as *Anisops wakefieldi* (backswimmers), *Rhantus rhantus* larvae (diving beetles), and *Onychohydus hookeri* (water beetles) were visually observed and counted. Predators were inspected and identified *in situ*, as they were easily identified to genus level. To estimate mosquito densities we used a conventional dipping technique (Russell 1993, Service 1995). The water surface was stirred using a stick before the sample was taken. A total of ten liters of water was sampled by dipping at equal intervals around the water trough. The water samples were strained through 0.10 mm mesh and the filtered water was then returned to the trough. Mosquitoes collected in the strainer were placed separately in containers with 500 ml of water and brought back to the laboratory for identification. If any predators were caught in the strainer, they were released back into the trough. Mosquito larvae were identified using a key by Snell (2005). The dipper and strainer were thoroughly rinsed with distilled water before sampling the next water trough.

The water volume for each trough was recorded. Water volume was used rather than water depth because there were different shapes of water troughs located in the field. Environmental data (temperature, wind speed, rainfall, pressure, and humidity) were provided by the New Zealand National Institute for Water and Atmospheric Research from Paraparaumu Aero Station, which is approximately 4.5 km from our study site.

We analyzed the data with logistic regression using R version 2.10.1 (Ihaka and Gentleman 1996). The presence and absence of mosquitoes served as the response variable. The predictor variables were predator, water volume, week of observation, temperature, rainfall, wind speed,

and humidity. This logistic regression approach was used because prior work has suggested that the sole presence of predators or kairomones can influence mosquito communities by altering mosquito behaviors such as adult oviposition (Blaustein et al. 2004, Blaustein et al. 2005). We then used Pearson's correlation tests in SPSS 15.0 (2006) to examine the relationship between the environmental factors and water volume, and the abundance of predators and mosquito populations in water troughs. If, as in our experiments, the presence or absence of predators does not influence mosquito behaviors such as oviposition, an analysis of the influence of predator abundance is more appropriate.

Predator translocation experiment

In order to examine the effectiveness of the predator *A. wakefieldi* in altering mosquito populations, we transferred these predators among troughs. *Anisops wakefieldi* was used in this study because it was the most common predator species at our study site. We transferred between one and nine predators, and 3rd instar *Cx. pervigilans* mosquito larvae, in each water trough as follows; (A) zero predators (control) with 20, 40, or 70 mosquito larvae, (B) one predator with 10, 15, or 20 mosquito larvae (C) three predators with 20, 30, or 40 mosquito larvae (D) nine predators with 30, 50, or 70 mosquito larvae. Experiments were replicated four times. Data were recorded two days after each transfer.

In order to test the effects of predator density and prey number on mosquito survival rates in the translocation experiments, we ran a two-way ANOVA in SPSS 15.0 (2006). The percentage of surviving prey served as the dependent variable, whereas type of treatment and initial number of prey offered were fixed factors. Survival rates were expressed as a percentage and were therefore transformed using arcsine square root prior to analysis to satisfy the assumptions of ANOVA.

RESULTS

The relationship between predator and mosquito populations

The only mosquito species that was observed utilizing animal drinking troughs during the entire study was *Culex pervigilans*. The three predator species observed in same habitat were *Anisops wakefieldi* (91.8%), *Rhantus rhantus* larvae (6.6%), and *Onychohydus hookeri* (1.6%). The total number of *Cx. pervigilans* larvae collected in 2007/2008 was 3,293, with only 715 individuals in 2008/2009 over the same sampling duration. The total number of predators was high in 2007/2008 with 916 individuals, but only 419 individuals were recorded in 2008/2009.

In 2007/2008 the highest numbers of predators (300) were observed after the first week of sampling during summer but densities declined closer to autumn (Figure 1A). In the same trough in the following year (2008/2009), the situation changed with a high abundance of mosquitoes. However, only two predators were observed for the entire study period (Figure 1A). In most troughs the pattern of

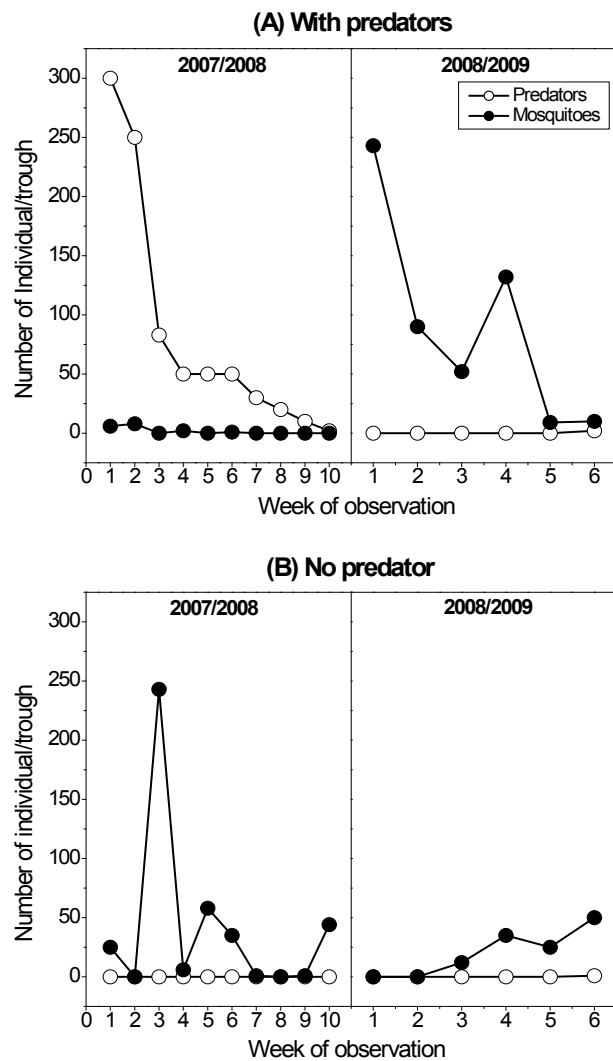


Figure 1. Examples of the relationship between predators and mosquitoes in water troughs at Queen Elizabeth II Park, Waikanae, New Zealand. Two of the 13 troughs examined in the study are shown, one trough with predators (A) and one without predators (B).

population dynamics of mosquitoes and predators was similar to that shown in Figure 1B.

The model resulting from the logistic regression indicated that with each increase in the abundance of predators, the probability that mosquito populations existed also increased, but it is not statistically significant (Figure 2). For each increase in the abundance of predators, the odds of mosquitoes being present (vs absent) positively increased by a factor of 1.0059. The probability of mosquito presence significantly decreased with increasing water volume and week of observation ($p < 0.05$; Table 1).

Predator abundance increased significantly with increasing water volume in both years of the study ($r^2 = 0.176$, $p = 0.011$; Table 2 and Figure 3), but was not correlated with any other environmental variable nor the abundance of mosquitoes ($p \geq 0.232$). In comparison, mosquito abundance decreased significantly with increasing water volume ($r^2 = -0.159$, $p = 0.022$; Table 2) and wind speed ($r^2 = 0.142$, $p = 0.041$) but was not correlated with any other environmental variable ($p \geq 0.220$).

Predator translocation experiment

The translocated predators had a major effect on mosquito populations. The maximum number of mosquito prey left alive was one mosquito two days post-treatment. Even at the highest number of 70 prey, with the largest water volume of 0.72 m^3 , the predator successfully consumed all of the prey (Figure 3). The ANOVA showed that the mosquito larvae survival rate in treatments with predators was significantly lower than in the control treatment without predators ($F = 443.92$, $df = 3$, $p < 0.001$). There was no significant effect of initial mosquito number on mosquito survival rate ($F = 0.87$, $df = 6$, $p = 0.524$), and the interaction between treatments and mosquito abundance was also non-significant ($F = 0.53$, $df = 6$, $p = 0.780$). Post-hoc comparisons showed that treatments with predators (one, three, and nine predators) were not significantly different from each other ($p \geq 0.704$) but had a significantly lower prey survival (Tukey, $p < 0.005$) than in the control treatment without

	Estimates	SE	z-value	p-value
Intercept	4.937	1.561	3.163	0.002**
Predator	7.217^{e-03}	6.343^{e-03}	1.138	0.255
Water Volume	-2.194^{e-06}	1.114^{e-06}	-1.969	0.049*
Water trough	5.201^{e-02}	6.036^{e-02}	0.862	0.389
Temperature	1.196^{e-01}	7.143^{e-02}	-1.675	0.094
Wind speed	3.939^{e-02}	2.376^{e-02}	1.658	0.097
Rainfall	2.658^{e-02}	2.062^{e-02}	1.289	0.197
Humidity	-1.477^{e-02}	9.551^{e-03}	1.289	0.197
Week	-1.976^{e-01}	5.084^{e-02}	-3.887	<0.001***

Table 1. Logistic regression analysis for the predictor variables included in the model that affect the presence or absence of mosquito populations in the water troughs. d.f. = 202 (** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$).

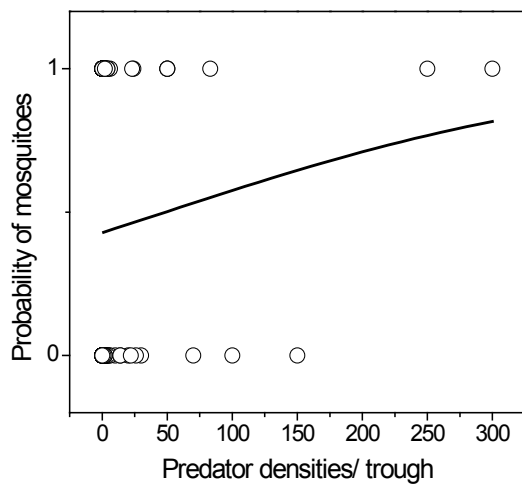


Figure 2. Logistic regression of the presence or absence of mosquitoes in association with predator abundance.

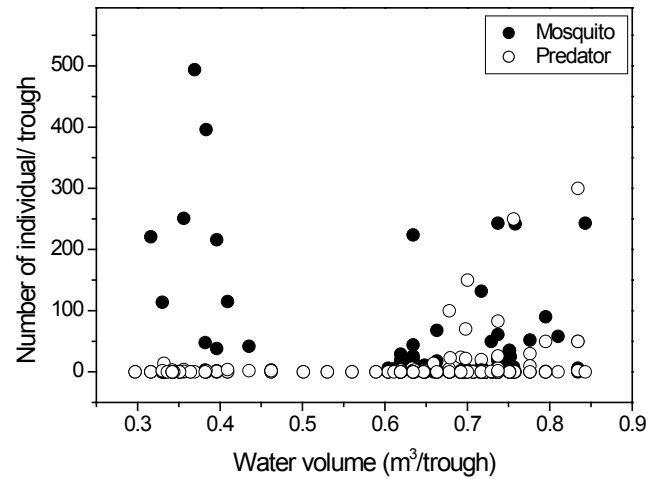


Figure 3. Abundance of mosquito larvae and predators recorded in animal drinking troughs compared to cumulative water volume.

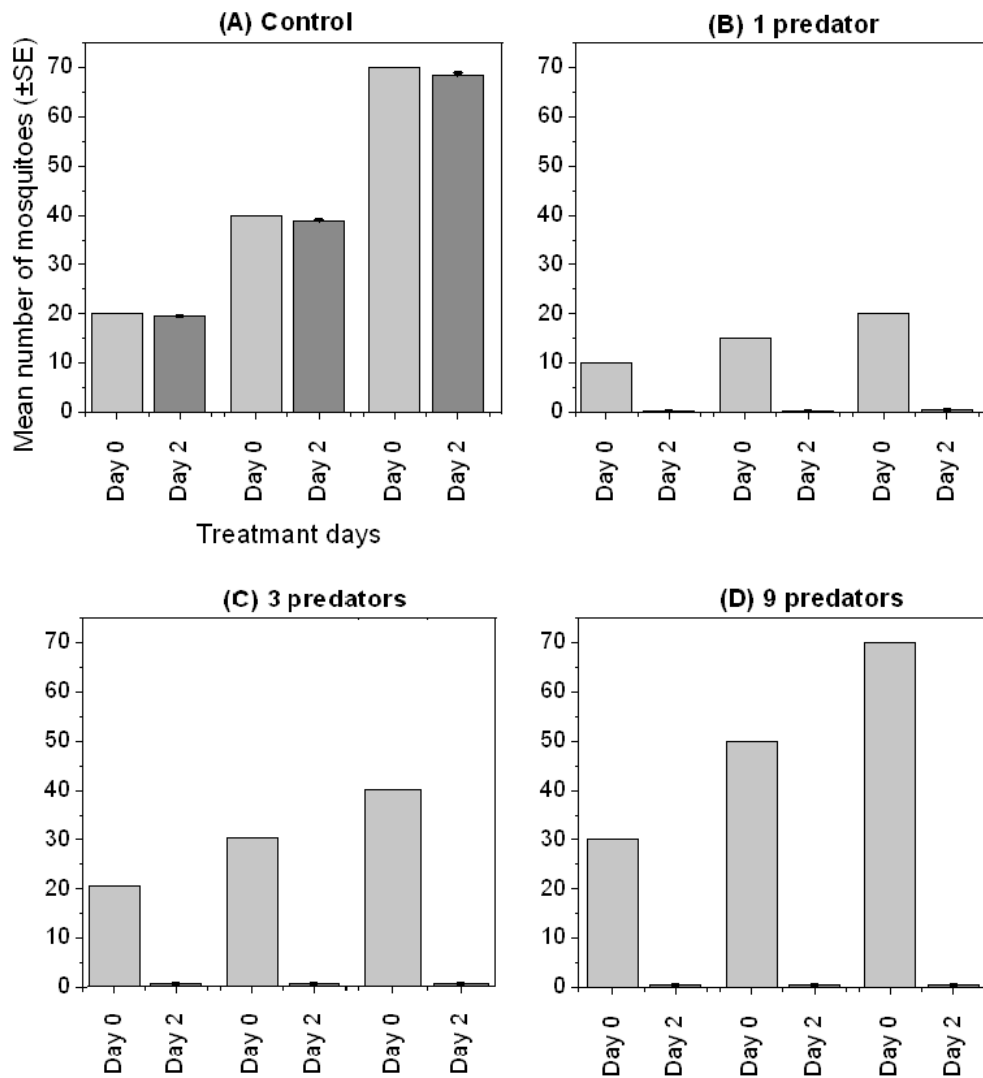


Figure 4. Results of the pre- (day 0) and post-treatment (day 2) experiment after mosquito prey were exposed to (A) control (with the absence of predators), (B) one free-roaming predator, (C) three free-roaming predators, and (D) nine free-roaming predators.

Table 2. Results of Pearson correlation tests between *Anisops wakefieldi* or mosquito populations, and environmental factors (temperature, rainfall, wind speed, humidity, and pressure) or water volume. Data were collected from animal water troughs during the Southern Hemisphere summer (December to February 2007/2008 and 2008/2009) for two consecutive years. Significant values are in bold with df= 207. Data represent Pearson's 2-tailed correlation.

	Predator	Mosquito
Mosquito	-0.052, 0.454	
Water volume	0.176, 0.011	-0.159, 0.022
Temperature	-0.020, 0.773	-0.085, 0.220
Rainfall	-0.041, 0.558	0.049, 0.484
Wind speed	-0.063, 0.365	0.142, 0.041
Humidity	-0.023, 0.741	0.064, 0.357
Pressure	0.083, 0.232	0.022, 0.748

predators. These results clearly demonstrate the ability of this predator to reduce the number of mosquitoes in water troughs within only two days. In the control treatments, where mosquito larvae were present in the water troughs without predators, the mortality rate of mosquitoes was less than 5%.

DISCUSSION

The only mosquito species observed in this study was *Cx. pervigilans*. *Culex pervigilans* is the most common mosquito in New Zealand, utilizing many water containers and habitat types (Graham 1939, Belkin 1968, Laird 1990, 1995). It prefers organically rich water bodies (Graham 1939, Dumbleton 1968, Urbinatti 2001) but has wide tolerance for a variety of water qualities (Graham 1929, Derraik and Slaney 2004). The predators *A. wakefieldi*, *R. rhantus*, and *O. hookeri* are also widely distributed throughout the North Island of New Zealand (Brown 1943, Winterbourn et al. 2006). At our sampling site, *A. wakefieldi* was the most abundant predator observed; only 2% of all predators were *R. rhantus* and *O. Hookeri*. In our study, most of the water troughs utilized by predators had low densities of mosquitoes. Some of the water troughs that were utilized by large numbers of predators in 2007/2008 had few predators and large numbers of mosquitoes in 2008/2009. There are at least two hypotheses to explain these results. Firstly, predators may consume large numbers of mosquitoes and control their population numbers. Secondly, adult mosquitoes may have an ability to detect the presence of a predator and will not oviposit in troughs with predators.

To test the hypothesis that the predators consumed a large number of mosquitoes, we undertook a translocation experiment in which *A. wakefieldi* and mosquitoes were placed together in the same environment. Our translocation experiment showed that within two days, all mosquitoes

were consumed by predators. Other laboratory studies confirm that a single *A. wakefieldi* predator in 500 ml of water can kill about 18 individual mosquitoes within 24 h (Zuharah, unpublished data). In our study, it appears that even in large water volumes *A. wakefieldi* are voracious predators. Both adults and nymphs of *A. wakefieldi* prey upon mosquito larvae. This predator is believed to be most effective against 3rd and 4th instar larvae (Bay 1974, Lee 1967, Ellis and Borden 1970). Additionally, *Anisops* spp. prefers mosquito larvae over other prey when given a choice (Ellis and Borden 1970).

Our second hypothesis to explain the relationship between predators and mosquitoes was that adult mosquitoes may have the ability to detect the presence of a predator and consequently do not oviposit in troughs with predators. Predators may therefore regulate prey abundance without directly killing mosquitoes (Brown et al. 1997). Stav et al. (1999) suggested that the distribution and abundance of a mosquito species is dependent on their ability to selectively oviposit according to predator abundance. Other mosquitoes such as *Culiseta longiareolata* can detect chemicals from *Notonecta* predators for periods of up to eight days after their removal from the pool (Blaustein et al. 2004) and this period is as low as two days for *Culex* species (Blaustein et al. 2005). It thus seems reasonable to expect that the mosquitoes would have shown a response to this predator. With increasing predator densities, the concentration of kairomones also increase and may result in reduced oviposition (Eitam and Blaustein 2004). A reduction in oviposition activities by mosquitoes may be expected with increasing numbers of predators, due to the ability of these prey species to detect the presence of a predator above a threshold density (Angelon and Petranka 2002) or a specific concentration of kairomones (Eitam and Blaustein 2004). In a separate experiment we examined the oviposition of *Cx. pervigilans* in response to the presence of

A. wakefieldi and kairomones. Unlike work elsewhere (e.g., Blaustein et al. 2004, Blaustein et al. 2005), we observed no response of mosquitoes to the presence of these predators (Zuharah and Lester, 2010).

Thus, it appears that the primary effects of predators are through predation rather than sub-lethal effects such as a change in oviposition behavior. However, our logistic data indicated a positive relationship between mosquito and predator densities, though this relationship was not significant ($p < 0.10$). Adult *Anisops* predators frequently disperse and may fly up to 1.6 km between habitats (Brier, unpublished data). It is possible that these predators periodically move between habitats and are more likely to stay longer in habitats with high numbers of mosquitoes. Further work is needed to confirm this hypothesis.

The abundance of mosquitoes was observed to be dramatically different between the two years of this study. None of the environmental factors we examined correlated with the abundance of predator and mosquito populations. Wind speed correlated only with mosquito abundance. Mosquitoes need 10-15 min to oviposit, therefore, slow velocity winds are important as they allow female mosquitoes to place their eggs without disturbance in water containers (Service 2000). The lack of any other relationship between mosquito abundance and predators with environmental variables may be a result of averaging. The environmental data analyzed are the average conditions over each weekly period of sampling, but within any one week there may have been conditions suitable for brief periods of oviposition or predator movement.

Water volume was highly correlated with predator and mosquito abundance in water troughs. Larger water troughs may be advantageous to *A. wakefieldi* colonies because they are less likely to dry out or overflow (Wilton 1968, Frank and Curtis 1977). A larger space offered by bigger water troughs can increase larval survivorship and reduce mortality risks such as cannibalism (Sota 1998, Sunahara et al. 2002). We also found that mosquito populations were negatively correlated with water volume. Female mosquitoes may oviposit in all types of habitats even if the water has evaporated. Browne (unpublished data) observed female mosquitoes ovipositing in full and nearly empty containers, suggesting that these mosquitoes are incapable of assessing water depth. Similarly *Cx. pervigilans* appear incapable of assessing water depth (Derraik and Slaney 2004). The positive relationship between water volume and predators, and the negative relationship between depth and mosquitoes, is in accordance with the observations that predators influence mosquito populations.

Our results indicate that the predator, *An. wakefieldi*, can control *Cx. pervigilans* densities even in relatively large volumes of water. However, the fluctuation in environmental changes from year-to-year, and even within a year, may indirectly affect the effectiveness of this predator in controlling mosquito populations.

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