MATEUS RAMOS DE ANDRADE

CONTAINER-DWELLING MOSQUITOES: HABITAT SIZE, DIRECT AND INDIRECT EFFECTS OF PREDATION

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

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APROVADA: 10 de Abril de 2015.

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Eraldo Rodrigues de Lima(Orientador)

“No animal on the earth has touched so directly and profoundly the lives of so many human beings. For all of the history and all over the globe she has been a nuisance, a pain and an angel of death. Mosquitoes have felled great leaders, decimated armies, and decided the fates of nations. All this and she is roughly the size and weight of a grape seed.”

Andrew Spielman - 2001

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SUMÁRIO

ABSTRACT

ANDRADE, Mateus Ramos, D. Sc., Universidade Federal de Viçosa, April, 2015. **Container-dwelling mosquitoes: habitat size, direct and indirect effects of predation.** Adviser: Eraldo Rodrigues de Lima.

Predation is important selective force acting in the evolution of organisms. Predators can directly influence the population dynamics of prey, through consumption. Furthermore, only the presence of predator cues suggesting a threat of predation may exert an indirect effect by changing the characteristics of the species detected. The main objective was to assess the direct and indirect effects of predation on mosquitoes that inhabit environments with very particular characteristics, the containers. We note that the container size influences the composition of species, and predators found more often in larger containers. Depending on the species of prey, the result of direct interaction with the predator may be negative for the prey (increased mortality) or neutral, and this may be related to behavior and habitat use by species. As indirect effects, the presence of predator may increase the mortality of prey, even when unable to consume them. In general, this thesis adds important evidence about the effect of predation on containers.

RESUMO

ANDRADE, Mateus Ramos, D. Sc., Universidade Federal de Viçosa, Abril, 2015. **Mosquitos em containers: tamanho do habitat, efeitos diretos e indiretos da predação.** Orientador: Eraldo Rodrigues de Lima.

A predação é uma importante força seletiva que atua na evolução dos organismos. Predadores podem influenciar diretamente a dinâmica populacional das presas, através do consumo. Além disso, apenas a presença de pistas dos predadores sugerindo uma ameaça de predação pode exercer um efeito indireto, alterando características das espécies que as detectam. O principal objetivo foi verificar a os efeitos diretos e indiretos da predação em culicídeos que habitam ambientes com características bastante particulares, os containers. Observamos que o tamanho do container influencia a composição das espécies, sendo os predadores encontrados com maior frequência em containers maiores. Dependendo da espécie da presa, o resultado da interação direta com o predador pode ser negativo para a presa (aumento da mortalidade) ou neutro, e isso pode estar relacionado ao comportamento e uso do habitat pelas espécies. Como efeitos indiretos, a presença de um predador pode aumentar a mortalidade das presas, mesmo quando impossibilitados de consumi-las. Em geral, a tese contribui com importantes evidências dos efeitos da predação em containers.

GENERAL INTRODUCTION

Mosquitoes are dipterous belonging to the family Culicidae, which constitute a monophyletic taxon. It is a diverse group with about 3,500 known species, and abundant in virtually all regions of the globe (Harbach 2007). Besides being annoying insects to humans, mosquitoes are vectors of many important diseases such as malaria, dengue fever, yellow fever, Chikungunya virus and West Nile virus (Chase & Knight 2003, Pialoux *et al.* 2007). The immature stages of these insects are aquatic and inhabit a spectrum of environments. Among these, we highlight the containers, which are environments with unique ecological properties (Washburn 2005). Containers are small breeding sites that support few species with reduced population size and high rates of extinction. Also have almost no internal productivity, and the energy intake is mainly based on the decomposition of organic detritus (Vezzani 2007). A controversial aspect of the containers is related to the factors regulating the populations. Some authors suggest that populations in containers are regulated by food limitation or competitive interactions rather than predation. Others assume that predators are important in the regulation of populations in these environments, and so may limit the success of the invasion of exotic species (Kesavaraju *et al.* 2008) and can be used in biological control programs (Shaalan & Canyon 2009).

Because many of these systems are related to human activities (artificial containers), they are closely related disease transmission (Yee *et al.* 2012). Therefore, a better understanding of these systems is required. In addition to contributions to the general ecological theory, the study of communities that inhabit containers can provide valuable information on how to manipulate these systems to the monitoring and control of mosquito populations. And that is the purpose of this thesis, which is divided into 3 chapters.

Chapter 1 discusses the role of the size of the containers in the structuring of mosquito communities. For many systems, the habitat size is reported as an important factor in determining the richness, abundance and species composition. In a field experiment using containers of different sizes, we see the influence of habitat size on mosquito communities, with a focus on predator-prey relationship. Determine which containers sizes are preferred by each species and the breeding sites where the occurrence of predation is more likely. Chapter 2 covers the direct effects of predation (consumptive effects). We show that the result of predation for the populations of prey can be completely different and investigate the relationship of the result of predatory activity with the behavior of prey species. Chapter 3 has as subject the indirect effects of predation (non consumptive effects). Here it was observed that predators may adversely affect the fitness of prey even when they are unable to consuming them. Finally, we summarize the conclusions of the results obtained in this thesis.

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CHAPTER 1

Habitat size structures container-dwelling mosquito communities?

Abstract

Artificial containers are important breeding sites for several species of mosquitoes. General theory for ecology of freshwater systems leads to the hypothesis that colonization of containers by mosquitoes should be affected by the size of containers. Likewise, the container size should affect the number and kinds of predators able to colonize these environments. Here we tested the influence of the size of the breeding sites on the mosquito communities in a field experiment, using containers ranging from 0.15L to 200L of volume capacity. Fortnightly samplings were carried out for 16 weeks. The container size had a strong impact on species richness, larval abundance and larval density. Mosquito species differ with regard to the preferred container size. Predators are found more frequently in larger containers, where the ratio predator/prey is lower, suggesting a higher risk of predation in these breeding sites. These results yield a better understanding of how habitat scale affect colonization by mosquitoes and which container sizes are likely to produce particular vector species.

Keywords: artificial container, mosquito, habitat size, predator-prey interaction.

Introduction

Ecologists have long recognized that habitat size has important consequences on the organization, size and persistence of resident biological communities (Washburn 1995). Some of these observations were formalized in a conceptual framework with the publication of “The Theory of Island Biogeography” (MacArthur & Wilson 1967). This book introduced a theoretical model for predicting the species composition of oceanic islands, and the size of the island is one of the main factors to be considered in the model. Since the original publication, principles of this theory have been applied to explain a wide range of biological events of size and scale in other environments that are not oceanic islands. (Whittaker & Fernández-Palacios 2007).

Ecological context, specifically habitat size, can also influence the species composition and interactions across freshwater communities (Juliano 2009). Freshwater bodies form a gradient to small and very ephemeral to large and permanent systems. Lentic aquatic environments can be classified at least within two discrete habitats categories: containers (e.g., tree holes, bromeliad axils and human-made containers) and pools (e.g., puddles, wetlands and ponds) (Welborn *et al.* 1996). Among these habitats, human-made containers are particularly important for often be inhabited by species of mosquitoes. In addition to being a general nuisance to humans, mosquitoes are vectors-borne diseases (Norris 2004). The number of mosquitoes that are vectors of diseases can be proportional to the availability of such artificial larvae habitats (Wang et. al 2000).

Mosquito species differ in preference of breeding sites. The artificial containers used by mosquitoes span a wide range of sizes: from small discarded containers like bottle caps and cups holding few milliliters, to large tires, water tanks and rain barrels holding more than a hundred liters. The composition of the mosquito community found in a water body is a result, firstly, of the oviposition behavior of the species. Ovipositing insects should select sites that improve the survival, growth, and reproductive potential of the offspring, especially for species in which juveniles are incapable of migrating away from low quality habitats (Peckarsky *et al.* 2000). Once inside of the breeding site with favorable abiotic conditions, the successful development of immature depends on several other factors such as food availability, competitors and predators.

Investigations on preferred artificial breeding sites of mosquitoes were performed, comparing containers of different materials, shapes, colors and heigths (Vezzani & Schweigmann 2002, Collins & Blackwell 2000, Jones & Schreiber 1994, Obenauer *et al.* 2009), but only two investigations tried to isolate the container size as the main variable (Lester & Pike 2003, Harrington et. al 2008). Furthermore, most of the studies on mosquitoes breeding in artificial containers involve the invasive species *Aedes aegypti* and *Aedes albopictus* (Juliano 2009). Investigations on resident species are important as these may be the main inhabitants of artificial containers and also act as pathogen vectors, especially the *Culex* genus. Even those unrelated to the transmission of diseases deserve attention as it has important role as competitors or predators, impacting the populations of vectors or even as barriers against invasive species (Juliano & Lounibos 2005).

Mosquito control is fundamentally the practical application of population dynamic theory, and reducing the density of biting adults is the fundamental goal to mosquito control (Juliano 2007). Control strategies may focus on preventing adult mosquito emergence by source reduction by killing the larvae, or the adults. Targeting larval habitats for control is usually regarded as both more cost-effective and more environmentally desirable than targeting adults, in part because preventing adult emergence can prevent, rather than manage, a disease outbreak (Focks *et al.* 2000, Floore 2006). Targeting only those species that are vectors, rather than broadly targeting all mosquitoes is a way to minimize environmental impacts (Mosquito Control Colaborative 2005). Thus, for control purposes, it is vital to identify species-specific source habitats.

Different species of mosquitoes are unlikely to be uniformly spread among artificial containers of different sizes. Some species are likely to predominate in large containers, whereas others are likely to predominate in small containers (Carrieri *et al.* 2003). These differences may be related to the oviposition behavior of species, competitive abilities and the presence of predators. This chapter focuses on the container size as a factor influencing mosquito communities. As aquatic habitats become larger they are expected to become more permanent, with greater abundance and impact of predators. A gradient of habitat size, then, is expected to include a shift from small ephemeral habitats with high levels of competition and little predation, to large, permanent habitats with little competition and high levels of predation. Along this gradient, the interaction of predation and prey density is expected to chance, and modify the impact of natural enemies on populations. Here, we determine how larval habitats sizes are important in the composition and functioning of mosquito communities and the major ecological reasons that justify the patterns found.

Material and Methods

Study area

Fieldwork was carried out at Mata do Paraíso Ecological Station, Federal University of Viçosa, Minas Gerais state, Southeast of Brazil (20°48′08 S 42°51′31 W). This station covers an area of 194.36ha, and average elevation of 690m. The natural vegetation was classified as a semidecidual forest remnant (Veloso *et al.* 1991). The climate is classified as humid subtropical (Cwa), according to Köppen-Geiger classification (Peel *et al.* 2007).

Field experimental setup

On 21st of November 2012, at beginning of the rainy season, a total of 186 black plastics containers of five different sizes, ranging from 0.15L to 200L were established. These containers were distributed in six transects of 310m long, 10m far from edge of the main trail. In each transect had containers of all sizes, spaced 10m apart. Along each transect, the following number of containers were distributed: size 1 = 16 containers; size 2 = 8 containers; Size 3 = 4 containers; size 4 = 2 containers and size 5 = 1 container. As was expected a larger variation between the smaller containers, they had a greater number of replicates. In order to avoid that containers of the same size got too close to each other, the sequence of containers within transects was defined as: 1-2-1-3-1-2-1-4-1-2-1-3-1-2-1-5-1-2-1-3-1-2-1-4-1-2-1-3-1-2-1 (figure 1.1). Each container was filled with spring water, with a maximum of 70% of total capacity, reaching the following initial volume: Size 1 = 0.08L; Size2 = 0.35L; Size 3 = 4L; Size 4 = 40L and Size 5 = 140L. In each container was added an amount of organic matter (*Cecropia* sp. dried leaves) proportional to its capacity (2g/L).

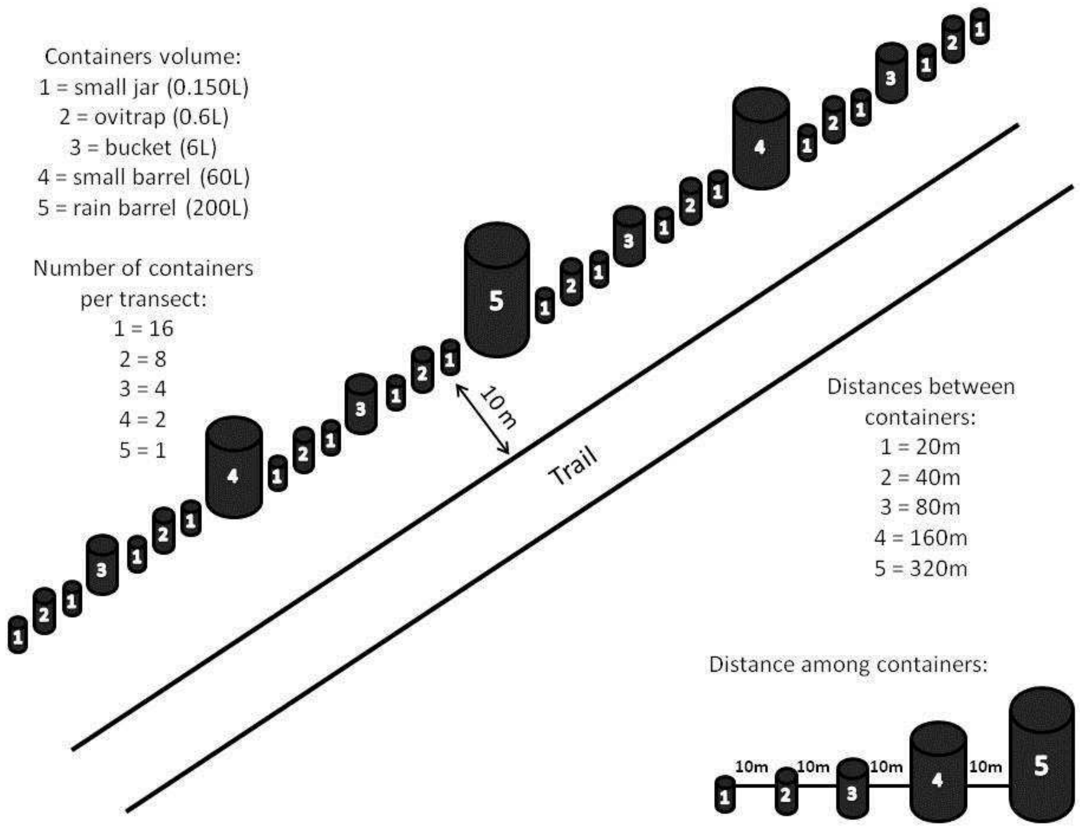

Figure 1.1: Field experimental design representation.

Sampling of communities

Beginning 2 weeks after the containers were established (5 December), the containers were sampled fortnightly during the next 16 weeks. For small containers (sizes 1 and 2), only half of the replicate was sampled every two weeks. The container content was sieved (100μm mesh) returning the water to it. This sampling plan was adopted to try to reduce system disruption. Each small container was sampled every four weeks, improving the ability to interpret the results. Medium container (size 3) was sampled placing a flat circular magnet into the container, waiting 3 minutes for the disturbed community to resettle, than plunged a 7.5 cm-diameter into the container and onto the magnet. The magnet then adhered to the end of the tube, sealing it and allowed the removal of a sample of the water column. The sample content was sieved, animals removed and the water was returned to the container. Larger containers (sizes 4 and 5) were sampling placing a 12 cm-diameter zooplankton net (100 μm mesh) into the container, waiting 3 minutes and drawing the net, collecting the animals. This procedure was repeated twice for size 4 and four times for container size 5. For all containers, water column was measuring using a meter. All animals collected were taken to the laboratory. Culicidae larvae were identified to either species according Forattini (1996) keys, and classified according to Harbach (2007).

Statistical analysis

The richness was determined by counting the number of species in each container. The density in each container was calculated by dividing the number of individuals sampled for the water volume of the sample. The abundance was estimated multiplying the density of individuals by the container volume of water. Because the data covered repeated measurements over time, it was analyzed using mixed-effects models. We determine the size and time as fixed effects, as well as the interaction between them (size * time). As random effects, we used transect and each container unit (transect/container_id). For all analyses, the dependent variable was log-transformed. All statistical analyses were conducted in R version 3.1.1 (R Core Team 2013), using the lmer or glmer function of the package lme4 and anova function of the package lemrTest.

Results

Richness

The mosquito community in our experiment was composed for 10 species (*Ochlerotatus terrens*, *Culex mollis*, *Culex iridescens*, *Culex eduardoi*, *Culex dolosus*, *Lutzia bigoti*, *Lutzia* sp. *Limatus durhamii*, *Haemagogus leucocelaenus* and *Toxorhynchites theobaldi*), belonging to 6 different genera. Mosquito richness was significantly higher in the two largest containers ($F_{4,181} = 301.81$, $p<0.001$) (Table 1.1, Table1.2, Figure 1.2).

Larval density

To calculate the larval density, we use an estimate of the number of mosquitoes larvae collected per liter of water. Larval density was significantly different among all five container sizes, with size 3 (6L) the container with higher density and size 5 (200L) the container with lower density ($F_{4,181} = 26.33$, $p<0.001$) (Table 1.1, Table1.2, Figure 1.3).

Abundance

To calculate the abundance, we multiply the larval density by water volume (liters), measured at the sampling time. Mosquito abundance was significantly higher in the two largest containers (Sizes 4 and 5) ($F_{4,181} = 640.20$, $p<0.001$) (Table 1.1, Table1.2, Figure 1.4).

Table 1.1: Mosquitoes larval richness, density and abundance means ± EP. Data log-transformed. Different letters indicate significant difference among container sizes.

	Size 1 (0.15L)	Size 2 (0.6L)	Size 3 (6L)	Size 4 (60L)	Size5 (200L)
Richness	0.025±0.004(a)	0.094±0.011(a)	0.327±0.014(a)	0.500±0.013(b)	0.527±0.017(b)
Density	0.469±0.043(a)	0.659±0.052(b)	1.252±0.038(c)	0.801±0.035(d)	0.447±0.035(e)
Abundance	0.202±0.020(a)	0.505±0.043(a)	1.914±0.052(a)	2.366±0.041(b)	2.322±0.054(b)

Table 1.2: Results of the linear mixed effects model analysis testing fixed effects for larval richness, density and abundance, with “transect/container_id” as random effects. Response variable log-transformed. ***P<0.001, **P<0.01, *P<0.05.

		df	F	P
Richness	Size	4	301.81	<0.001 ***
	Time	7	2.04	0.044 *
	Size:Time	28	3.30	<0.001 ***
Density	Size	4	26.33	<0.001 ***
	Time	7	1.95	0.059
	Size:Time	28	3.88	<0.001 ***
Abundance	Size	4	640.20	<0.001 ***
	Time	7	3.38	<0.001 ***
	Size:Time	28	5.00	<0.001 ***

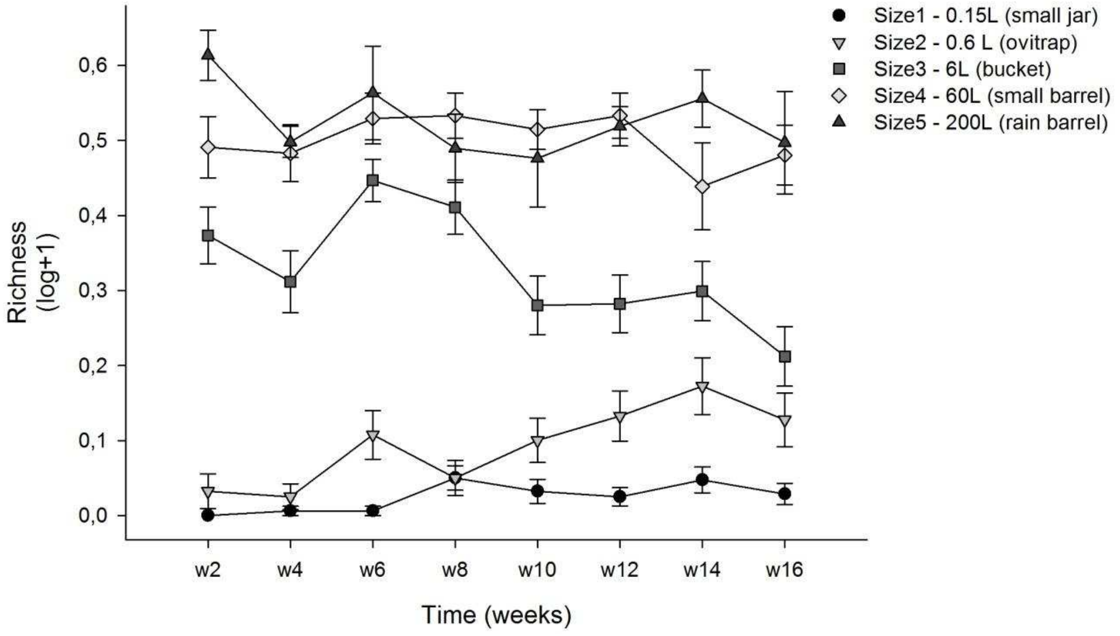

Figure 1.2: Mosquito species richness in each sampling week. Containers were sampled fortnightly for 16 weeks. Symbols indicate log-transformed means with error bars for each container size.

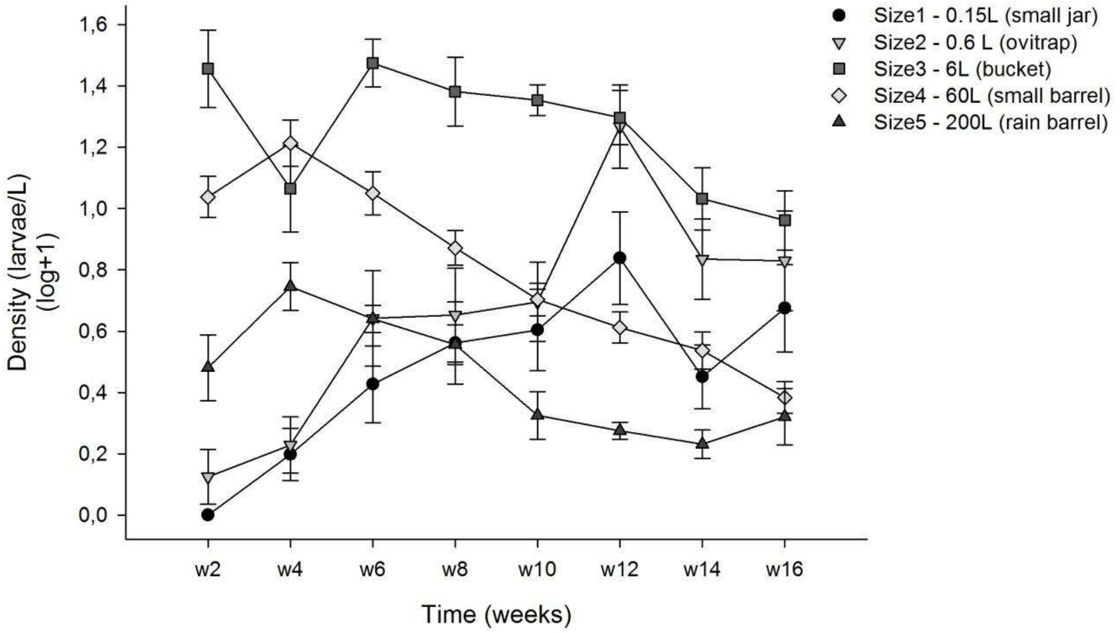

Figure 1.3: Mosquito larval density in each sampling week. Containers were sampled fortnightly for 16 weeks. Symbols indicate log-transformed means with error bars for each container size.

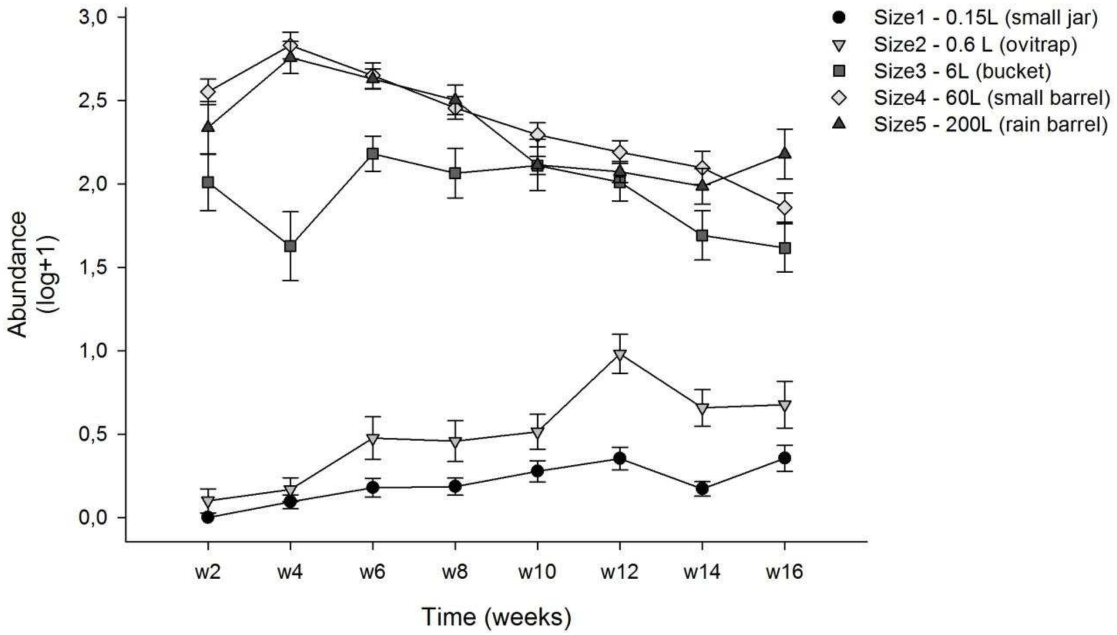

Figure 1.4: Mosquito abundance in each sampling week. Containers were sampled fortnightly for 16 weeks. Symbols indicate log-transformed means with error bars for each container size.

Density by species

Culex iridescens

C. iridescens was the prevailing species, found in 43.8% of all sampled containers, with an average density of 17.5 larvae per liter. *C. iridescens* density was significantly higher in the medium-sized container (Size 3 – 6L). Size 5(200L) was by far the container with less larval density ($F_{4,181} = 26.76$, $p<0.001$) (Table 1.3, Table1.4, Figure 1.5).

Culex mollis

C. mollis was the second most sampled species, present in 28.4% of all observations, with an average density of 3.06 larvae per liter. *C. mollis* density was significantly higher in the Size 4 (60L) container, rarely found in low volume containers ($F_{4,181} = 98.70$, $p<0.001$) (Table 1.3, Table1.4, Figure 1.6).

Limatus durhamii

L. durhamii was the third most sampled species, present in 11.9% of all observations, with an average density of 1.56 larvae per liter. *L. durhamii* shows great preference for medium-sized containers (Size 3 – 6L) being found in low density in all other sizes ($F_{4,181} = 55.78$, $p<0.001$) (Table 1.3, Table1.4, Figure 1.7).

Toxorhynchites theobaldi

T. theobaldi was the prevailing predator and the fourth most sampled species, found in 12.9% of all observations, with an average density of 0.63 larvae per liter. *T. theobaldi* density was significantly higher in Size 2 (0.6L), compared with other four ($F_{4,181} = 4.27$, $p=0.003$) (Table 1.3, table1.4, Figure 1.8).

Ochlerotatus terrens

Found in 10.9% of all observations, with an average density of 0.42 larvae per liter, *O. terrens* was the fifth most sampled species. This species density was significantly higher in Sizes 3 (6L) and 4 (60L), compared with other three ($F_{4,181} = 11.19$, $p=0.002$) (Table 1.3, Table1.4, Figure 1.9).

Table 1.3: Larval density means ± EP for the five more prevailing mosquito species. Data log-transformed. Different letters indicate significant difference among container sizes.

	Size 1 (0.15L)	Size 2 (0.6L)	Size 3 (6L)	Size 4 (60L)	Size 5 (200L)
C.iridescens	0.375±0.040 (a)	0.530±0.053 (b)	0.980±0.040 (c)	0.227±0.023 (a)	0.029±0.008 (d)
C. mollis	0.011±0.008 (a)	0.056±0.020 (a)	0.513±0.041 (b)	0.630±0.044 (c)	0.394±0.038 (b)
L. durhamii	0.011±0.008 (a)	0.038±0.014 (a)	0.323±0.034 (b)	0.033±0.009 (a)	0.013±0.009 (a)
T. theobaldi	0.051±0.013 (a)	0.108±0.015 (b)	0.019±0.006 (a)	0.013±0.002 (a)	0.015±0.002 (a)
O. terrens	0.017±0.009 (a)	0.009±0.007 (a)	0.099±0.015 (b)	0.101±0.017 (b)	0.010±0.005 (a)

Table 1.4: Results of the linear mixed effects model analysis testing fixed effects for the five most prevalent species, with “transect/container_id” as random effects. Response variable log-transformed. ***P<0.001, **P<0.01, *P<0.05.

		df	F	P
C. iridescens	Size	4	26.76	<0.001 ***
	Time	7	4.63	<0.001 ***
	Size:Time	28	1.72	<0.001 ***
C. mollis	Size	4	98.70	<0.001 ***
	Time	7	20.99	<0.001 ***
	Size:Time	28	7.72	<0.001 ***
L. durhamii	Size	4	55.78	<0.001 ***
	Time	7	11.23	<0.001 ***
	Size:Time	28	10.13	<0.001 ***
T. theobaldi	Size	4	4.27	0.003 **
	Time	7	1.18	0.312
	Size:Time	28	1.37	0.097
O. terrens	Size	4	11.19	<0.001 ***
	Time	7	3.34	0.002 **
	Size:Time	28	1.88	0.004*

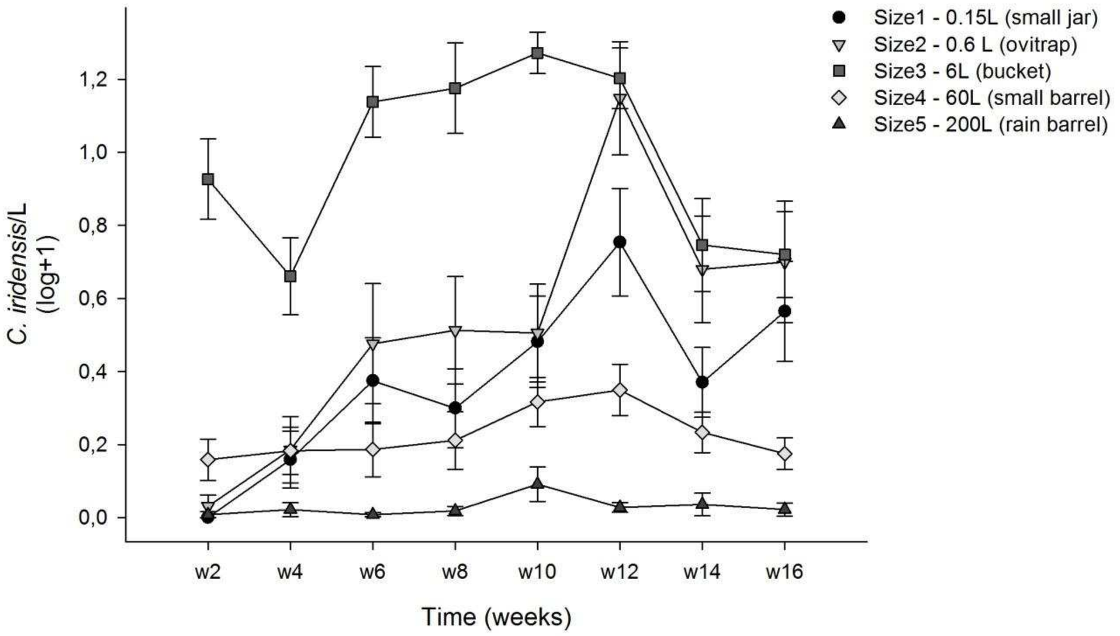

Figure 1.5: *Culex iridescens* density in each sampling week. Containers were sampled fortnightly for 16 weeks. Symbols indicate log-transformed means with error bars for each container size.

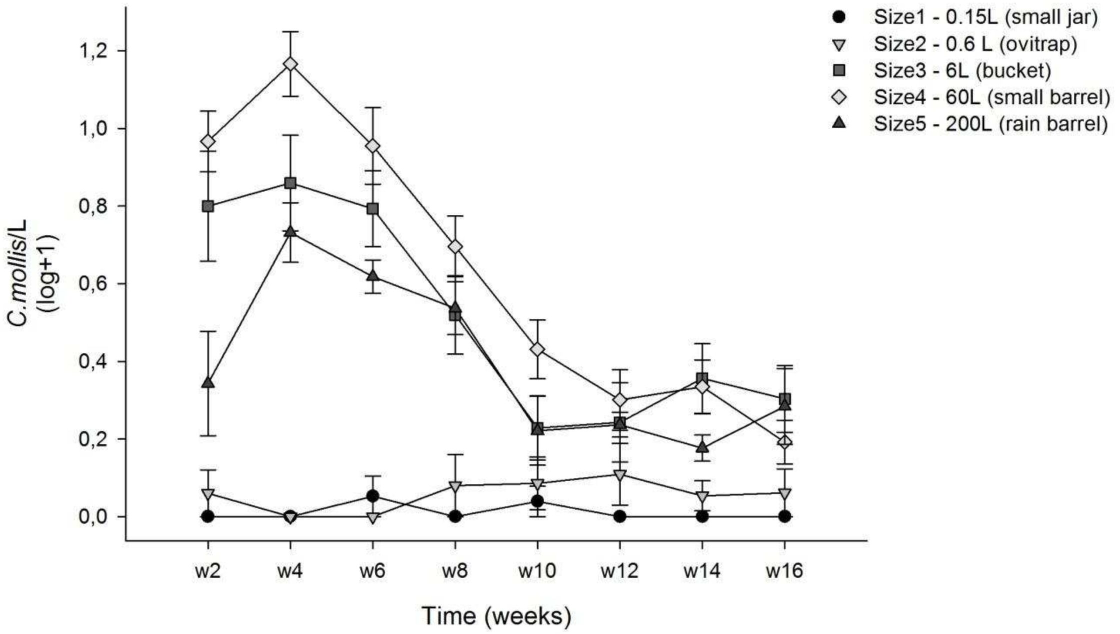

Figure 1.6: *Culex mollis* density in each sampling week. Containers were sampled fortnightly for 16 weeks. Symbols indicate log-transformed means with error bars for each container size.

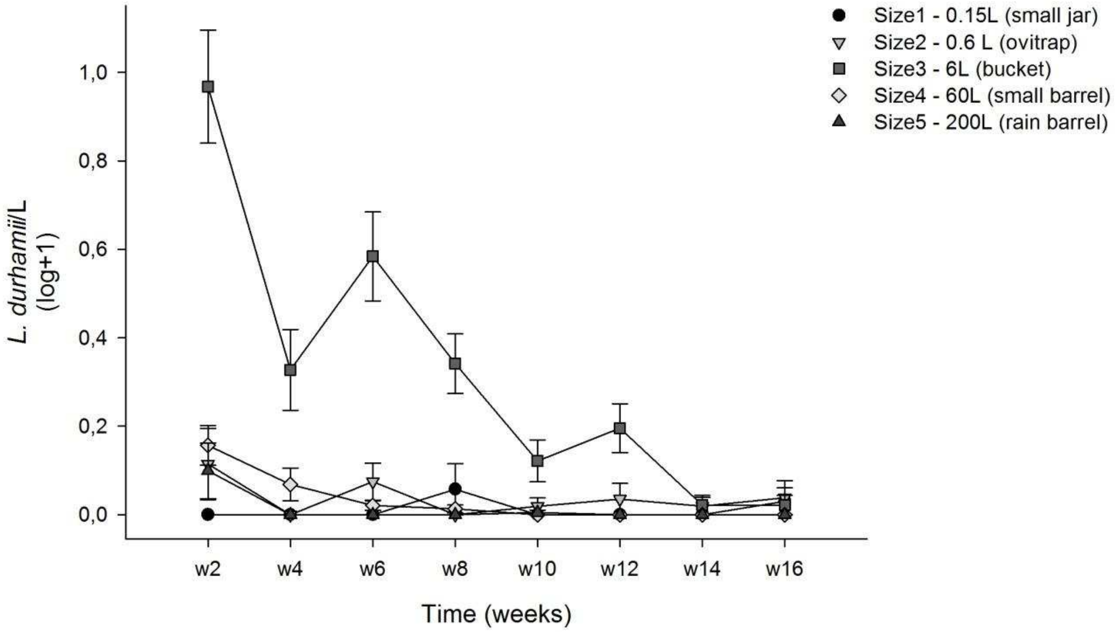

Figure 1.7: *Limatus durhamii* density in each sampling week. Containers were sampled fortnightly for 16 weeks. Symbols indicate log-transformed means with error bars for each container size.

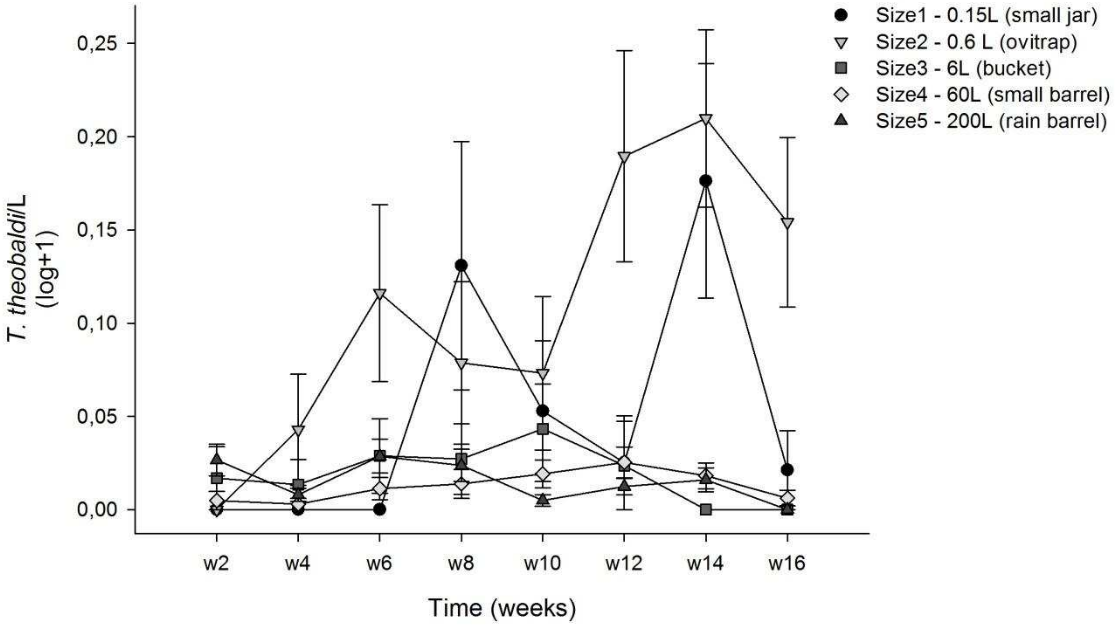

Figure 1.8: *Toxorhynchites theobaldi* density in each sampling week. Containers were sampled fortnightly for 16 weeks. Symbols indicate log-transformed means with error bars for each container size.

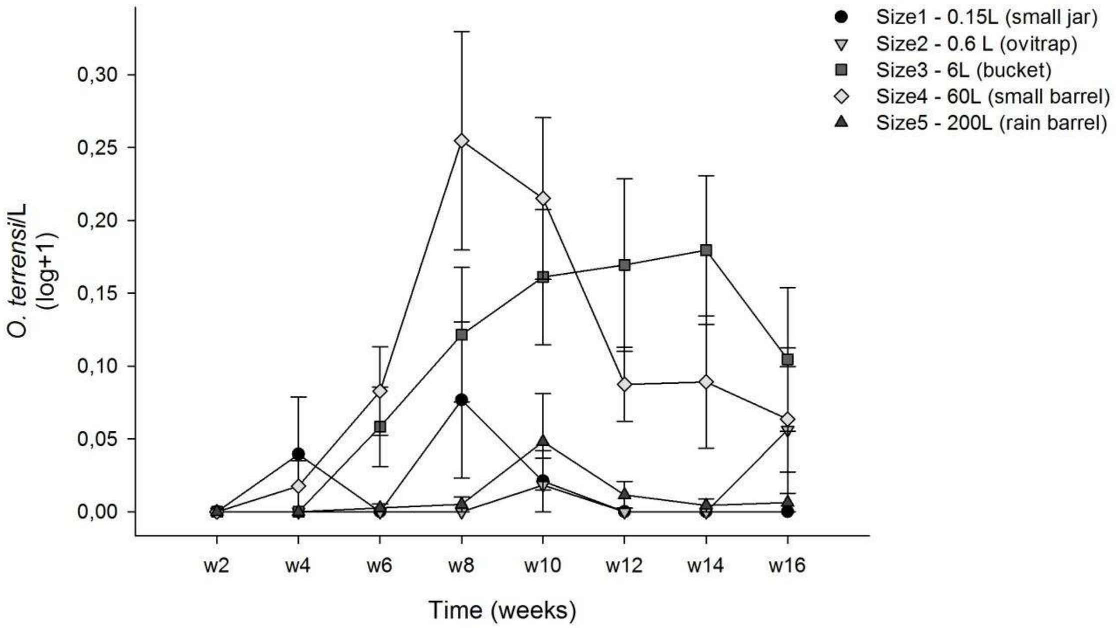

Figure 1.9: *Ochlerotatus terrens* density in each sampling week. Containers were sampled fortnightly for 16 weeks. Symbols indicate log-transformed means with error bars for each container size.

Predation risk

To calculate predation risk in each size, we used a ratio of prey density to predator density for each week. This ratio is lower in the largest containers (sizes 4 and 5), followed by the container size 2 (0.6L). In the small jar (0.15L) and the bucket (6L), we found a higher prey/predation value ($F_{4,181} = 13.49$, $p<0.001$) (Table 1.3, Table 1.4, Figure 1.10). We also calculated the proportion of containers with predators, relative to the total. Predators are more common in the larger container, followed by sizes 2 and 4, which the frequency of predators was statistically the same. Sizes 1 and 3 showed a lower risk, on which predators are found less often ($F_{4,181} = 13.24$, $p<0.001$) (Table 1.3, Table 1.4, Figure 1.11).

Table 1.5: Ratio of prey density to predator density (larvae/L) means (data log-transformed); and percentage of positive containers for predators. Different letters indicate significant difference among container sizes.

	Size1(0.15)	Size 2(0.6L)	Size 3(6L)	Size 4(60L)	Size5(200L)
Prey/Predators	1.582±0.108(a)	0.919±0.079 (b)	1.338±0.028(c)	0.771±0.036 (d)	0.424±0.034 (d)
Predators presence(%)	3.906±2.032(a)	20.833±7.256(b)	9.895±6.098(c)	35.416±12.96(b)	60.416±19.03(d)

Table 1.6: Results of the linear mixed effects model analysis testing fixed effects, with “transect/container_id” as random effects. Response variable log-transformed. ***P<0.001, **P<0.01, *P<0.05.

		df	F	P
Prey/Predators	Size	4	13.49	<0.001 ***
	Time	7	6.44	<0.001 ***
	Size:Time	28	3.58	<0.001 ***
Predators presence(%)	Size	4	13.24	<0.001 **
	Time	7	2.56	<0.026 *
	Size:Time	28	1.05	<0.398

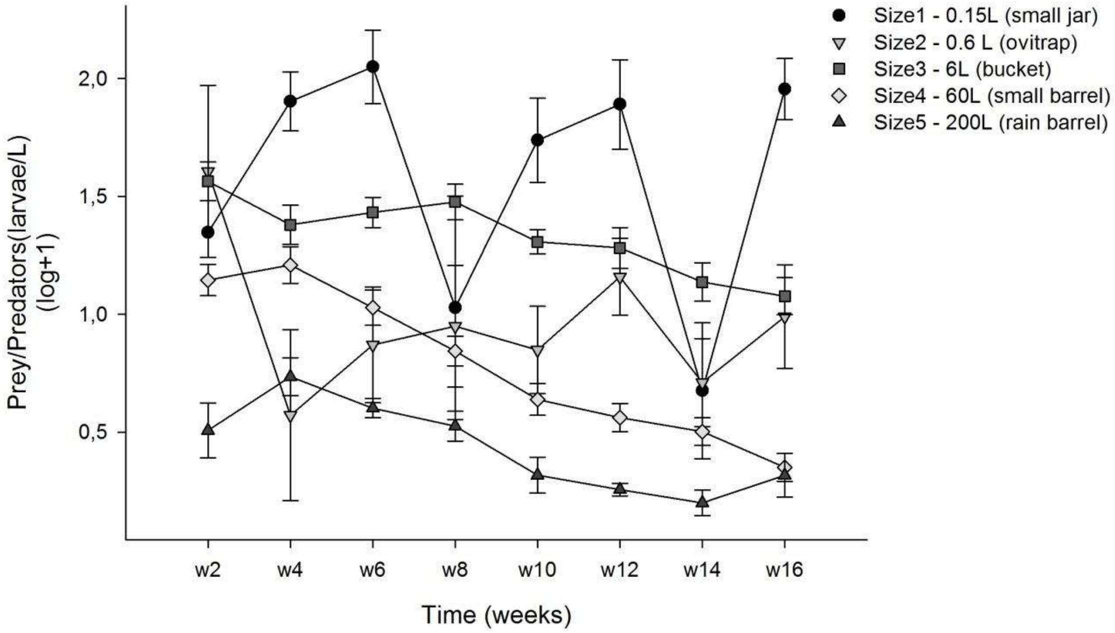

Figure 1.10: Ratio of prey density to predator density in each sampling week. Containers were sampled fortnightly for 16 weeks. Symbols indicate log-transformed means with error bars for each container size.

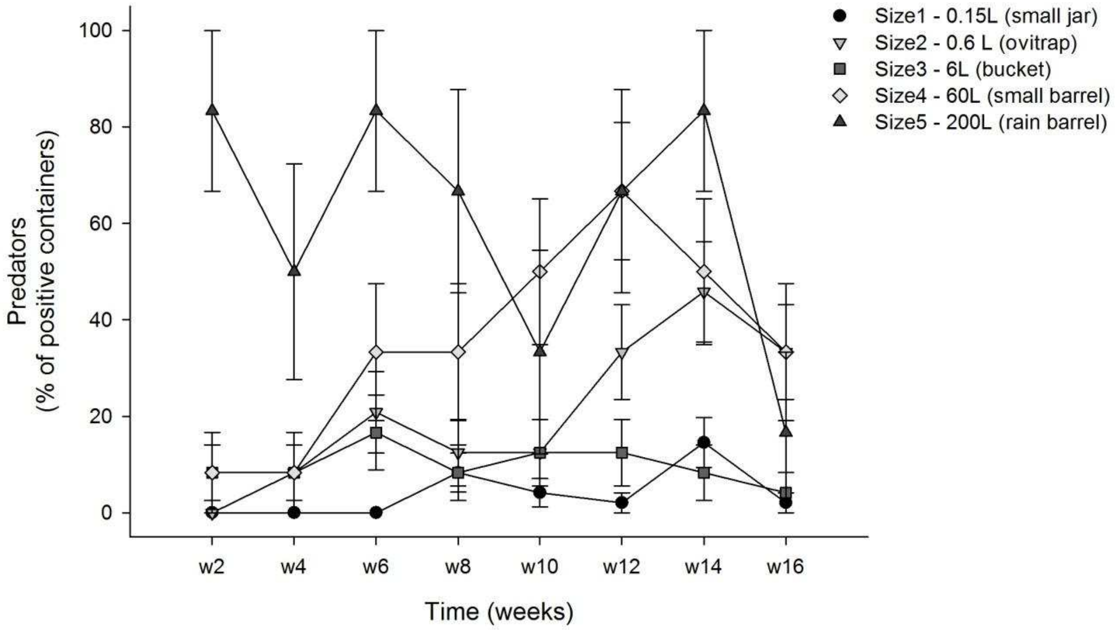

Figure 1.11: Percentage of positive containers for predators in each sampling week. Containers were sampled fortnightly for 16 weeks. Symbols indicate means with error bars for each container size.

Discussion

“The Theory of Island Biogeography’’ proposes that island size, remoteness and length of isolation are the major factors in determine its species richness. Since all containers are in a same area, equidistant from each other and placed at the same time, in this study the last two factors may be excluded. So, the positive relationship observed between container size and mosquito species richness is consistent with the first factor (size) of this theory. However, despite the rain barrel (size 5) is more than 3 times larger than the small barrel (size 4) richness difference between these two containers was not observed. In general, the existence of more species on large islands than small islands may be related to higher immigration rates and/or the higher available niches (Angermeier & Scholosser 1989). In our case, immigration and the availability of niches may be related to oviposition preference and the heterogeneity of the environment in the containers. The size difference between these containers may not be sufficient to affect these two factors in order to change the species richness.

Something similar occurs in relation to species abundance. Similar larval abundances are found in environments with capacity of 60 and 200L. This can be explained by the fact that the 200L containers have a lower larval density compared to all other sizes. Large containers are rarely found in nature. Perhaps, for this reason, few species of mosquitoes were able to colonize the rain barrels efficiently. Furthermore, the most commonly found species in containers in our study area (*Culex iridescens*) demonstrated low preference for large containers, contributing to the lower density.

Another point is that, in larger containers, predators were found in a higher frequency and proportion relative to prey number. Many species of predators are effective in reducing the population of prey in aquatic environments (Bay 1974). For this reason, the pressure exerted by predation may have contributed to reduce the larval density in containers with larger volume of water. Wellborn *et al.* (1996) predicted that predation should be more important in relative permanent large bodies of water. In addition, we must consider the indirect effects of the presence of predators. Chemical cues released by the predators can significantly reduce the oviposition of some species of mosquitoes (Blaustein *et al.* 1995, Blaustein 1998, Spencer *et al.* 2002), thus promoting a lower larval density in environments with increased risk of predation.

Local habitat size has been shown to influence colonization and extinction processes of species in patchy environments. However, species differ in body size, behavior and trophic level, and may not respond in the same way to habitat size (Petermann *et al.* 2014). Many aspects of the mosquito oviposition behavior and breeding sites preferences are known (Bentley & Day 1989). Despite this, the full spectrum of cues that gravid mosquitoes use to assess potential oviposition sites, and the scale at which these cues become important, have not been fully understood (Willians *et al.* 1999). In this study, we were able to demonstrate that different species have different preferences for the size of container used as breeding sites. This is observed even for closely related species.

As one example, the two most abundant species observed in our containers (*Culex iridescens* and *Culex mollis*), despite belonging to the same genus, demonstrate a completely opposite habitat preference. While *C. iridescens* was rarely found in rain barrels (200L) and showed significant densities in small containers, *C. mollis* was sampled at very low densities in the containers with less than 1 liter of capacity (small jars and ovitraps), and at high densities in larger containers. One aspect that may explain the observed difference is related to the oviposition behavior of this species. *C. mollis*, as most species of the genus *Culex*, females deposit all their eggs assembled into rafts on the water surface (Clements 1992). Larger containers should favor this behavior because they prevent overcrowding and intense competition among siblings that would be caused by deposition of more than a hundred eggs in a small container at the same time. *C. iridescens* lays its eggs singly on the surface or in the container wall above the waterline (personal observation). As this species can distribute their eggs between several breeding and smaller containers are more easily flooded, reaching the eggs deposited on the wall, breeding sites with lower water capacity seem to be better suited to this oviposition behavior.

Limatus durhamii is one of the few species of Sabetini tribe able to colonize artificial containers, being found in a variety sorts of containers. *L.*

durhamii showed higher preference for buckets (6L), being found at higher density in this containers than in all the others. Buckets were the containers that had a higher total density of larvae, making this container size a very competitive environment. In the absence of availability of food resources, such as environments with high competitiveness, *L. durhamii* can adopt an optional predatory habit (Lopes 1999), consuming larvae that are in the early development instars. This species is also capable of using carcasses of dead larvae as a food item in such situations (personal observation). In environments with higher larval density and consequently increased competition, mortality rates are higher, and the larval development time is longer (Moore & Fischer 1969). Thus, intermediate-sized containers such as buckets should promote species with greater plasticity related to feeding behavior, such as *L. durhamii*.

Ochlerotatus terrens showed preference for buckets (6L) and small barrels (60L). Interestingly, *O. terrens* was the only species among the five most representative that was not found in any of the containers in the first sampling (week 2). In the second sample (week 4) was observed at low densities, and reached its maximum density only at the fourth sampling (week 8). This may be due to the oviposition behavior of this species. *O. terrens* female lays its eggs on the wall of the breeding, above the waterline requiring an increase in the water level to larvae hatching. However, according to Alencar *et al.* (2014) for this species, eggs may need more than one flood event for hatching. This event is known as installment hatching (Gillet 1955). This seasonal quiescence ceases after exposure of eggs to appropriate stimuli for hatching (multiple floods in this case) is considered as a reproductive strategy that avoids exposure of larvae to unfavorable environmental conditions (Vinogradova 2007). Due to less water volume fluctuation in larger containers (200L), perhaps many eggs deposited did not reach a sufficient number of flood events to trigger the larval hatching, which would explain the low density found in rain barrels.

While often found inhabiting artificial containers, larvae of the genus *Toxorhynchites* are reported as specialists in colonizing natural containers such as tree holes and bromeliads (Steffan & Evenhuis 1981). In this study, this species was sampled at higher densities in ovitraps (0.6L). This result coincides with the water storage capacity of the natural breeding, as well tree holes and bromeliads store a volume of about 0.6L of water (Sota *et al.* 1994, Cogliatti-

Carvalho 2010). While *T. theobaldi* was the predominant predator in our samples, a lower ratio prey / predators and a higher frequency of containers predators was found in rain barrels. In addition to *T. theobaldi*, other mosquito predator species (*Lutzia bigoti*) was found in our containers. Unlike *T. theobaldi, L. bigoti* showed preference for larger containers and was never sampled in small jars and ovitraps. Surprisingly, beyond these two species of mosquitoes, no other predator was sampled. Although not very common, predators of other orders as Hemiptera, Coleoptera and Odonata have been reported inhabiting artificial containers, particularly those with larger water volume.

In this study, we demonstrated that the size of the breeding sites is an important factor in structuring mosquito communities and discuss some mechanisms that make different sizes of containers have different characteristics such as richness, abundance, larval density, species composition and predation risk . From these results, we suggest caution should be exercised in efforts to control and surveillance of container-breeding mosquitoes. The use of traditional sampling methods as ovitraps is not suitable to sample all species, especially when dealing with insects of epidemiological importance. As an example *C. mollis*, recently reported as a potential vector of West Nile Virus (Morales-Betoulle 2012), was one of the most abundant species, although rarely found in small containers. *Haemagogus leucocelaenus*, an important vector of yellow fever, was found in our artificial containers in a few opportunities, but are commonly observed in natural containers, highlighting the importance of the survey of natural breeding too. The same goes for the use of predators in biological control programs. Among other factors, similar preferences between predator and target prey species in relation to the size of breeding sites should be considered.

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CHAPTER 2

Relationship between prey behavior and the predation outcome for populations of container-dwelling mosquitoes

Abstract

The importance of the factors that regulate populations is a central issue in the ecology of organisms inhabiting containers. Predation is a major factor or regulation is mainly determined by the availability of resources and competition? The activity of natural enemies can produce three basic outcomes in prey populations, which are additive mortality, compensatory or overcompensatory. This chapter aims to verify in the field the result of predatory activity for different prey species of mosquitoes. For this, experimentally manipulate containers with and without predators (*Toxorhynchites theobaldi* larvae) and daily follow the development of prey species. We observed that predators had a completely different effect on the prey species. While *Limatus durhamii* populations experienced a strong additive mortality, predators resulted in a compensatory mortality to *Culex iridescens*. Thus, we investigated whether this differential mortality may be related to the behavior of prey species. In fact, *L. durhamii* exhibit behaviors of high predation risk more frequently than *C. iridescens*.

Keywords: regulatory factors, mosquito behavior, oviposition.

Introduction

Predation is an ecological interaction that can strongly affect the structure and dynamics of ecological populations and communities (Schmitz 2005). When a predator is inserted in an environment, it is expected that the mortality of prey increases, compared to natural mortality that would occur in a predator-free environment (additive mortality). For this reason, predators are often used to control populations of target species in biological control programs. A group of organisms with great interest as a target for biological control are mosquitoes, (Diptera: Culicidae) due to its potential as a vector of several diseases (Floore 2007). However the additive mortality is not the only possible outcome. Sometimes, predators do not affect prey populations (compensatory mortality), or even benefit them (overcompensatory mortality) (Washburn 1985). This is one of the reasons why many biological programs can fail in controlling mosquito populations (Kumar *et al.* 2006).

Studies show that, for two mosquitoes prey species that share the same predator, the effect of predation on populations can be completely different (Linden & Cech 1990, Blaustein & Byard 1993, Blaustain 1998, Quiroz-Martinez *et al.* 2005). While the mortality of individuals can be drastic in some species, in other this may be imperceptible. A more likely common mechanism is that the most vulnerable species serve as the main prey source for the predator, thus diluting predation intensity on the other species in the absence of numerical predator responses (Blaustein 2007). The result of predator-prey interactions for the populations depends on several factors, such as physical and chemical characteristics of the environment, and biological characteristics of the organisms involved, such as morphology, physiology and behavior of predators and prey (Endler 1986). Explanations for the reasons of such divergence in mortality (some prey species being more vulnerable than others) are discussed, but rarely investigated (McPeek 1990).

When different species inhabiting the same environment and share the same predator, particularities of prey should be the determining factor in the outcome of predator-prey interaction. When the prey species have morphological and physiological similarities, it is expected that behavioral differences play a key role in vulnerability, as has been shown for mosquito larvae. The capture probability increases depending upon the behavior displayed by the prey and its position in the water column (Juliano & Reminger 1992).

In this chapter, we investigated the result of the addition of predators to an artificial container in a field experiment. We tested the hypothesis that mosquito populations that share the same environment, and consequently the same predator, may respond in distinctive ways to predation. For this purpose, predatory mosquito larvae (*Toxorhynchites theobaldi*) were added to artificial containers, and the development of prey that colonized these environments was monitored daily, and compared to predator-free containers. We also recorded the behavior of the two most abundant mosquito larvae found in containers, suggesting that differences in the outcome of predator-prey interactions for populations can be explained by behavioral differences between prey species.

Material and Methods

Experiment 1: Larval development in the field

Study area

Fieldwork was carried out at Mata da Biologia, Federal University of Viçosa, Minas Gerais state, Southest of Brazil (20°45′29 S 42°51′43).

Field experimental setup

On 5st of March 2014, a total of 20 artificial breeding sites were established. As breeding sites, 2-L black plastic containers were used (20 x10 cm), with a hole that keep the container holding until 1 liter of water. There were two treatments: two predatory larvae (*T. theobaldi*) present, or no predators. For all containers, 1L of deionized water with 2g of organic matter (*Cecropia* sp. dried and milled leaves) was added to stimulate mosquito oviposition. These containers were distributed in pairs 0.5 meters apart and at least 15 meters away from another pair. For the treatment with predatory larvae, 3 days after the containers establishment (8st of March), two newly-hatched larvae were placed inside one container of each pair.

Sampling of mosquitoes

All containers were inspected every day. All found pupae were removed and date and the source container were recorded. *T. theobaldi* eggs were also removed. The individuals were taken to the laboratory for identification. If any larva of *T. theobaldi* was lost, it was immediately replaced by another of the same age, reared in the laboratory under similar conditions. When necessary, more water was added, keeping the volume of the container in 1L. For each pair, the experiment was finished when both *T. theobaldi* reached the pupal stage. Then, the container was taken to the laboratory and all larvae were counted and identified.

Statistical analyses

Were counted the number of prey completing the larval stage, the number of prey which remained in the containers after the end of the experiment and the number of *T. theobaldi* eggs found during the tests.

Analysis was carried out using generalized linear models (GLM) with quasipoisson distribution. This distribution was used to correct the data over-dispersion diagnosed by residue analysis for the poisson distribution. All analyses were performed using statistical software R version 3.1.1 (R Core Team 2013).

Experiment 2: Record of larval behavior

C. iridescens and *L. durhamii* fourth instar larvae were collected in artificial containers placed in the Mata da Biologia and taken to the laboratory. Experimental containers consisted of 700-ml plastic pots with 200 ml of deionized water. *Cecropia* sp. dried and milled leaves were added to this water (2g/L), 24 hs before the start of the experiment. For each replication, one larva was placed inside the container. After a period of acclimatization (5 min) behaviors were record using a camera (Panasonic BP334), connected to a computer with the software (Ethovision XT). Manual behavior function was used to record the positions and activities. The activities were allotted into 3 categories: resting (not moving larva), browsing (movement using its mouthparts) and thrashing (movement through the water column by side flexations of the body) (Juliano & Reminger 1992). The positions were also categorized into three types: surface (larvae siphon in contact with the water-air interface), middle and bottom. After each run, the larva and the water were replaced. For each species, this procedure was repeated 20 times.

Statistical analyses

The time spent by the larvae in each activity and position were record and converted into percentage. We compare each behavior category among the two species. Analysis was carried out using generalized linear models (GLM) with quasibinomial distribution. This distribution was used to correct the data over-dispersion diagnosed by residue analysis for the binomial distribution. All analyses were performed using statistical software R version 3.1.1 (R Core Team 2013).

Results

Experiment 1: Larval development in the field

Culex iridescens

Predators had no effect for *C. iridescens* that completed larval development. The mean was 2.8 ± 0.3 individuals reaching pupal stage in treatment with predators and 0.7 ± 0.1 in the treatment without predators ($F_{1,19}$ = 3.33, p = 0.086) (figure 2.1A). At the end of the experimental period (when both predators larvae reached the pupal stage), the mean of *C. iridescens* larvae did not differ between the treatment with predators (97.1 ± 3.5 larvae), and the treatment without predators (83.2 ± 4.2 larvae) ($F_{1,19}$ = 0.64, p = 0.424) (figure 2.1B*).*

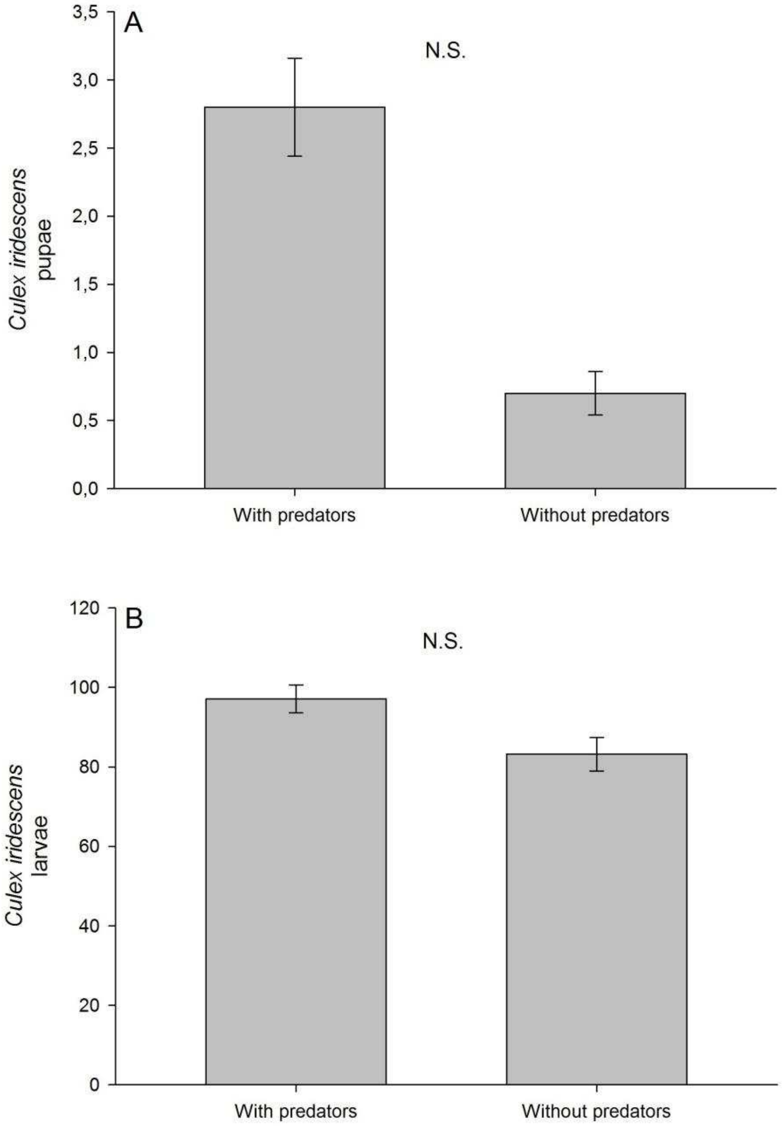

Figure 2.1: A) *Culex iridescens* individuals that reached the pupal stage during the experiment 1. B) *C. iridescens* larvae found inside the container in the end of experiment 1. Mosquitoes colonized the containers naturally in the field, containing one of two treatments: two predatory larvae (*T. theobaldi*) or no predators. Bars are means ± SE; *n*=10 replicates per treatment. ***p<0.001, **p<0.01, *p<0.05, N.S=not significant.

Limatus durhamii

Regarding the number of individuals who reached the pupal stage throughout the experimental period, the treatment with predators had a large effect ($F_{1,19} = 86.21$, $p < 0.001$), since the mean of individuals reaching the pupal stage in the treatment with predators (0.9 ± 0.1) was over 20 times less than the treatment with no predators (20.3 ± 0.87) (figure 2.2A). *L. durhamii* larval mean inside the containers in the end of experiment was also much lower (almost 40 times) in the treatment with predators (larvae 2.7 ± 0.2) compared to the treatment without predators (100.4 ± 2.7 larvae) ($F_{1,19} = 255.99$, $p < 0.0001$) (figure 2.2B).

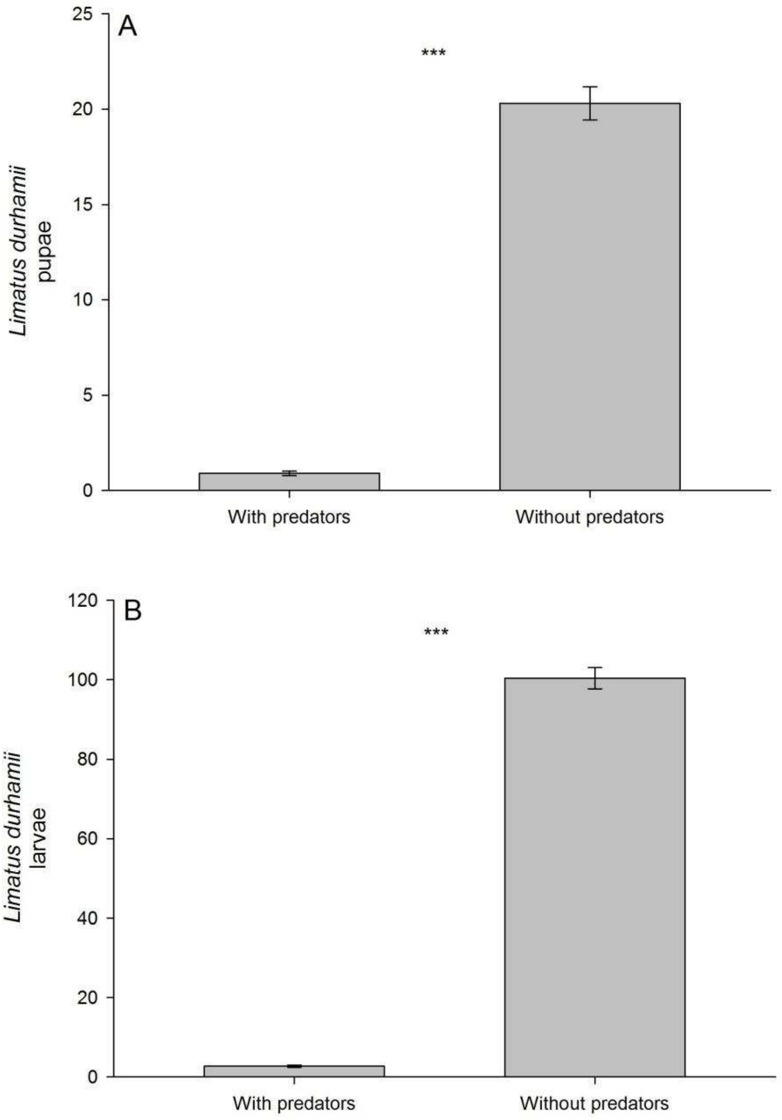

Figure 2.2: A) *Limatus durhamii* individuals that reached the pupal stage during the experiment 1. B) *L. durhamii* larvae found inside the container in the end of experiment 1. Mosquitoes colonized the containers naturally in the field, containing one of two treatments: two predatory larvae (*T. theobaldi*) or no predators. Bars are means ± SE; *n*=10 replicates per treatment. ***p<0.001, **p<0.01, *p<0.05, N.S=not significant.

Experiment 2: Larval behavior record

C. iridescens spent more time browsing compared to *L. durhamii*. However, *L. durhammi* spends about 2.5 times longer thrashing than *C. iridescens*. For the time at rest, there was no significant difference (Table 2.1) (Figure 2.4). Regarding the location of the larvae in the water column, significant differences were found for the three categories. *C. iridescens* have a preference for container surface while *L. durhamii* spends about 60% of the time on the bottom (Table 2.1) (Figure 2.5).

Table 2.1: Time spent in each activity and position by *C. iridescens and L. durhamii* larvae. Numbers are means (%) ± SE, with df, F and p values for the GLM analyses (quasibinomial distribution).

	Culex iridescens	*Limatus durhamii*	df	F	p
Activity					
Resting	31.56±0.533	29.77±0.589	38	0.2536	>0.05
Browsing	60.46±0.564	50.37±0.688	38	6.395	<0.05
Thrashing	7.98±0.150	19.87±0.357	38	54.617	<0.001
Position					
Surface	37.43±0.431	26.14±0.445	38	16.174	<0.001
Middle	27.86±0.303	13.61±0.232	38	68.573	<0.001
Bottom	34.72±0.465	60.25±0.483	38	68.541	<0.001

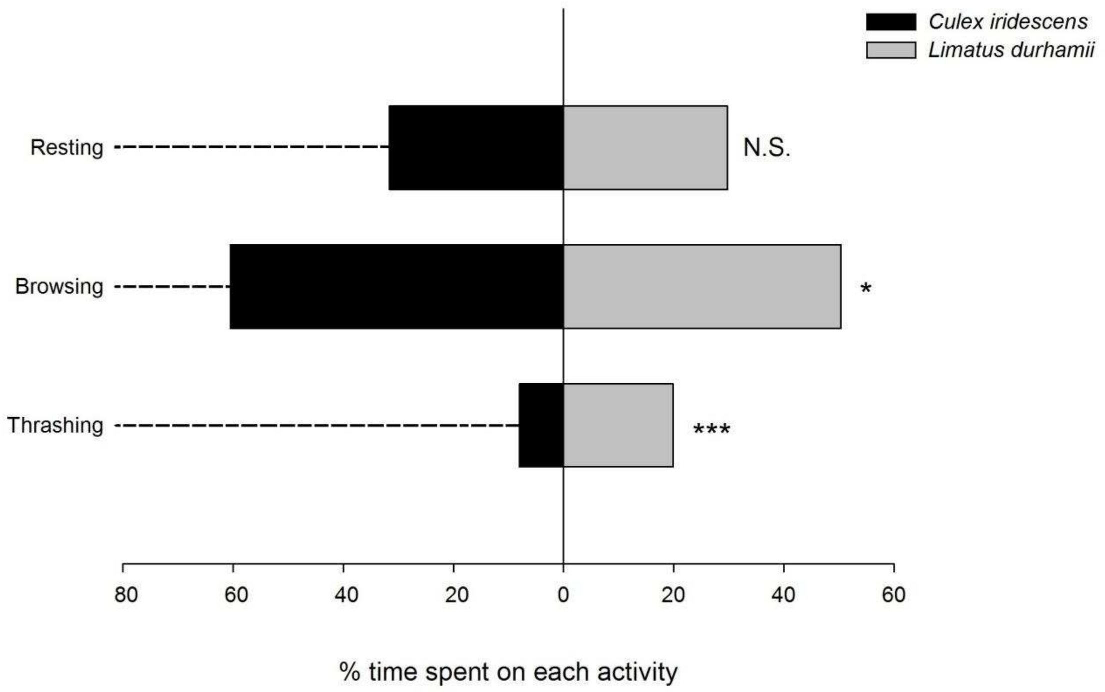

Figure 2.4: Percentage of time spent by *Culex iridescens* and *Limatus durhamii* larvae on each activity. Larval behavior was recorded for 10 minutes. Bars are means (black for *C. iridenscens* and gray for *L. durhamii*); *n* = 20 replicates per treatment for each species. ***p<0.001, **p<0.01, *p<0.05, N.S=not significant.

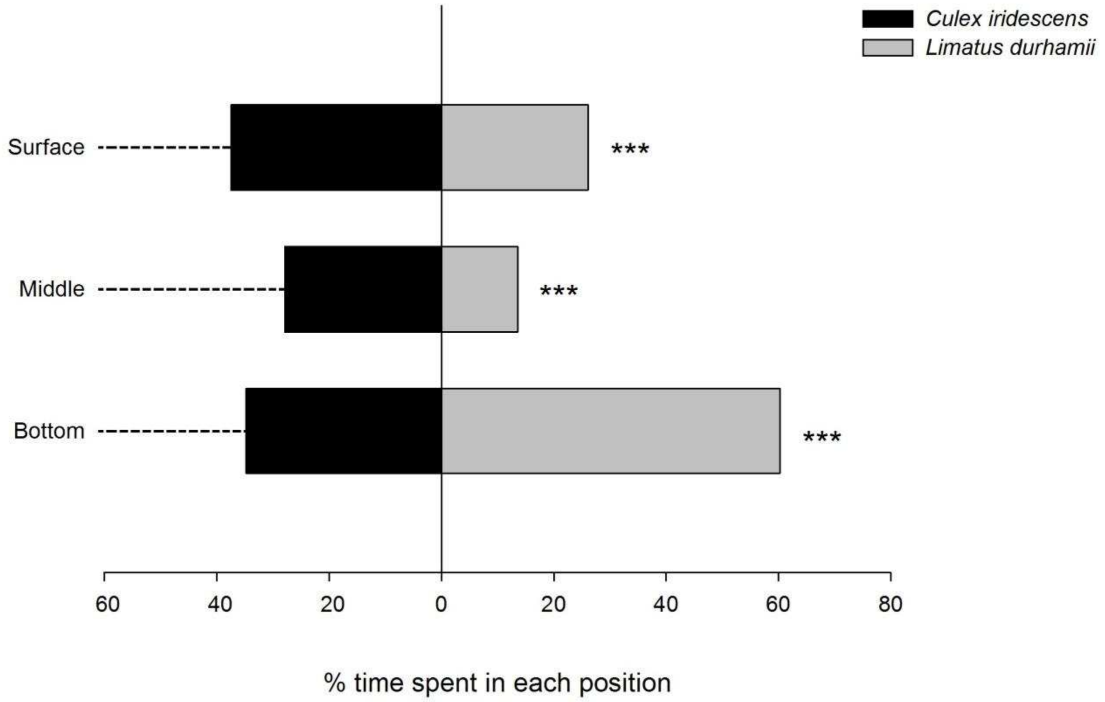

Figure 2.5: Percentage of time spent by *Culex iridescens* and *Limatus durhamii* larvae in each position. Larval behavior was recorded for 10 minutes. Bars are means (black for *C. iridenscens* and gray for *L. durhamii*); *n*=20 replicates per treatment for each species. ***p<0.001, **p<0.01, *p<0.05, N.S=not significant.

Discussion

The effect of *T. theobaldi* predatory larvae on the populations of prey species was very dissimilar. For *L. durhamii*, the presence of *T. theobaldi* drastically reduced the number of larvae that reached the pupal stage. Meanwhile, the number of *C. iridescens* larvae that reached the pupal stage was not affected by predation. A hypothesis that explains many cases of differential predation between prey species is the frequency-dependent predation (the effect of predation is stronger on the most abundant species). Although this effect is commonly shown in arthropods (Sherratt & Harvey 1993), it does not explain our results, because the abundance of the two species in the containers without predators was similar.

Since abundance of species seems not to interfere in the differential outcome of predation in this study, it is expected that the characteristics of prey species can explain the results. One of the prey traits that interfere in vulnerability to predation is the body size (Thompson 1975, Semlitsch 1989). For mosquitoes, Kumar & Rao (2003) demonstrated that predatory cyclopoid copepods preyed more heavily on first-instar mosquito larvae compared to medium-sized cladocerans. When fourth-instar mosquito larvae were offered, the preference of predators changed and cladocerans have become more vulnerable. However, it is likely that the body size of the prey is not a determining factor in our experiment, since the sizes of their prey species is similar and even a newly hatched *T. theobaldi* larva is able to prey on fourth-instar larvae of both prey species (personal observation).

Other prey features which may influence vulnerability to predation is the behavior (Lima & Dill 1990) and habitat use (Brown 1999). We found that *L. durhamii* and *C. iridescens* have significant differences with respect to these two characteristics. *L. durhamii*, use abrupt movements (thrashing) more frequently than *C. iridescens*, and spend more time on the bottom of the container. Juliano and Reminger (1992) observed the category of activity and the prey position (*Aedes triseriatus*) in the container immediately before capture by the predator (*Toxorhynchites rutilus*). They found that trashing and bottom are respectively the activity and position more dangerous for prey. As *Toxorhynchites* are ambush predators, is expected that more active larvae experience a greater risk of predation than those that avoid turbulent motions. Regarding the position, larvae utilizing shredding feeding mode, which feed on organic surfaces and sediments, tend to spend more time in the background, as is where is deposited most of these items (Merrit 1992). In this study the predator behavior has not been evaluated, but it is possible that *T. theobaldi* larvae remain on the bottom of the container more frequently when they are waiting for prey, because the probability of find larvae moving vigorously searching for food is greater on the bottom.

These findings contribute to a better understanding of the role of predation on mosquito communities, both for ecological theory and for biological control. The ability of predatory larvae of the genus *Toxorhynchites* in reducing mosquito populations has already been demonstrated in several studies (eg : Bradshaw & Holzapfel 1983, Lounibos *et al.* 1997, Adytia *et al.* 2006). The success of biological control efforts using *Toxorhynchites* is variable (Collins & Blackwell 2000), but the failure is not usually linked to compensating or overcompensating mortality. Most studies on *Toxorhynchites* as biological control agents have the *Aedes aegypti* as target. As *L. durhamii*, larvae of *A. aegypti* are shredded feeders and this seems to be a feeding behavior that imposes a great vulnerability against ambush predators. Thus, it is expected that the control of the collecting-filtering larvae using ambush predators is not appropriate. As seen for *C. iridescens*, a collecting-filtering larva, the predator had no effect on the population.

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CHAPTER 3

Fitness cost as indirect effects in container-dwelling mosquitoes

Abstract

Predation is an important selective force acting on in the evolution of organisms. Studies have shown that the mere threat of predation may be sufficient to modify the behavior and the phenotype of prey, affecting the fitness and the population dynamics. In preliminary studies, we observed that *Culex mollis* larvae reduce foraging time in the presence of chemical cues from the predatory *Toxorhynchites theobaldi* larvae. Here we analyze the possible fitness costs due to this behavioral change. Development of *C. mollis* larvae were evaluated in the presence of *a* free predator; a caged predator; and with no predator. Larvae reared in pots with caged predator had a longer development time comparing to the two other treatments. Treatments with predators, free or caged, influence the size of adult females, but not males. The presence of predators, even caged, resulting in higher mortality of prey. These results provide further evidence of the strength of non-consumptive effects on prey species.

Keywords: predation risk, non consumptive effects, mosquito.

Introduction

Predators capture, kill and consume their prey. This is the only approach considered in most studies of predator-prey interaction (Preisser & Bolnick 2008). However, recent investigations have been concerned with the indirect effects of predation. It has been shown that the fear or risk effects of predators (also known as nonlethal or non-consumptive effects) can have as great, or even greater, impacts on population regulation (Werner and Peacor 2003, Schmitz *et al.* 2004, Trussell *et al.* 2006, Creel & Christianson 2008).

Animals exhibit specific behavioral responses from a set of possible alternatives (Dill 1987). Thus, all the time, individuals are driven to take crucial decisions with consequences for their fitness (Conradt & Roper 2005). Under predation risk prey can change their behavior, reducing your chances of death. However, low risk behavior may have costs such as the decrease in foraging rate, resulting in high losses for individuals (reviewed in Lima & Dill 1990, Lima 1998, Preisser *et al.* 2005).

When a choice has costs and benefits in relation to one another there is a conflict, which in the context of behavioral ecology is characterized by tradeoff. And the tradeoff between growth and mortality is one of the classic examples of this. Studies have shown that, in a population, very active individuals with higher growth rates experience higher predation mortality (Werner & Anholt 1993, Anholt 1995, Mangel & Stamps 2001). This is because the search for food increases the individual exposure to predators. However, reducing exposure may result an energy deficit and damage to the development, since animals that change their diet or reduced food intake can experience malnutrition, reducing the survival and fecundity (Peckarsky *et al.* 1993, Schmitz 1997, Nelson *et al.* 2004).

As investigations on non-consumptive effects are recent, we know little about the relative importance of the attributes of predators, prey, and the environment in determining the strength of antipredator responses, and still less about the manner in which these responses carry costs that affect prey dynamics (Creel 2011). In aquatic habitats prey organisms frequently perceive predation risk by chemical cues, and exhibit diverse behavioral, physiological, morphological, and life-history responses to these cues (Larsson & Dodson 1993, Kats & Dill 1998, Lass & Spaak 2003).

In this chapter, we investigated some of these aspects on non-consumptive effects through laboratory experimental manipulations in artificial containers, using mosquito larvae. *Culex mollis* (Diptera: Culicidae) was chosen as prey and *Toxorhynchites theobaldi* (Diptera: Culicidae) as predator. Both species are native to South America and often found cohabiting natural and artificial containers. *Toxorhynchites* are primarily ambush predators, and prey is apparently detected by mechanoreceptors (Steffan & Evenhuis 1981, Russo 1986). Behavioral patterns of prey are, in short term, the main determinant of the likelihood of predation by *Toxorhynchites* larvae (Juliano *et al.*, 1993). In this case, more active individuals that move and feed more often, experience higher probability to be victims of predation (Russo & Westbrook 1986, Grill & Juliano 1996).

In a preliminary study, we observed that *C. mollis* stay longer at rest and avoid turbulent movements in the presence of *T. theobaldi* chemical cues. Here we tested the hypothesis these behavioral change, considered a low risk strategy, have costs for the prey fitness. Larvae of *C. mollis* were evaluated in three situations: in the presence of *T. theobaldi* free in container (predation was possible); in a cage inside the container (predation was not possible); and in the absence of predator. As measures related to fitness, we evaluated the larval development time, the size of the adults and the mortality rate, supposing that non-consumptive effects should influence the population dynamics of prey.

Material and Methods

Insects rearing

The study was conducted at the Laboratory of Semiochemicals and Insect Behavior, Department of Entomology, Federal University of Viçosa, MG, Brazil. *T. theobaldi* predatory larvae naturally co-occur with *C. mollis* in both natural and artificial containers. *T. theobaldi* larvae were reared and maintained at 26 ± 3 °C, 80 ± 15 % relative humidity and a 12:12-h light:dark (L:D) photoperiod, kept in 50-mL glass vials and fed with *A. aegypti* larvae ad libitum. *C. mollis* larvae were obtained from egg rafts collected in artificial containers placed in the woods of the Federal University of Viçosa. Rafts were individualized and larvae were used in the experiments 2 days after hatching.

Experimental setup

Sixty nine 750-mL polypropylene pots were filled with 500 mL deionized water and was added 1g of dried and milled *Cecropia* sp. leaves, to provide nutrients to larvae. Inside each pot has also been added a cylindrical cage of polypropylene (8 cm x 4 cm diameter) with the bottom covered by a net (100 microns). The cage allows the passage of water, chemicals and microorganisms, but blocks the passage of the larvae. The containers were incubated under insectary conditions (see above) for 48 h.

Containers were divide 23 sets of 3 containers. In each one were placed 30 *C. mollis* larvae in the first instar of development, outside the cage. All *C. mollis* larvae from each set were siblings. The pots were divided into three treatments: A) Free predator: 1 *T. theobaldi* (fourth instar larvae) outside the cage B) Caged predator: 1 *T. theobaldi* (fourth instar larvae) inside the cage. C) Control: No predator.

Larval development

C. mollis larvae were counted every day until they reach the pupal stage. The pupae were identified in separate glass vials. When emerged, adults were frozen for preservation.

Adult size

The left wings of all emerged adults were removed and photographed by a digital camera Leica TFC295 attached to the microscope stereoscopic Leica S8APO. The length of the wings was measured from the end of alula to the the distal edge of the wing, using the Leica Application Suite software.

Statistical analyses

Development time data were taken as the number of days it took for larvae to turns into pupae. For this, data were subjected to censored survival analysis with a Weibull distribution (Crawley 2007), performed with survival package in R (R Core Team 2013). Survival analysis, or failure time data analysis, means the statistical analysis of data where the response of interest is the time, t, from a well-defined time origin to the occurrence of some given event (end-point) (Martinussen & Scheike, 2006). In our case, the time origin is the moment that larvae were placed inside the containers, and the end point is the day that the larva turns into pupae. Adult wing length analysis was carried out using generalized linear models (GLM) with normal distribution (Crawley, 2007). Larval mortality analyses were performed using GLM with quasibinomial distribution. This distribution was used to correct the data over-dispersion diagnosed by residue analysis for the binomial distribution. All analyses were performed using statistical software R version 3.1.1 (R Core Team 2013).

Results

Larval development

Larvae reared in pots with caged predator had a longer development time (9.58 ± 0.28 days) comparing to the pots with free predator (7.99 ± 0.18 days) (p <0.0001). The difference between the average development time between the pots with a caged predator (9.58 ± 0.28 days) and without predator (8.48 ± 0.19 days) (treatments A and C) was not significant (p> 0.05) (Figure 3.1).

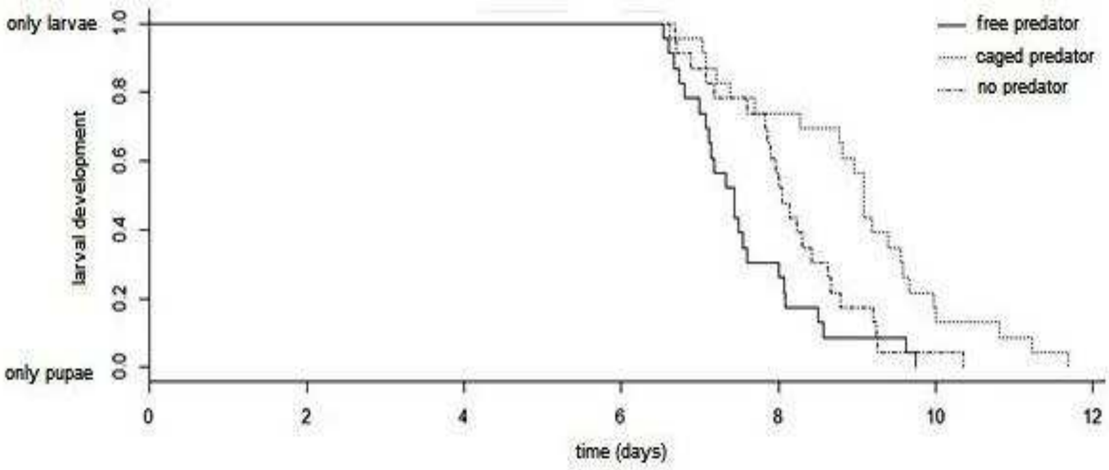

Figure 3.1: *C. mollis* larval development time comparison among all 3 treatments (free predator, caged predator and no predator). *Toxorhynchites theobaldi* fourth instar larvae were used as predators.

Adult size

Females that spent the larval phase in the absence of predators had the wing length (2.686 ± 0.016 mm) significantly higher ($F_{2,66}$ = 5.915, p = 0.004) than those reared in the presence of *T. theobaldi*, free (2.561 ± 0.035 mm) or caged (2.598 ± 0.033 mm)(Figure 3.2). For males, there was no significant difference ($F_{2,66}$ = 1.158; p = 0.320) when the wings length was compared among larvae reared with predator free (2.131 ± 0.019 mm), caged (2.123 ± 0.017 mm) or absent (2.155 ± 0.010 mm) (Figure 3.3).

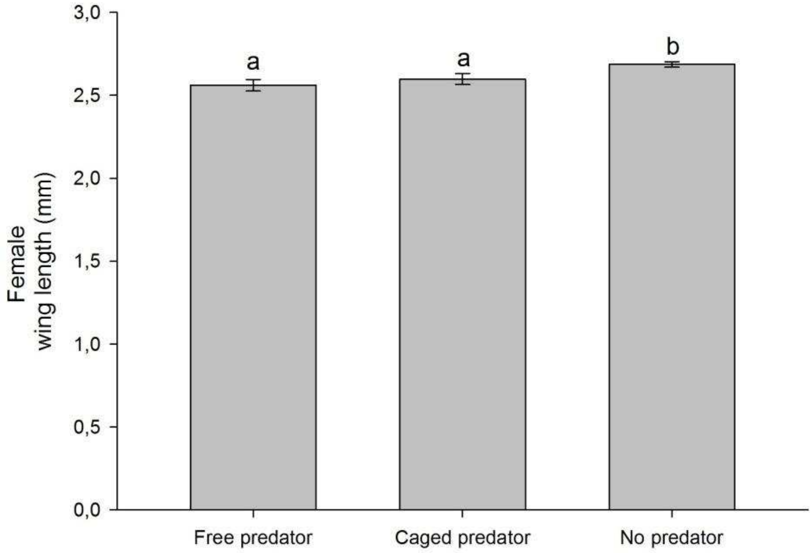

Figure 3.2: *C. mollis* female adult size. Bars indicate wing length means (mm) with standard error for all 3 treatments (free predator, caged predator and no predator). Different letters indicate significant differences among treatments (p<0.05).

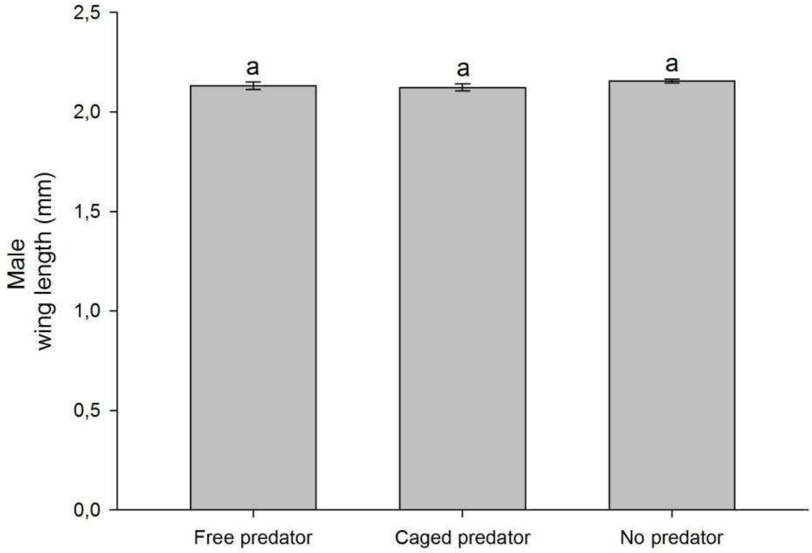

Figure 3.3: *C. mollis* male adult size. Bars indicate wing length means (mm) with standard error for all 3 treatments (free predator, caged predator and no predator). Different letters indicate significant differences among treatments (p<0.05).

Larval mortality

Larval mortality was higher in the free predator treatment (53.6 ± 4.7 %), followed by the treatment with caged predator (14.6 ± 1.1%). When the predator was absent, a lower mortality rate was observed (8.1 ± 1.0 %) ($F_{2,66}$ = 118.298; $p < 0.001$) (Figure 3.4).

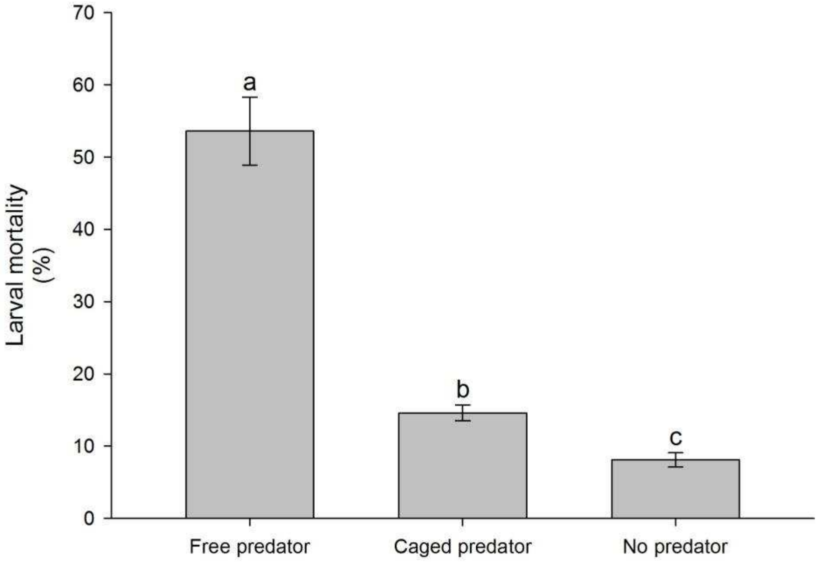

Figure 3.4: *C. mollis* larval mortality. Bars indicate bars dead larvae means (%) throughout the experiment with standard error for all 3 treatments (free predator, caged predator and no predator). Different letters indicate significant differences among treatments (p<0.05).

Discussion

In previous experiments, it was observed that *C. mollis* remained at rest in average 30% longer in the presence of possible *T. theobaldi* chemical cues, compared to the control (pure water) (unpublished data). Therefore, it was expected that *C. mollis* had a considerable delay in development time in predator presence, because of shorter time spent in foraging activities. However, in this study, there was no difference in the duration of the larval stage, comparing caged predator to no predator treatments. One cue for this result would be the *C. mollis* feeding mode. Like other species of the subgenus (*Culex*), *C. mollis* has collecting-filtering behavior, feeding on microorganisms and small suspended particles in the water column (revised in Merrit 1992). In a more detailed observation is remarkable that even at rest, *C. mollis* moves the mouth parts, probably creating feeding currents and ingesting small particles. Thus, even at rest, *C. mollis* is likely to be able to feed efficiently, minimizing the development costs in the presence of predator cues. Regarding to the shorter development time comparing the treatment with free predator to the other treatments, one explanation is the increased availability of nutrients and less intraspecific competition for *C. mollis*, when the consumption by *T. theobaldi* is allowed. Larval predation by *Toxorhynchites* increases the availability of micro-organisms in the environment (Albeny 2014), which are a food source for the prey larvae. Furthermore, as many *C. mollis* are consumed, a reduced number of larvae share the same resources, alleviating the competition and reducing development time for survivors (Preisser *et al.*, 2009).

Although we do not found non-consumptive effects in the larval development time, a possible effect of predation risk can be found when we analyzed the size of adults. Using the length of the wings as an estimate it was observed that adult females emerged from containers without predator had wings significantly longer in relation to the treatments with predator. This result is consistent with the theory of trade-off predation risk/growth. When under predation threat, individuals may exhibit growth deficiency (Benard 2004) due to factors such as reduced intake of nutrients and stress. However, the same result was not observed in adult males. Sex-biased predation is a widespread phenomenon and has been found in a number of animals. Additionally, malebiased predation is more common than female-biased predation: among 81 predator-prey pairs, male bias was 2.3 times as common as female bias (Boukal *et al.* 2008). This can be related to behavioral differences and perceived risk between genders. For example, male damselfly larvae are more active than female larvae (Mikolajewski 2005). For snails, females are able to detect chemical cues related to predation more efficiently than males, showing antipredator behaviors more frequently, being less vulnerable to predation, compared to males (Xu *et al.* 2014). Although behavioral differences between genders were not investigated in our experiments, perhaps *C. mollis* females are more careful than males in predation risk situations, resulting in adult females with small size. Furthermore, males develop considerably faster than females, spending less time under threat of predation. This reduced contact time in the larval stage may not be enough sufficient to cause a significant response in the size of adult male.

The treatments with free predator had the highest mortality rate, which was already expected due to the consumption of *C. mollis* larvae by *T. theobaldi*. However, even when the predator was caged, the mortality rate was increased 1.8 times compared to the treatment with no predator. This difference in mortality rate can be attributed to the non-consumptive effects. In a study of dragonfly larvae (*Leucorrhinia intacta*) reared in the presence of caged predators, McCauley *et al.* (2011) found that prey mortality increased 2.5 times in the presence of *Anax junius* and more than four times in the presence of fish in laboratory experiments. The mechanisms responsible for increased mortality in predation risk situations are not known, but some studies provide evidence of how it occurs. An important factor is associated with stress. Stress response to the presence of predators has been demonstrated in numerous animals (Hawlena and Schmitz 2010). Stress involves increases in stress hormones (Travers *et al.* 2010) and can negatively affect animals in a variety of ways, including compromised immune response and antioxidant defense, (Slos *et al.* 2009) and altered behavioral patterns involving trade-offs that affect survival (Werner and Peacor 2003, Preisser *et al.* 2005), and may generate a cascade of negative physiological responses. Organisms under stress are often more vulnerable to a range of mortality causes that often interact synergistically. For example, a small nutritional deficit caused by decreased foraging that would not prejudice the organism, could become lethal if combined with the cell damage caused by oxygen reactive species or pathogens confronting a weakened immune system. Another example of synergism is the interaction between predation risk and pesticides. The addition of predator chemical cues to sub lethal concentrations of common pesticides causes a large increase in mortality of amphibian larvae (Sih *et al.* 2004) and crustaceans (Qin *et al.* 2011).

Our experimental results suggest that behavioral changes induced by predation risk have costs for the mosquito larvae fitness and can be an important component for structuring communities and ecosystem processes. Also a better understanding of non-consumptive effects in vector-borne diseases could stimulate the study and development of new control alternatives for these insects. As demonstrated in this and other studies, only the threat of predation can be lethal to organisms. If this response is triggered by chemicals, the identification of these compounds can be an opportunity for the development of a new class of insecticides, applying ecological knowledge to minimize environmental problems.

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GENERAL CONCLUSIONS

The importance of predators in the animal communities living in containers, especially in relation to mosquitoes is today a controversial issue. Are the effects of predation in containers as strong as in aquatic environments of high volume? The results presented in this study suggest that predators can have a major impact on these systems. In Chapter 1 we demonstrated that it is not the type of environment (containers or pools), but the habitat size that influences the frequency and abundance of predators. Although rare in nature, artificial containers of large volume are fairly distributed in urban environments. Depending on the size and location, containers can be permanent bodies of water and function as pools, with substantial influence of predators. In addition, some predators such as *Toxorhynchites* can be found frequently in small containers. And as demonstrated in Chapter 2, these predators can have a great impact on prey populations. Among other factors, this vulnerability to predation can be directly related to the behavior of the species. Therefore, a prediction of the outcome of predator-prey interaction can be provided only by knowing the behavior of the species. In the Chapter 3, we found that predators can interfere with the population dynamics of prey, without consuming a single individual. These results, combined with other studies that also show non-consumptive effects of predators in mosquitoes, provide new perspectives for the use of predation lanes in integrated mosquito control programs. I hope this thesis contributes in some way in understanding the nature of mosquitoes and their interactions with the environment in the search to soften the damage caused by these insects.