DANIEL ALBENY SIMÕES

PREDADOR E PRESA DE CULICIDAE: A INTERAÇÃO EM MICROCOSMO AQUÁTICO MEDIADA POR BACTÉRIAS

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: 31 de Julho de 2012.

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Dedicatória

À família pelo apoio imensurável
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e à minha mãe Milene e à minha irmã Tatiana

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Biografia

Daniel Albeny Simões, filho de Milene Albeny Roque Simões e Clóvis Simões, nasceu no municipio de Coronel Fabriciano, estado de Minas Gerais, às 22:28 minutos no dia 14 de Abril do ano de 1980.

Em Julho de 2004, graduou-se em Ciências Biológicas Licenciatura Plena, pelo até então Centro Universitário do Leste de Minas Gerais (UNILESTE MG), Ipatinga-MG.

Durante o período de graduação, de 2000 a 2004 foi estagiário do Laboratório de Estudos em Invertebrados (LEI) do Curso de Ciências Biológicas (CCB).

Em Março de 2005 iniciou a especialização em Saúde Pública e Meio Ambiente pelo UNILESTE MG, defendendo o trabalho de conclusão de curso em Março de 2006.

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Resumo

SIMÕES, Daniel Albeny, D. Sc., Universidade Federal de Viçosa, julho de 2012. **Predador e presa de Culicidae: a interação em microcosmo aquático mediada por bactérias.** Orientador: Evaldo Ferreira Vilela. Coorientadores: Eraldo Rodrigues de Lima, Gustavo Ferreira Martins e Simon Luke Elliot.

Em criadouros de mosquitos a riqueza ou abundância de micro-organismos pode ser afetada negativamente pela presença de larvas dos mesmos. Contudo, efeitos indiretos de predadores sobre micro-organismos não são bem conhecidos. Sabe-se que fêmeas de mosquitos são atraídas para ovipositar em criadouros onde micro-organismos são abundantes. A resposta destas fêmeas a estes sinais de oviposição é uma importante parte da ecologia e controle de mosquitos. Esta tese é composta por dois capítulos desenvolvidos através de um desenho experimental laboratorial. Capítulo I: Larvas do predador *Toxorhynchites rutilus* se alimentam de larvas de *Aedes triseriatus*, os quais se alimentam de bactérias. Nós postulamos que surge uma cascata trófica da predação de larvas de *A. triseriatus* por *T. rutilus* impactando a abundância bacteriana. Como predito abundância bacteriana foi maior nos tratamentos com predação do que nos demais. Abundância bacteriana não diferiu entre os tratamentos com a presença do predador e aqueles com sinais de predação. Foi testada a hipótese de que presas comidas parcialmente estavam presentes nos tratamentos com o predador e nos tratamentos que receberam os sinais de predação, aumentando assim a abundância bacteriana. Surpreendentemente a abundância bacteriana foi maior em tratamentos onde houve a predação real do que nos demais. Foi sugerido que fezes (tanto do predador quanto das presas) ou ainda redução do forrageamento das presas, induzido por sinais do predador, contribuíram para o cresimento bacteriano. Capítulo II: A espécie invasiva *Aedes aegypti*, geralmente oviposita em recipientes com grande abundância de nutrientes. Contudo, diferentemente de outras espécies de mosquitos *A. aegypti* parece não perceber sinais de predadores como larvas de *Toxorhynchites*, e no entanto não evita ovipositar em locais onde estes predadores estão presentes. Se a predação por *Toxorhynchites* pode potencialmente aumentar a abundância bacteriana em criadouros, seja pela redução do número de larvas de mosquitos ou pela adição de substratos que permitem o crescimento bacteriano, e desde que *A. aegypti* prefira ovipositar onde bactérias, as quais são alimento para larvas, são abundantes, é possível que *A. aegypti* oviposite em recipientes onde *Toxorhynchites* são abundantes. Foi conduzido um experimento laboratorial no qual à fêmeas de *A. aegypti* foram oferecidos dois locais para oviposição, combinados em 6 tratamentos. Fêmeas de *A. aegypti* preferiram ovipositar em recipientes onde houve predação ativa e predação simulada, não exibindo nanhuma preferência de oviposição para os demais tratamentos. Estes resultados suportam a hipótese de que fêmeas de *A. aegypti* não são atraídos para locais com coespecíficos ou *Toxorhynchites* por sí, mas são atraídas para locais com grande abundância bacteriana.

Abstract

SIMÕES, Daniel Albeny, D. Sc., Universidade Federal de Viçosa, July, 2012. **Predator and prey of Culicidae: the microcosm aquatic interaction mediated by bacteria** Adviser: Evaldo Ferreira Vilela. Co-Advisers: Eraldo Rodrigues de Lima, Gustavo Ferreira Martins and Simon Luke Elliot.

Feeding by container-dwelling mosquito larvae may negatively affect microorganism richness or abundance. However, indirect effects of predators of mosquitoes on microorganisms are poorly studied. It is known that mosquito females are attracted to oviposit in high microorganism abundance containers. The response of ovipositing mosquitoes to chemical cues is an important part of mosquito ecology and control. This thesis consists of two chapters developed through experimental laboratory design. **Chapter I:** Larvae of the predator *Toxorhynchites rutilus* prey on larval *Aedes triseriatus*, which feed on bacteria. We postulated that a trophic-cascade arises from *T. rutilus* predation on *A. triseriatus* larvae, impacting bacterial abundance. As predicted, bacterial abundance was greater in the predator treatment than in others. Bacterial abundance did not differ between predator and predatory cues treatments. We hypothesized that predator and predatory cues treatments contain partially eaten prey, increasing bacteria abundance. Surprisingly, bacterial abundance was greater with real predation than in all other treatments. We suggest that feces (from either predator or prey) or reduced foraging induced by predator cues contributed to bacterial growth.

Chapter II: The highly invasive *Aedes aegypti* preferentially oviposits in containers with high nutrient abundances; however, unlike many native species, *A. aegypti* does not appear to detect chemical cues of predaceous *Toxorhynchites* larvae, and therefore does not avoid ovipositing at sites containing these predators. Since predation by *Toxorhynchites* can potentially increase bacterial abundance in containers by reducing numbers of mosquito larvae and by adding substrates for bacterial growth, and since *A. aegypti* may prefer to oviposit where bacteria, which are the food of larvae, are abundant, it is possible that *A. aegypti* preferentially oviposit in containers where *Toxorhynchites* are abundant. We conducted a laboratory study in which gravid *A. aegypti* were offered two oviposition sites, combined in one of 6 treatments. Female *A. aegypti* preferentially oviposited in containers with active *T. theobaldi* predation, and also preferentially oviposited in containers with crushed *A. aegypti*, but displayed no oviposition preference in any other treatments. Our study supports the hypothesis that ovipositing *A. aegypti* are not attracted to sites with conspecifics or *Toxorhynchites* per se, but rather are attracted to sites with greater bacterial abundance.

1 Introdução Geral

Detritos orgânicos, principalmente folhas em decomposição, é um componente crítico de diversos habitats larvais, formando a base de muitas teias alimentares (Merritt *et al.*, 1992; Moore *et al.*, 2004). Contudo, microorganismos como bactérias, protozoários e fungos tem um importante papel na ciclagem e quebra de grandes moléculas tais como a celulose, lignina e quitina (Sinsabaugh & Linkins, 1990) tornando-as mais facilmente assimiláveis para organismos aquáticos tais como larvas de mosquitos (Merritt *et al.*, 1992), principalmente aquelas pertencentes à família culicidae. Em decorrência disto, comunidades microbianas decompositoras apresentam uma relevante contribuição para a dieta de larvas de culicídeos, que acabam por ingerir-las juntamente com detritos orgânicos (Merritt *et al.*, 1992; Cochran-Stafira & von Ende, 1998; Kaufman *et al.*, 1999; Eisenberg *et al.*, 2000). Neste sistema, larvas de mosquitos juntamente com microorganismos (protozoários, fungos e bactérias) formam uma teia trófica onde mudanças na sua estrutura e diversidade são preditas como afetando o fluxo de nutrientes e energia através dos níveis tróficos (de Ruiter *et al.*, 2005). Contudo, larvas de mosquitos são capazes de causar alteração na biota microbiana de criadouros (Walker *et al.*, 1991; Merritt *et al.*, 1992; Kaufman *et al.*, 1999; Kneitel & Miller, 2002; Trzcinski *et al.*, 2005).

Em microhabitas aquáticos (ex: criadouros de mosquitos), a presença de um predador de topo é reconhecida como um importante fator na estruturação de comunidades (revisado em Juliano (2009)). Em um sistema produtores-consumidores-predador a predação pode reduzir a abundância de consumidores que são competidores superiores (consumo direto) (Dolan & Gallegos, 1991; Eitam & Blaustein, 2010; Schwenk *et al.*, 2010) ou ainda desencadear alteração comportamental nos consumidores no sentido de se evitar o predador (Werner, 1992; Werner & McPeek, 1994; Werner & Peacor, 2003), tendo um efeito positivo indireto sobre competidores menos adaptados ou ainda sobre riqueza/abundância de produtores (Cochran-Stafira & von Ende, 1998; Kneitel & Miller, 2002). Este processo pode então aumentar a riqueza e/ou a abundância de espécies de uma comunidade (Paine, 1966).

Efeitos não destrutivos que um predador pode exercer sobre a estruturação de uma comunidade são denominados efeitos indiretos da predação (Werner & Peacor, 2003; Preisser *et al.*, 2005) e tem sido levados em consideração em diversos estudos posteriores às equações predador-presa sugeridas por Lotka-Volterra (Volterra, 1928) onde discute-se que os efeitos de predadores sobre a população de presas são diretamente relacionados ao consumo direto de presas pelos predadores. Contudo, nos dias atuais, sabe-se que efeitos indiretos da predação podem até ser maiores que aqueles relacionados ao consumo direto (Werner, 1992; Werner & McPeek, 1994; Lima, 1998; Werner & Peacor, 2003; Preisser *et al.*, 2005).

Em relação a família Culicidae, trabalhos demonstram que a percepção do risco de predação induz alterações comportamentais nas larvas de algumas espécies de mosquitos (Kesavaraju & Juliano, 2004; Kesavaraju *et al.*, 2007).

Tais alterações podem ter um efeito negativo no *fitness* do indivíduo (ex: diminuição do movimento e consequentemente na obtenção de alimento). Estas alterações comportamentais geralmente apresentam custos, pois reduzem o tempo e a energia que poderiam ser empregadas em forrageamento (Relyea & Werner, 1998; Van Buskirk, 2000; Stoks *et al.*, 2005). Contudo, espera-se que, em um sistema bactérias-larvas de mosquitos-predador, a diminuição do consumo individual de alimento pelas presas (larvas de mosquitos) devido à ameaça de predação (ex: presença física do predador ou pistas químicas que indiquem a presença do mesmo) poderá afetar a abundancia dos níveis tróficos inferiores (ex: bactérias).

Por outro lado a alteração da abundância bacteriana em criadouros de mosquitos pode afetar o comportamento de oviposição dos mesmos (revisado em Ponnusamy *et al.* (2010)). Algumas espécies são capazes de avaliar o possível local de oviposição antes de decidir ovipositar e este comportamento pode maximizar sua prole. Assim, ambientes com alta produtividade bacteriana indicam para a fêmea um local ideal para o desenvolvimento de suas larvas (Revisado em Bentley & Day (1989)). Contudo, se um predador é capaz de alterar a estrutura trófica presente em criadouros de mosquitos, isso possivelmente terá um efeito sobre a preferência de oviposição de fêmeas.

Em decorrência da atratividade exercida sobre fêmeas que procuram um local para depositar seus ovos, a abundância, biomassa e a composição de bactériana em criadouros naturais e artificiais tem sido sugeridas como sendo importantes determinantes ambientais da ocorrência, distribuição e abundância de mosquitos (Ponnusamy *et al.*, 2010; Murrell *et al.*, 2011). Contudo evidências indicam que outros determinantes ambientais são levados em consideração por fêmeas antes da oviposição. Por exemplo, algumas espécies de mosquitos podem evitar oviposição em criadouros onde predadores estão presentes ou onde há risco de predação para a sua prole (Chesson, 1984; Blaustein *et al.*, 1995; Angelon & Petranka, 2002; Blaustein *et al.*, 2004, 2005; Arav & Blaustein, 2006; Silberbush *et al.*, 2010). Emtretanto, existem espécies que não evitam ovipositar em criadouros onde predadores estão presentes (Dam & Walton, 2008; Hurst *et al.*, 2010) e ainda existem algumas espécies que são atraídas por criadouros onde predadores estão presentes (Torres-Estrada *et al.*, 2001; Pamplona *et al.*, 2009). Contudo, acredita-se haver um balanço entre escolher um local ideal para o desenvolvimento de seus descendentes, e evitar ou não a presença de possíveis predadores, principalmente se é levada em consideração a possibilidade de que um predador indiretamente possa afetar a abundancia bacteriana em criadouros.

Esta tese é composta por dois capítulos desenvolvidos através de experimentações laboratóriais realizadas em microcósmos artificiais e envolvendo as espécies de mosquitos *Aedes triseriatus* e *Aedes aegypti* e as espécies de predadores *Toxorhynchites rutilus* e *Toxorhynchites theobaldi.*

O capítulo 1 aborda os efeitos diretos e indiretos do predador de topo *Toxorhynchites rutilus* sobre as populações do mosquito *Aedes triseriatus* e bactérias, respectivamente, em criadouros artificiais. Foram testadas as hipóteses de que (A) a adição de larvas predadoras (*Toxorhynchites*) ou (B) sinais químicos de predação ao sitema bactérias-consumidores (larvas de mosquito)-predador, diretamente reduziria a abundância de consumidores (via predação direta), ou poderia desencadear mudança comportamental nos consumidores, consequentemente afetando diretamente a abundancia bacteriana. Foi predito que o ato de predação e sinais de predação poderiam adicionar materia orgânica animal ao sistema, criando um ambiente ideal para o crescimento bacteriano.

O capítulo 2 aborda os efeitos diretos e indiretos da predação de larvas do mosquito *Aedes aegypti* por larvas predadoras de *Toxorhynchites* sobre o comportamento de oviposição de fêmeas grávidas de *A. aegypti*. Foram testadas as hipóteses de que (A) Fêmeas grávidas de *A. aegypti* são atraídas para ovipositar em recipientes onde o predador *T. theobaldi* esteve presente, (B) Esta atração é resultado do aumento da abundância bacteriana nestes recipientes. Foi predito que (A) *A. aegypti* iria preferir recipientes onde ocorreu a predação real (*T. theobaldi* se alimentanto de *A. aegypti*) ou predação simulada (larvas de *A. aegypti* mortas mecanicamente), (B) Esta atratividade desapareceria com a adição de antibiótico ao sistema, (C) Tratamentos preferidos para oviposição por *A. aegypti* teriam maior abundância bacteriana que tratamentos não preferidos.

Chapter I

A trophic cascade effect of *Toxorhynchites rutilus* (Diptera: Culicidae) predation on aquatic bacteria

Resumo

Feeding by container-dwelling mosquito larvae may negatively affect microorganism richness or abundance. However, indirect effects of predators of mosquitoes on microorganisms are poorly studied. Larvae of the predator *Toxorhynchites rutilus* prey on larval *Aedes triseriatus*, which feed on bacteria. We postulated that a trophic-cascade arises from *T. rutilus* predation on *A. triseriatus* larvae, impacting bacterial abundance within water-filled containers. Experimental microcosms (450ml plastic cups holding 300ml of deionized water) received 100ml of oak leaf infusion (35mg/L, 9 days old) as a bacterial source and 100 first-instar *A. triseriatus* larvae. Treatments were: prey alone; prey with one *T. rutilus* larva; and prey with water-borne predatory cues, which consisted of water that had held one *T. rutilus* larva feeding on *A. triseriatus* larvae for 5 days. Controls were: infusion alone; or infusion plus predatory cues. We measured bacterial production via 3H leucine incorporation every 7 days 14 days. As predicted, bacterial abundance was greater in the predator treatment than in others. Bacterial abundance did not differ between predator and predatory cues treatments. We hypothesized that predator and predatory cues treatments contain partially eaten prey, increasing bacteria abundance. We tested this in an experiment in which *A. triseriatus* were either crushed, crushed and removed, or subject to real predation. Controls received 100 first instar *A. triseriatus* larvae. Surprisingly, bacterial abundance was greater with real predation than in all other treatments. We suggest that feces (from either predator or prey) or reduced foraging induced by predator cues contributed to bacterial growth.

Keywords: *Aedes triseriatus*, *Toxorhynchites*, predation, trophic cascade.

2 Introduction

Although the trophic cascade term has first coined by Paine (1980), its concept already appeared in Darwin's view that plants and animals *"are bound together by a web of complex relations"* (Darwin, 1859). May (2001) postulated that species interactions may be related to trophic links, and the number of such links per consumer measures its connectedness. However, because trophic cascades have been defined in several different ways (Persson, 1999), we define them as a direct or indirect effects of predators on prey that alter the abundance of the basal level population through the food web system (Persson, 1999).

There are in the literature many examples of trophic cascades within different systems (Begon *et al.*, 2006). In aquatic detritus based systems, addition of a second level trophic to the system (i.e. mosquito larvae), negatively impacts the abundance of the basal trophic level [e.g. rotifers (Kneitel & Miller, 2002), Kinatoplastids (Trzcinski *et al.*, 2005), microeukaryotes (Kaufman *et al.*, 1999) and bacteria (Walker *et al.*, 1991)]; these effects exist in natural and artificial systems, and under laboratory and field conditions.

It is known that in a multi-species system (i.e. producers-prey-predators) keystone predators, by reducing the abundance of prey, may have indirect positive effects on the lower trophic level organisms, possibly increasing their abundance or preventing predation by prey (Paine, 1966). Thus, predators can affect the prey density by both i) direct consumption "density-mediated interaction - **DMI**", described as: (direct impact on the prey density and indirect impact on the third-level food chain of the trophic cascade - Density mediated by indirect interaction **DMII**) and by ii) simulating costly defensive traits "trait-mediated interaction - **TMI**", described as: (changes on the prey behavior causing indirect impact at the third-level food chain of the trophic cascade - Trait mediated by indirect interaction **TMII**) (*reviewed from* Werner & Peacor (2003); Preisser *et al.* (2005)).

DMI are well described in a variety of systems. For example: Rotifers predators reduced microflagellates density (DMI) and indirectly increased the bacterioplankton abundance (DMII) (Dolan & Gallegos, 1991). In an artificial pool experiment the density of the preferred prey *Daphnia magna* decreased with the predator *Notonecta maculata* density, while densities of the smaller cladocerans *Moina brachiata* and *Cerio-daphnia spp.* increased (Eitam & Blaustein, 2010). This same pattern was observed in an birds-arthropods-host plants system where the reduction of herbivores arthropods abundance by birds predation, indirectly reduced the striped maple *Acer pensylvanicum* leaf damage (Schwenk *et al.*, 2010).

In the last decades studies have shown that the non-destructive effects of predators on the prey population (i.e., TMI) might be greater than consumption effects (Werner, 1992; Werner & McPeek, 1994; Lima, 1998; Preisser *et al.*, 2005), perhaps because predation risk perception by prey induces behavioral changes such as reduction of foraging and consequently, food consumption (Preisser *et al.*, 2005). For example, in order to avoid the predator wolf spiders (Lycosidae) (TMI), grasshoppers *Eritettix simplex* and

Melanoplus femurrubrum, reduced their grass consumption in the presence of mouthparts-glued spiders (TMII) (Schmitz *et al.*, 1997). In the Amazon forest, in a four-leveled trophic system, the myrmecophyte *Hirtella myrmecophila* (Chrysobalanaceae) hosts an ant *Allomerus octoarticulatus* that protects the plant against herbivory. In the presence of the spider *Dipoena bryantae* (specialized ant predator) ants reduced foraging, allowing herbivores to feed on the plant leaves (Rosa & DeSouza, 2011).

In general, trophic cascades were well studied in plant-herbivores-predator system and several are the examples, such as plant-beetles-ants (Messina, 1981), plant-caterpillars-ants (Fritz, 1983), plant-grasshoppers-spiders (Schmitz *et al.*, 1997; Schmitz, 1998; Gastreich, 1999) etc. Moreover, in the aquatic environment, the largest literature body is mostly related to streams [e.g. algal resources-mayflies-fishes (McIntosh & Townsend, 1996), algal resources-mayflies-stonefly (Peckarsky & McIntosh, 1998; Diehl *et al.*, 2000)] and ponds [e.g. isopods-salamanders-fishes (Huang & Sih, 1991), zooplankton-bluegill-piscivorous bass (Turner1990)] systems. However, trophic cascade effects, specially those related to TMII, in small water bodies such as, phytotelmata (water-filled depressions in or on plants, such as tree-holes) and artificial containers (man-made, such as used automobile tires and plastic buckets), have been receiving less attention. Although investigations of how detritus-derived productivity affects species richness and abundance in different trophic levels (producers and consumers) within natural (Yee *et al.*, 2007b; Yee & Juliano, 2007) and artificial (Yee & Juliano, 2006) mosquito breeding places was well defined, no studies has examined the role of an keystone predator in a three level trophic system (microorganism-grazer-predator), affecting both, microorganism community and detritus decomposition.

Detritus is a source of energy and nutrients to living organisms supporting many food webs systems (Wetzel, 1995; Moore *et al.*, 2004). It can support higher diversity, larger predator biomass, longer food chains (Hairston Jr & Hairston Sr, 1993) and it can stabilize the dynamics of consumer population, alter habitat complexity, stabilize food webs (Moore *et al.*, 2004) and alleviate or promote competition among organisms (Yee *et al.*, 2007b; Murrell & Juliano, 2008; Juliano, 2009). In aquatic systems organic detritus, especially decomposing leaves, are considered a critical component of habitats forming the basis of several food webs (Merritt *et al.*, 1992; Moore *et al.*, 2004). Microorganisms such as bacteria, protozoans and fungi also play key roles in nutrient cycling and breakdown of large molecules such as cellulose, chitin, and lignin, into smaller compounds (Sinsabaugh & Linkins, 1990) that can be more easily assimilated by others aquatic animals, such as mosquito larvae (Merritt *et al.*, 1992), having a relevant contribution to their diet (Merritt *et al.*, 1992; Cochran-Stafira & von Ende, 1998; Kaufman *et al.*, 1999; Eisenberg *et al.*, 2000). This detritus-bacteria-grazers-predators system constitutes a four-level trophic web system where changes in structure and diversity are predicted to change the nutrient flow and energy throughout the trophic levels and consequently change its structure (de Ruiter *et al.*, 2005).

Our experimental system (container mosquitoes), compared with other systems (e.g. lakes, streams and rivers), has a less complex food web where trophic cascades are predicted as likely occurring (Strong, 1992). Furthermore, containers mosquitoes are an ideal system for investigating detritus-based trophic cascades, because such containers are small and can be abundant, are easily replicated in the lab setting, have well-defined borders, simple food webs, restricted movement and fast temporal dynamics (Blaustein & Schwartz, 2001; Srivastava *et al.*, 2004). The physical boundaries between air-water in these systems represents a natural constraint for biota, which facilitates the addiction or removal of species, or even the reassembly of the entire community from scratch (Blaustein & Schwartz, 2001; Srivastava *et al.*, 2004). In addition, bacterial production can be quantified (Yee & Juliano, 2006; Yee *et al.*, 2007a) and the trait-mediated effects of predators on larval mosquito behavior can be manipulated (Kesavaraju & Juliano, 2004; Kesavaraju *et al.*, 2007, 2011).

Tree hole food web system in United States usually host a four-level trophic cascade, with detritus supporting bacteria growth, which in turn supports invertebrate consumers such as the tree hole mosquito larvae *Aedes triseriatus* (Say) (Yee *et al.*, 2007a). This mosquito species is perhaps the most common tree hole mosquito distributed throughout the eastern United States (Bradshaw & Holzapfel, 1985). Larvae of the mosquito *Toxorhynchites rutilus* (Coquillett) are the dominant predators of invertebrates, although this species is seasonal in tree holes (Bradshaw & Holzapfel, 1985). The community structure system occurring in the Midwest USA works as a simple food web, allowing us to test the relationship among the top predator *T. rutilus* and the lower levels of the trophic cascade.

In this study we tested the hypothesis that introducing predatory larvae (A) or predatory cues (B) to the system would directly reduce the abundance of consumers (DMI), or it would trigger consumer behavioral changes (TMI), hence indirectly positively affecting the bacterial abundance (DMII or TMII). We predicted that the predation act and/or the predatory cues would add animal organic matter to the system, creating a profitable environment for bacterial growth. These hypotheses were tested by carrying out two laboratory experiments.

3 Material & Methods

3.1 Insect Colonies

Aedes triseriatus and *Toxorhynchites rutilus* were reared and maintained at 25±3°C, 80±15% relative humidity and a 14:10 L:D photo-period. *A. triseriatus* larvae were kept in 25 x 30cm plastic trays at a density of approximately 1,000 immatures/L of deionized water and fed every other day standard volumes of a liver powder suspension prepared with 0.4 g of liver powder per 1000 ml of DI water. *Toxorhynchites rutilus* larvae were individually raised in 20-ml glass tubes (filled with 10-ml DI water) and allowed to feed on *A. aegypti* larvae ad libitum until reaching its fourth instar. Upon eclosion, both *Aedes triseriatus* and *Toxorhynchites rutilus* adults were kept in 60 x 60 x 60 cm and 30 x 30 x 30 cm cages, respectively, and provided continuously with water-sugar solution (20%). In order to provide eggs for the experiment *A. triseriatus* females were blood fed on anesthetized mice (1 h) four to seven days after emergence and *T. rutilus* females were submitted to induced-mating technique two to four days after emergence (Baker *et al.*, 1962). All of the *A. triseriatus* and *T. rutilus* larvae used on these experiments were supplied by eggs from laboratory colonies.

3.2 General laboratory experimental design

Experimental microcosm consisted of 450 ml plastic cups, filled with 300 ml of deionized water plus 100 ml oak leaves infusion (35 g/l) aged for nine days. The leaves were oven dried at 50°C for 48 hours. The infusion provided organic mater and bacterial food source for *A. triseriatus* larvae. The *T. rutilus* and *A. triseriatus* eggs were hatched in a 20-ml glass tubes filled with 10-ml DI water, individually and in a group of $\simeq$ 100 eggs/tube respectively. The *A. triseriatus* eggs were hatched in a solution of 10-ml DI water plus 0.4 g/l of lac-albumin. Both the *T. rutilus* and *A. triseriatus* eggs were hatched 24 hours before starting the experiment. To assess the effects of predator presence, predatory-cues, prey larvae and prey larvae carcasses on bacterial abundance, we tested different combinations of treatments in laboratory bioassays (*see Treatments*).

3.3 Laboratory experiments

Experiment I

3.3.1 *Assessment of predation and predatory-cues effects on bacterial abundance*

This experiment was designed to assess the effect of the predation act, predatory cues and prey larvae on bacterial abundance within artificial mosquito larval containers. One hundred first-instar *A. triseriatus* larvae were added to each treatment experimental cup. Control cups received no larvae. Experimental cups were incubated under insectary conditions (see above) for 14 days. At the 07^{th} and 14^{th} days, after the first and second weeks, the number of surviving *A. triseriatus* larvae and bacterial abundance (*see Bacterial Productivity*) were recorded. This was done to assess the effect of the predator, predatory cues and prey larvae on bacterial abundance within artificial mosquito larval containers. Predatory-cues were obtained holding one *T. rutilus* fourth instar for 5 d in a 50-ml cup with 50 ml of water and 20 *A. triseriatus* fourth instar larvae (Kesavaraju & Juliano, 2004). Prey larvae were counted daily and any missing or dead larvae were replaced with additional larvae. Some detritus (e.g., feces, bits of eaten prey) accumulated during the 5 days period, and this solid material remained in the treatment water during the trial. Both, the non-predatory cue treatments and control treatments were supplied with 50 ml of 5 days aged deionized water.

Treatments

Infusion plus prey alone (IP): To assess the direct impact of *A. triseriatus* larvae predation on bacterial abundance (DMI).

Infusion plus prey plus predatory cues (IPC): To assess the direct impact of water borne predatory-cues on *A. triseriatus* larvae (TMI) and the indirect impact on bacterial growing (TMII).

Infusion plus prey plus predator (IPR): To assess the direct impact of *T. rutilus* larva predation on *A. triseriatus* larvae (DMI) and the indirect impact on bacterial abundance (DMII).

3.3.2 *Controls*

Infusion alone (IA): To allowed the bacterial growing without both, *A. triseriatus* direct predation pressure or *T. rutilus* indirect predation effects interference.

Infusion plus predatory cues (IC): To assess the impact of water-borne predatory-cues on bacterial growing.

Experiment II

3.3.3 *Assessment of predation act, induced predation and conspecifc carcasses on bacterial abundance*

This experiment was designed to assess the effect predation act (infusion + predation + prey = IPR), simulated predation (infusion + crushed prey larvae removed from the water = ICR), animal carcasses (infusion + crushed prey larvae = IC) and prey larvae (infusion + prey larvae alone = IP) on bacterial abundance within artificial mosquito larval containers. Both treatment and control cups received one hundred first instar *A. triseriatus* larvae. The predation act treatment received the prey larvae plus one first-instar *T. rutilus* larva. In order to evaluate both the induced predation and conspecifc carcass effect, every experimental day the number of prey larvae consumed by predation was recorded and the average consumed prey number was removed from the ICR and IC treatments. In the ICR and IC treatments, prey larvae were killed by crushing the middle of their bodies using a forceps. Experimental cups were incubated under insectary conditions (see above) for 10 days. Prey larvae survivorship was measured every experimental day, however in order to run the statistical analysis we used the prey larvae survivorship recorded on the 05^{th} and 10^{th} days after the first and second weeks. The reason for that was because those were the bacterial abundance measurement days.

3.4 Bacterial Productivity

Production of new bacterial biomass was quantified by estimating protein synthesis (hereafter PS) using a tritiated L-leucine (4,5-3H, 50 Ci mmol-1) incorporation assay. The L-leucine incorporation assay technique is specific to bacteria in aquatic systems (Riemann & Azam, 1992) and has been used to quantify bacterial productivity in container mosquito experiments (Kaufman *et al.*, 2001; Yee *et al.*, 2007a,b; Murrell & Juliano, 2008). We measured water column PS following Kirchman (1993) and refined by Kaufman *et al.* (2001) for container systems. To a 1mL fluid sample, [H]-leucine was added, incubated for 30 min, and then protein was precipitated in trichloracetate. [H]-Leucine incorporation was quantified by liquid scintillation (Beckman LS-6500 scintillation counter). This procedure measures the leucine incorporation into microbial biomass, which quantifies microbial growth (Kirchman, 1993).

3.5 Statistical Analyses

Differences among the treatments (independent variables) over the two sampling weeks, on the fist and second experiments, were analyzed using repeated measures multivariate analysis of variance (MANOVA) (PROC GLM, SAS Institute Inc, 1990). On first experiment the effect of the treatments on leucine incorporation rate was analyzed. On the second experiment NANOVA's were used to evaluate the response of the dependent variables survival and leucine incorporation rate to the treatments (independent variables). Significant MANOVA effects were interpreted using standardised canonical coefficients (SCC) (Scheiner, 1993), which quantify the contributions of the individual dependent variables to significant multivariate effects.

4 Results

4.1 Experiment I - *Predation act and predatory-cues effects on bacterial abundance*

All treatments: Bacterial abundance has significantly changed over time (Pillai's Trace = 0.18, $F_{1,34}$ = 7.54, p = 0.0096). In addition there was a significant effect on the treatment/week interaction showing that variation in bacterial abundance over the weeks depended on treatment (Pillai's Trace = 0.47, $F_{1,34}$ = 9.40, $p < 0.0001$). The presence of prey larvae did not impact the bacterial abundance over the weeks (Pillai's Trace = 0.02, $F_{1,34}$ = 9.40, p = 0.3808). However, there was a significant effect of predation cues on bacterial abundance in both prey larvae absence (contrasting IA and IC treatments, Pillai's Trace = 0.35, $F_{1,34} = 18.97$, $p < 0.0001$) and presence (contrasting IPC/IPR group and IP treatment, Pillai's Trace = 0.33, $F_{1,34} = 16.99$, $p = 0.0002$). Adding predatory cues to prey larvae containers (IPC treatment) did not differ from real predation (IPR treatment) (Pillai's Trace = 0.03, $F_{1,34} = 1.24$, $p = 0.2732$). The bacterial abundance trend for IA, IP and IPR was the opposite of the trend for IC treatments (Fig. 1). The real predation (IPR treatment) kept the bacterial abundance level higher than prey larvae alone (IP treatment) during the whole experimental period (Pillai's Trace = 0.21, $F_{1,34} = 9.18$, $p = 0.0046$, Fig. 1).

Prey larvae x cues effects as a factorial design: Regarding the factorial design (Table 1) neither prey larvae (Pillai's Trace = 0.00, $F_{1,34} = 0.22$, $p = 0.6388$) or predatory cues (Pillai's Trace = 0.03, $F_{1,34} = 1.29$, $p = 0.2640$) presence and/or absence (IP, IPC vs IA, IC contrast), impacted the bacterial abundance over week. However, we did find a significant interaction effect between prey larvae and predatory cues presence/absence on bacterial abundance (Pillai's Trace = 0.50, $F_{1,34} = 34.96$, $p < 0.0001$). The effects of prey larvae presence depended on whether or not predatory cues were added to the system. Bacterial abundance decreased over time after the 7^{th} day in the IC treatment. Therefore, IA, IPC and IPR treatments positively increased the bacterial abundance over time reaching the same level as IC treatment by the 14^{th} day. The addition of prey larvae with no predatory cues depressed the bacterial abundance and kept it low by the day 14^{th}. In addition, both, the prey larvae ($F_{1,34} = 33.95$, $p < 0.0001$) and the predatory

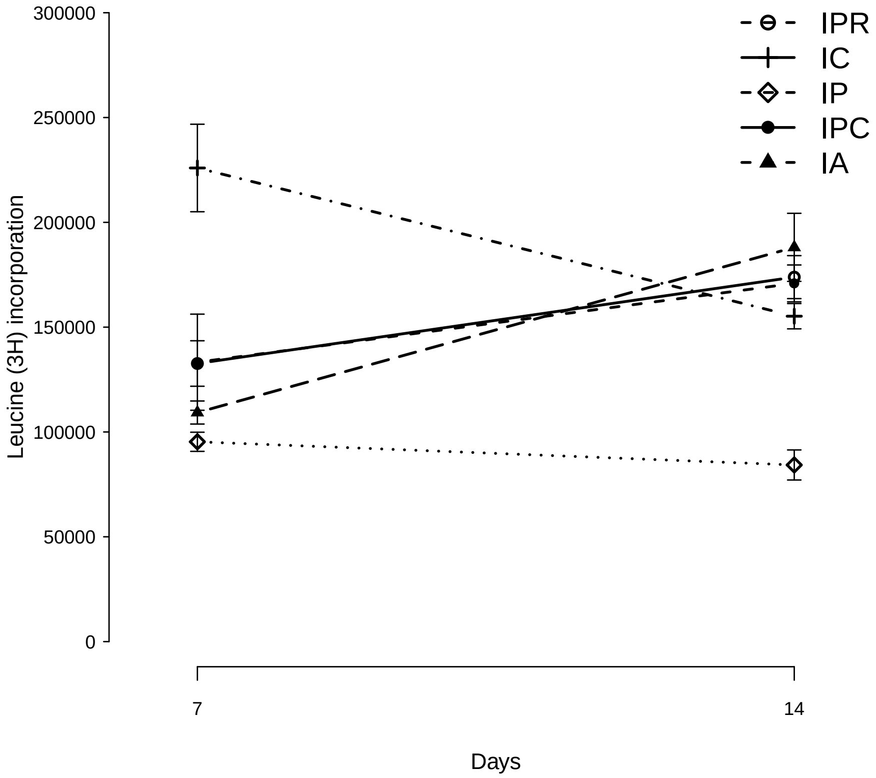

Figura 1: Production of new bacterial biomass based on measurement of protein synthesis (quantified by measuring incorporation of tritiated H-leucine (4,5-[H])) in mosquito containers measured after 7^{th} and 14^{th} days after the first and second weeks. Controls are: IA: infusion alone, IC: infusion + cues of predation. Treatments are: IP: infusion + prey larvae, IPC: infusion + prey + cues of predation, IPR: infusion + prey + predator.

Tabela 1: Factorial design formed by ignoring actual predation treatment (IPR)

Cues	Yes	No
Larvae	*IPC*	*IP*
No larvae	*IC*	*IA*

cues ($F_{1,34} = 25.99$, $p < 0.0001$) absence/presence displayed significant main effects on bacterial abundance when the trend over time was ignored.

4.2 Experiment II - *Predation act, induced predation and conspecifcs carcasses effects on bacterial abundance*

First week (5 days): In the first experimental week the variation among the treatments was related mostly to *A. triseriatus* larvae survival (97.7% of the variation). Standardized canonical coefficients (SCC) for prey larvae survivorship (SCC = 3.563) and bacterial abundance (SCC = 0.027) were not similar, indicating that prey larvae survivorship displayed a better contribution to the significant MANOVA effect (Pillai's Trace = 1.16, $F_{1,47} = 11.19$, $p < 0.001$).

The prey alone treatment (IP) differed almost totally in *A. triseriatus* larvae survivorship from predation act (IPR) (SCC = 3.553) (Pillai's Trace = 0.89, $F_{1,22} = 99.34$, $p < 0.0001$), crushed/removed (ICR) (SCC = 3.555) (Pillai's Trace = 0.90, $F_{1,22} = 115.22$, $p < 0.0001$) and crushed (IC) (SCC = 3.553) (Pillai's Trace = 0.88, $F_{1,22} = 90.43$, $p < 0.0001$) treatments (Fig.

2). The treatments crushed/removed (ICR) and crushed (IC) differed statistically in prey larvae survivorship (thought not after correction for n = 6 tests). The difference was mostly in prey larvae survivorship (SCC = 2.133) but somewhat in bacterial abundance (SCC = 0.860) (Pillai's Trace = 0.27, $F_{1,22} = 4.32$, $p < 0.0255$). The predation act treatment (IPR) did not differ statistically from crushed/removed (ICR) (Pillai's Trace = 0.20, $F_{1,22} = 2.98$, $p = 0.0708$) and crushed (IC) treatments (Pillai's Trace = 0.05, $F_{1,22} = 0.62$, $p = 0.5459$). The Brown and Forsythe's Test for homogeneity did not detect significant differences in variances in either bacterial abundance ($F_{1,2} = 0.83$, $p = 0.4922$) and prey larvae survivorship ($F_{1,2} = 0.14$, $p = 0.9355$) variables (Fig. 2).

Second week (10 days): At the second experimental week the IP, IPR, ICR and IC treatments differed along two significant axes. 90% of the variation was related to the *A. triseriatus* prey larvae survivorship (Pillai's Trace = 3.84 , $F_{1,45} = 39.92$, $p < 0.0001$) and 9.5% was related to the bacterial abundance (Pillai's Trace = 0.22, $F_{1,23} = 18.14$, $p < 0.0001$, Figure 2).

The IP treatment was significantly different, mostly in prey larvae survivorship, from IPR (SCC = 3.378) (Pillai's Trace = 1.16, $F_{1,22} = 105.28$, $p < 0.0001$), ICR (SCC = 3.943) (Pillai's Trace = 0.91, $F_{1,22} = 128.66$, $p < 0.0001$) and IC treatments (SCC = 3.944) (Pillai's Trace = 0.89, $F_{1,22} = 101.08$, $p < 0.0001$). Both, IC and ICR treatments were not significantly different (Pillai's Trace = 0.126, $F_{1,22} = 1.66$, $p = 0.2119$). The crushed/removed (ICR) and predation act (IPR) treatments were significantly different mostly in bacterial abundance (SCC = 2.032), but also in prey larvae survivorship (SCC = 1.407) (Pillai's Trace = 0.52, $F_{1,22} = 12.60$, $p = 0.0002$). The crushed

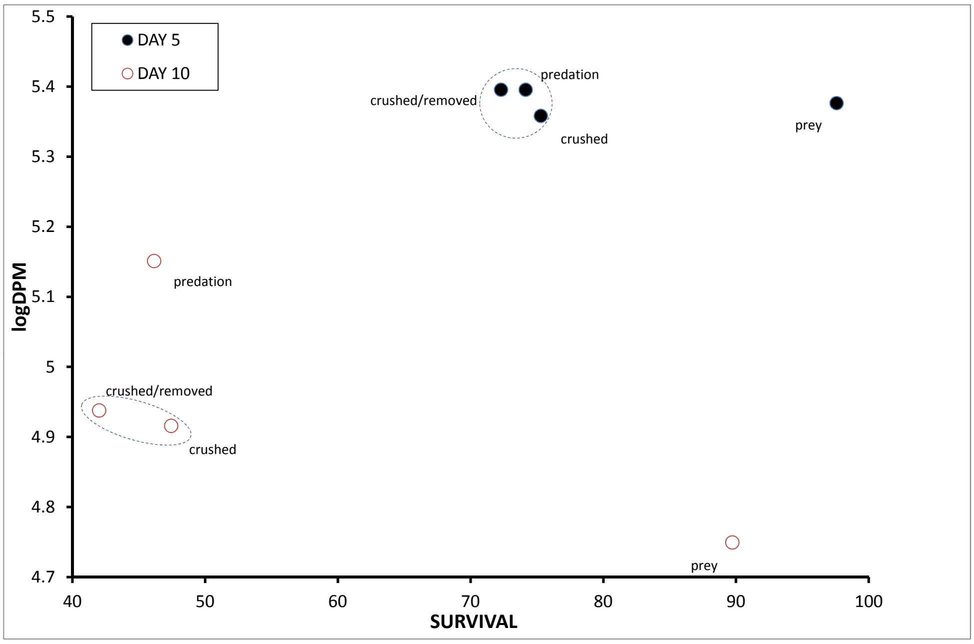

Figura 2: Production of new bacterial biomass based on measurement of protein synthesis (quantified by measuring incorporation of tritiated H-leucine (4,5-[H])) in mosquito containers measured after 5^{th} (*filled circles*) and 10^{th} (*open circles*) days after the first and second weeks as a function of prey larvae survival and the treatments prey, predation act, crushed, and crushed/removed.

(IC) and predation act (IPR) treatments were significantly different in both dimensions, prey larvae survivorship (SCC = 1.407) and bacterial production (SCC = 1.976). The SCC for those variables were similar, indicating that both contributed with the significant MANOVA effect (Pillai's Trace = 0.57, $F_{1,22} = 15.27$, $p < 0.0001$) (Figure 2).

5 Discussion

We tested two hypothesis concerning the effects of both predation action and predatory cues provided by predation action on trophic cascades in a laboratory microcosm simulating tree hole systems. In the laboratory experiment, single *A. triseriatus* prey larvae (PA treatment) did not impact bacterial abundance over weeks, and prey larvae kept the bacterial abundance at low levels during the whole experimental period (Figure 1). In general these results partially contrast with previous studies showing that mosquito larvae negatively impact microorganism abundance in detritus-based systems (Walker *et al.*, 1991; Kaufman *et al.*, 1999, 2001; Kneitel & Miller, 2002; Trzcinski *et al.*, 2005). It was expected that prey larvae decreased bacterial abundance over time. The reason bacteria did not decrease over time is because organic input from larvae as they become larger slightly enhanced bacterial growth, resulting in that same net abundance. Bacteria stayed in low abundance the whole time (Fig. 1), because larval consumption and not enough organic input to make a difference did not allow it to become very abundant.

Top-down theory, in which predators regulate species abundance, predicts negative effects on adjacent trophic levels and positive effects on nonadjacent trophic levels below the top predator (Hairston *et al.*, 1960; Oksanen *et al.*, 1981; Carpenter *et al.*, 1985; Persson, 1999). Based on this theory, we initially hypothesized that the presence of predators would indirectly increase bacterial abundance within mosquito containers, by the prey larvae consumption, triggering predator avoidance behavior on prey larvae or increasing animal organic material (uneaten parts of prey) that would enhance bacterial growth. Our first hypothesis was supported by our results, which show that predator presence (IPR) and predatory cues (IPC) treatments increased the bacterial abundance over weeks (Fig. 1). One possible explanation is that it was probable that the predator reduced the population of prey larvae (DMI), which in turn reduced predation pressure on the bacterial population (DMII), allowing it to grow. This result is consistent with others described in various systems. For example, in terrestrial environment ant foragers (Messina, 1981; Fritz, 1983), predatory nematodes (Preisser, 2003), spiders (Rosa & DeSouza, 2011; Schmitz *et al.*, 1997; Schmitz, 1998; Gastreich, 1999), mites (van Rijn *et al.*, 2002) and birds (Schwenk *et al.*, 2010) have been shown to decrease the abundance of potential herbivores of plants, indirectly decreasing leaf damage. In aquatic systems, such as rivers, streams and lakes, fishes (Flecker & Townsend, 1994; Mancinelli *et al.*, 2002), rotifers (Dolan & Gallegos, 1991) and microcrustaceans (Eitam & Blaustein, 2010) decrease prey survivorship and thus indirectly positively affect basal trophic levels. On a microcosm scale, studies with pitcher plant communities did detect indirect effects of the predatory mosquito *Wyeomiia smithii* on bacterial abundance

(Cochran-Stafira & von Ende, 1998; Kneitel & Miller, 2002). In a tree hole system, odonate larvae had an indirect effect on litter degradation under limiting resource levels (Yanoviak, 2001).

An alternative explanation is that the presence of the top predator could have affected the bacterial abundance by releasing nutrients into the system (Vanni & Layne, 1997; Vanni *et al.*, 1997; Cochran-Stafira & von Ende, 1998). It is known that predatory larvae of *Toxorhynchites*, during the predation act, can leave parts of the prey uneaten (Steffan & Evenhuis, 1981). This could have increased animal organic material in experimental containers, supporting greater microbial populations (Yee & Juliano, 2006; Yee *et al.*, 2007a,b). Supporting this explanation, is the fact that bacterial abundance in the predation act (IPR) and predatory cues (water that hold a predator feeding on prey for 5 d) (IPC) treatments slightly increased at the same rate over week (Fig. 1). Further, the greatest bacterial abundance in the treatment that received predatory cues only (Fig. 1, IC), suggesting that animal organic matter provided from predation act and/or predatory cues could have worked as a profitable environment for growing bacteria.

It is also possible that threat-induced predator avoidance behavior by the prey larvae caused bacterial abundance increment (Werner, 1992; Werner & McPeek, 1994; Lima, 1998; Preisser *et al.*, 2005). This trait mediated interaction (TMI) has been observed in birds and mammals [reviewed by Caro (2005)], anurans (Kats & Dill, 1998; Lawler, 1989; Skelly & Werner, 1990; Azevedo-Ramos *et al.*, 1992) and insects (Hassell & Southwood, 1978; Kerfoot & Sih, 1987; Kohler & McPeek, 1989; McPeek, 1990). Furthermore, it is known that the *A. triseriatus* mosquito larvae strongly change their behavior (more resting, less feeding and movement) when they are exposed to the *T. rutilus* predatory larva (Juliano & Reminger, 1992; Juliano & Gravel, 2002) and/or cues of predation (Kesavaraju & Juliano, 2004; Kesavaraju *et al.*, 2007, 2011). In our study *A. triseriatus* larvae, when exposed to predator presence and/or predatory cues, could have changed its behavior, moving less and feeding less (Preisser *et al.*, 2005) decreasing the predation pressure and allowing the bacterial population to grow. Our results clearly show that the predatory larvae of *T. rutilus* did change the trophic cascade within *A. triseriatus* artificial mosquito breeding containers. However it could have happened by prey density reduction, animal organic matter addition and/or threat-induced-predator-avoidance-behavior, or by any combination of the three. Our initial experimental design did not allow us to make conclusions about the mechanisms that allowed bacterial growth.

In our second laboratory experiment we isolated effects of predator presence, predation act and prey larvae carcasses on the bacterial abundance in artificial mosquito breeding containers. Our results have shown that predator presence strongly reduced the *A. triseriatus* prey larvae survival, and that the predator presence treatment had the highest level of bacterial abundance by the end of the experiment (Fig. 2). Although the impact of both predation (IPR) and mechanically killed prey (IC and ICR) on the prey larvae survival was similar (Fig. 2), there was a difference in bacterial abundance between these treatment groups. It is possible that the differences between these two could be due to treat-induced predator avoidance.

A possible explanation is that mechanically killed prey either could not add alarm cues to the water, or it was not enough to trigger prey avoidance behavior. Kesavaraju *et al.* (2007) have shown that behavioral responses of *A. triseriatus* larvae to *T. rutilus* predation risk cues decreased as the concentration of cues-laced water and suspended solids decreased via dilution with distilled water. In our experiment it could have happened due to the low concentration of alarm cues, especially when the larvae were crushed and removed from the water. We suspected that, because predation pressure on the bacterial population could have been higher in the mechanically killed prey treatments than in the real predation treatment (Fig. 2)

It is known that cues with which aquatic prey perceive the risk of predation can originate with the presence of predator (Chivers & Smith, 1998; Wisenden, 2000) or can be created by the act of the predation (Chivers & Smith, 1998; Relyea, 2001). Kesavaraju & Juliano (2010) demonstrated that *A. triseriatus* larvae increased low-risk behavior in water containing filtered solids from predation (uneaten body parts and predator feces), however low-risk behavior was not detected in the absence of such solids. They reached the conclusion that the contact of *A. triseriatus* larvae with uneaten conspecific body parts and predator feces provided predation-threat cues. In our mechanically killed prey treatments we had no solid residuals from predation act, thus we believed that artificial killing could not trigger anti-predator behavior in *A. triseriatus* larvae.

We did not find a difference in bacterial abundance between the crushed (IC) and crushed/removed (ICR) treatments (Fig. 2). One explanation is that the mechanisms that allowed bacteria growth are due to predator-avoidance behavior rather than larval carcass decomposition. Our results did show that both predator presence and predatory cues can indirectly positively affect bacterial abundance within artificial containers. The increase in bacterial abundance may be due threat-induced predator avoidance behavior, or by additional predator and prey excrements released into the water on the predation act treatment. Mosquito larvae excrement is composed primarily by ammonia. In tree hole systems, the ammonia derived from larval excrement strongly contributes to nitrogen levels in the system (Walker *et al.*, 1991), providing a profitable environment for bacterial growth (Kaufman & Walker, 2006).

Effects of predators on mosquito population may cause mortality that merely replaces mortality that would be caused by density dependence. When mortality due to an enemy result in equal production of adults, it is called compensatory mortality, and when it results in a grater production of adults it is called overcompensatory mortality (Juliano, 2009; Juliano *et al.*, 2010). Under certain developmental conditions (e.g., limited per capita food source), predators reduce the number of competing mosquito larvae, which may allow the production of more and larger adults (Washburn, 1995; Alto & Griswold, 2005; Juliano *et al.*, 2010). Juliano *et al.* (2010) have found that the native mosquito larvae *A. triseriatus* strongly increase the mean within-instar size after predatory reduction on itraspecific and interspecific competition. Alto & Griswold (2005) have shown that the predators *T. rutilus* and *Corethrella appendiculata* decreased both *A. triseriatus* and *A. albopictus* prey survivorship, shortened development time, and increased adult size compared with treatments where predators where absent.

Despite this, if the trophic cascade effect makes more bacterial food available (e.g., via predation act or predatory cues) and still reduces the density-mediated competition (e.g., direct reduction on prey number or prey behavioral change), we would expect more robust and higher quality mosquitoes being produced in containers where the trophic cascade is occurring. We predicted that in mosquito habitats, with hight density dependence among crowded larvae, introduction or enhancement of predators is likely to alleviate density dependence (i.e., reduce competition) and may produce counter intuitive results for prey: as many or more mosquitoes may be produced with predators presence than without, especially if the predators increase the availability of food for mosquito larvae.

Chapter II

Attracted to the enemy: *Aedes aegypti* prefers oviposition sites with predator-killed conspecifics

Resumo

The response of ovipositing mosquitoes to chemical cues is an important part of mosquito ecology and control. The highly invasive *Aedes aegypti* preferentially oviposits in containers with high nutrient abundances; however, unlike many native species, *A. aegypti* does not appear to detect chemical cues of predaceous *Toxorhynchites* larvae, and therefore does not avoid ovipositing at sites containing these predators. Since predation by *Toxorhynchites* can potentially increase bacterial abundance in containers by reducing numbers of mosquito larvae and by adding substrates for bacterial growth, and since *A. aegypti* may prefer to oviposit where bacteria, which are the food of larvae, are abundant, it is possible that *A. aegypti* preferentially oviposit in containers where *Toxorhynchites* are abundant. We conducted a laboratory study in which gravid *A. aegypti* were offered two oviposition sites, combined in one of 6 treatments: (A) the predator *Toxorhynchites theobaldi* alone vs. DI larvae high vs. low densities, (D) *A. aegypti* high density vs. *A. aegypti* low density with crushed *A. aegypti* larvae, (E) *A. aegypti* low density vs. *T. theobaldi* with *A. aegypti* larvae (F) *A. aegypti* high density vs. *A. aegypti* low density with crushed *A. aegypti* larvae and the antibiotic tetracycline. Female *A. aegypti* preferentially oviposited in containers with active *T. theobaldi* predation in treatment B, and also preferentially oviposited in containers with crushed *A. aegypti* in treatment D, but displayed no oviposition preference in any other treatments. Our study supports the hypothesis that ovipositing *A. aegypti* are not attracted to sites with conspecifics or *Toxorhynchites* per se, but rather are attracted to sites with greater bacterial abundance. These data suggest that the addition of *Toxorhynchites* to field containers may be doubly beneficial in controlling *A. aegypti* populations, because ovipositing *A. aegypti* show no predator avoidance and are attracted to containers with the bacterial by-products of *Toxorhynchites* feeding.

Keywords: *Aedes aegypti*, *Toxorhynchites*, predation, oviposition behavior

6 Introduction

In organisms that do not provide post-oviposition care to their young, the selectivity of the female in choosing an appropriate oviposition site may be vital for the survival of her offspring (Lima & Dill, 1989). Selective oviposition behavior has been well-documented in a number of insects, demonstrating that gravid females of some species actively seek sites that maximize larval growth (Resetarits, 2001; Mayhew, 2001; Rieger *et al.*, 2004; Müller & Arand, 2007), while other species will actively avoid sites in which the probability of larval mortality is increased (reviewed by (Blaustein, 1999)).

Mosquito oviposition behavior in particular has been studied extensively over the past 30 years. The oviposition patterns of mosquitoes are important for determining their population dynamics, and have important implications for mosquito control (Bentley & Day, 1989). Several mosquito species demonstrate strong oviposition preferences, many of which appear to be driven by the female's detection of the bacteria upon which larvae feed (Walker *et al.*, 1991; Allan & Kline, 1995; Navarro *et al.*, 2003; Trexler *et al.*, 2003; Ponnusamy *et al.*, 2010) or chemical cues (Hazard *et al.*, 1967; Allan & Kline, 1995; Sant'ana *et al.*, 2006; Ponnusamy *et al.*, 2010) present in potential oviposition sites. Some mosquito species exhibit both oviposition preference and avoidance behaviors. *Culex quinquefasciatus* oviposits preferentially in containers that have been used to rear conspecifics (Suleman

& Shirin, 1981) or that contain chemicals emitted by conspecific egg rafts (Laurence & Pickett, 1985), but will actively avoid sites that contain high densities of conspecific larvae (Wilmot *et al.*, 1987), or predaceous notonectids (Chesson, 1984; Blaustein *et al.*, 2005). *Anopheles gambiae* oviposit preferentially in water from natural development sites, but avoid sites that contain conspecific larvae (McCrae, 1984).

Avoidance behavior of sites with predators is particularly noteworthy, as predation on juvenile stages is an important factor in the population biology of many animals (Endler, 1986; Kerfoot & Sih, 1987). Therefore, it is expected that female mosquitoes can detect aquatic predators and avoid them when choosing oviposition sites. This has been supported in a number of studies (Chesson, 1984; Angelon & Petranka, 2002; Blaustein *et al.*, 2004; Arav & Blaustein, 2006; Silberbush *et al.*, 2010). Much like other organisms (reviewed by (Wisenden, 2000)) predator avoidance behavior of mosquitoes appears to be driven by their detection of chemical cues either emitted by the predator directly, or released during the act of predation (Bentley & Day, 1989; McCall, 2002). For example, *Culiseta longiareolata* strongly avoid pools inhabited by *Anisops sardea* (Eitam *et al.*, 2002) and *Anax imperator* (Odonata) (Stav *et al.*, 2000) when these predators are allowed to feed on heterospecific and conspecific mosquito larvae. Mosquitoes also respond to chemicals liberated from wounded conspecifics by avoiding ovipositing in water bodies with such chemical cues (Blaustein & Margalit, 1995).

Despite the theoretical importance of predator avoidance, and empirical evidence that it occurs, there are also mosquito oviposition studies that have failed to demonstrate predator avoidance behavior. *Culex pervigilans* do not avoid ovipositing in places where their key predator, the backswimmer, *Anisops wakefield* (Heteroptera), is present. *Culex quinquefasciatus* oviposition choice was unaffected by exposure to the predatory fish *Melanotaenia duboulayi* (Hurst *et al.*, 2010). These studies suggest the possibility that these species may not have a coevolutionary history with these predators so that potential prey may not be able to detect or to respond appropriately to these predators. Alternatively, it is possible that predators that have a coevolutionary history with particular prey may have evolved chemical camouflage that masks cues to their presence.

While direct predator avoidance in mosquito oviposition behavior has been well-studied, there is an additional indirect effect that has been less considered. The act of a predator feeding on larvae has the potential to increase bacteria in the water (the primary food of mosquito larvae) both directly via addition pieces of of uneaten prey and predator feces in the water, which provides a substrate for bacterial growth, and indirectly via consumption of larvae that would otherwise consume bacteria (i.e, trophic cascade; (Carpenter *et al.*, 1985)). If a mosquito species prefers to oviposit in containers with high bacterial abundance, but also is not simultaneously adapted to avoid predator cues, then females may actually be attracted to containers where a predator is present, due to the higher bacterial abundances in those containers.

One species in which this oviposition paradox is likely to occur is *Aedes aeygpti*, a highly invasive mosquito that has been established in tropical and subtropical locations worldwide (Tabachnick *et al.*, 1979; Juliano & Lounibos, 2005). *Aedes aegypti* may be sensitive to bacterial abundances, as it prefers to oviposit in containers prepared with nonsterile detritus over sterile detritus (Ponnusamy *et al.*, 2010), prefers to oviposit in bacteria-laden water over distilled water (Navarro *et al.*, 2003), and preferentially oviposits among containers with different detritus types (Santos *et al.*, 2010), some of which can differ in the microbial abundances they support (Yee & Juliano, 2006; Murrell & Juliano, 2008). However, *A. aegypti* does not avoid oviposition in water with the physical or chemical presence of several different predators, including predatory fish (Dam & Walton, 2008; Pamplona *et al.*, 2009), and is even attracted by chemical cues emitted by the copepod predator *Mesocyclops longisetus* (Torres-Estrada *et al.*, 2001). These studies strongly suggest that *Aedes aegypti* has not evolved oviposition avoidance behavior for multiple predators; therefore, we hypothesize any predator that can positively affect bacterial abundances in containers may actually attract oviposition by *A. aegypti*.

Larvae of the predatory mosquito *Toxorhynchites* are a good choice for testing this hypothesis. *Toxorhynchites* spp. are native to many areas of the world (Collins & Blackwell, 2000; WRBU, 2001; Albeny *et al.*, 2010), colonize containers like those favored by *A. aegypti*, and are highly predaceous upon other mosquito larvae, including *Aedes* (Peterson, 1956; Engber *et al.*, 1978). *Toxorhynchites* are capable of killing and consuming large numbers of prey larvae (Corbet & Griffiths, 1963; Rubio *et al.*, 1980; Machado-Allison, 1981; Steffan & Evenhuis, 1981; Albeny *et al.*, 2011), thus potentially having a large effect on the bacterial concentrations within containers. Some *Toxorhynchites* species (particularly in island regions) co-occur in the same habitats as *Aedes aegypti*, and have been employed in the past as a natural mosquito control agent (Bonnet & Hu, 1951; Sempala, 1983; Miyagi *et al.*, 1992; Collins & Blackwell, 2000; Lounibos & Campos, 2002; Schreiber, 2007).

In this study, we tested the hypotheses that (A) *Aedes aegypti* is attracted to containers where *Toxorhynchites theobaldi* has occurred, (B) This oviposition attraction is a result of increased bacterial abundance in containers where predation has occurred. We predicted that (A) *Aedes aegypti* would preferentially oviposit in containers filled with water where either actual (*T. theobaldi* feeding) or simulated (crushed *A. aegypti* larvae) predation has occurred, (B) These oviposition preferences would disappear if the bacteria are reduced or eliminated by antibiotic addition, (C) Treatments preferred by ovipositing *A. aegypti* would have higher bacterial abundances than treatments not preferred by *A. aegypti*.

7 Materials & Methods

7.1 Assessment of Oviposition Behavior of *A. aegypti*

Insect colony

Aedes aegypti and *Toxorhynchites theobaldi* were reared and maintained at 25°3C, 80±15% relative humidity and a 12L:12D photo-period. *Aedes aegypti* larvae were kept in a 25 x 30cm plastic tray at a density of 1,000 immatures/L of tap water and were fed with turtle food (ReptoLife 0.5g, São Paulo, SP). *Toxorhynchites theobaldi* larvae were kept in 100-ml polypropylene cups and fed with *A. aegypti* larvae ad libitum. Upon eclosion, both *A.*

aegypti and *T. theobaldi* adults were kept in 60 x 60 x 60 cm cages and provided continuously with water-honey solution (1:1). *Aedes aegypti* females were bloodfed on anesthetized mice (1 h) four to seven days after emergence. Naïve *A. aegypti* females 10 days old were used in the oviposition bioassays.

General experimental design

Two 250-ml polypropylene transparent cups (treatment and control) were filled with 200-ml deionized water and used for assays. Any larvae placed in oviposition containers were rinsed once in 100-ml of deionized water before being transferred to the experimental cups. Both containers were incubated under insectary conditions (see above) for 48 hours. As we were interested only in *A. aegypti's* response to predator-prey chemical cues released in the water, and not the physical presence of predators or conspecifics, we removed all prey or predator larvae via sieving prior to the beginning of the oviposition assays. To assess the effects of predator presence on oviposition choice of *A. aegypti* females, we tested different combinations of treatments in odor bioassays (see Treatments).

Oviposition Bioassays

A single gravid *A. aegypti* female was added to each bioassay cage (60 x 60 x 60cm) and allowed to oviposit for a period of 48 hours. Bioassays were conducted using individual females to reduce the potential influence of previously laid eggs on oviposition site preference (Chadee *et al.*, 1990; Allan & Kline, 1998). Two oviposition cups (control and treatment) were placed 50 cm apart at opposite sides of the cage. To ensure that the position of the oviposition cups inside the bioassay cages had no influence on oviposition choice, after every 10 replicates the positions of the oviposition cups in the cage were rotated clockwise. Two filter paper discs were placed into oviposition cups as an oviposition substrate (Gomes *et al.*, 2006). Assays were run under the same conditions as described for insect colony maintenance. In all cups, larvae used to prepare water for oviposition trials were fourth instar *T. theobaldi* or *A. aegypti*. The number of eggs on the filter papers from the control and treatment cups were counted after the oviposition period ended. Individual bioassays were replicated 21-36 times.

7.2 Bioassays

Predator without prey: To assess the impact of the predator itself on oviposition by *A. aegypti*, a single *T. theobaldi* fourth instar larva was placed in the treatment cups with deionized water. The control cups received only deionized water (Fig. 3A).

Predator feeding on prey: To assess the impact of predator larvae feeding on prey larvae on the attractiveness or repellency of oviposition cups, one *T. theobaldi* larva plus fifty *A. aegypti* larvae were placed in each treatment cup, allowing the predator to kill and eat prey. Each control cup received fifty *A. aegypti* larvae with no predator (Fig. 3B).

Effects of conspecific density: To determine whether any preferences among the previous treatments resulted from reduction in conspecific density by *T. theobaldi* predation, rather than cues from *T. theobaldi* predation itself, an experiment was conducted subtracting the mean number of prey eaten by experimental *T. theobaldi*, (17 *A. aegypti* larvae) from the total number of larvae used in control cups (50-17 = 33). Thus, each treatment cup received 33 *A. aegypti* fourth instar larvae and each control cup received 50 *A. aegypti* fourth instar larvae (Fig. 3C). To determine the lower conspecific density, we conducted a pilot study in which 10 individual *T. theobaldi* fourth instar larvae, starved for 48 hours, were each allowed feed on fifty *A. aegypti* larvae for 48 hours in an experimental set-up as described above. The mean number of consumed larvae (NCL) was 16.7±2.65 SE, which was rounded to seventeen.

Mechanical injury: To assess the impact of dead bodies of conspecific larvae on attractiveness or repellency of oviposition cups, 17 dead (*simulating NCL; see above*) plus 33 live *A. aegypti* larvae were placed in each treatment cup. The larvae were killed by inserting a pin into the head. Each control cup received fifty live *A. aegypti* larvae. This experiment was intended to simulate predation without the actual predator (Fig. 3D).

Mechanical injury vs. Predation: To assess the impact of dead conspecific larvae and predator larvae feeding on prey larvae on the attractiveness or repellency of oviposition cups, fifty *A. aegypti* larvae plus one *T. theobaldi* larva were placed in one cup, and another cup received 17 dead plus 33 live *A. aegypti* larvae (Fig. 3E).

Bacterial activity effects: To test whether bacterial activity resulting from insect death and cadaver decomposition was responsible for oviposition preferences, an experiment was conducted with addition of the antibiotic tetracycline to the water. Control and treatment cups received 25 mg/L of tetracycline (Navarro *et al.*, 2003). Treatment cups received 17 dead plus 33 live *A. aegypti* larvae plus antibiotic. Control cups received fifty live *A. aegypti* larvae plus antibiotic (Fig. 3F).

7.3 Assessment of Bacterial Production Effects

All of the experimental sections described above were conducted at the Laboratory of Insect Behavior and Pheromones, Department of Entomology, Federal University of Viçosa, Viçosa, MG, Brazil. However, due the lack of radioactive facilities at the Brazilian lab, the bacterial production experiment and assays were done at the Insect Ecology Research laboratory, School of Biological Sciences, Illinois State University, Normal, IL USA.

The Predator feeding on prey (prey+predator), Mechanical injury (prey crushed) and Control (prey alone) treatments were re-established at Illinois State University using both *A. aegypti* (Florida F_x strain) and *Toxorhynchites rutilus*, an ecologically similar North American congener of *T. theobaldi.* Both species were obtained from eggs from the Insect Ecology Research laboratory colonies. *A. aegypti* larvae were kept in 30x30cm plastic tray and fed every other day standard volumes of a liver powder suspension prepared with 0.40 g of liver powder per 1000 ml of DI water. The *T. rutilus* larvae were individually raised in 20-ml glass tubes (filled with 10-ml DI water) and allowed to feed on A. aegypti larvae ad libitum until reach its fourth instar. Mosquito maintenance and the experiment were conducted at 25±3°C, 70±15% relative humidity and a 14L:8D photo-period environmental chamber.

Because we used a different predator species, the same previous (NCL) pilot experiment described above was done for *T. rutilus.* The NCL values did not differ statistically between the predators (*T. rutilus* 23.2±4.52 SE and *T. theobaldi* 16.7±2.65 SE *A. aegypti* larvae consumed, $F_{1,19} = 1.55$, $p = 0.22$).

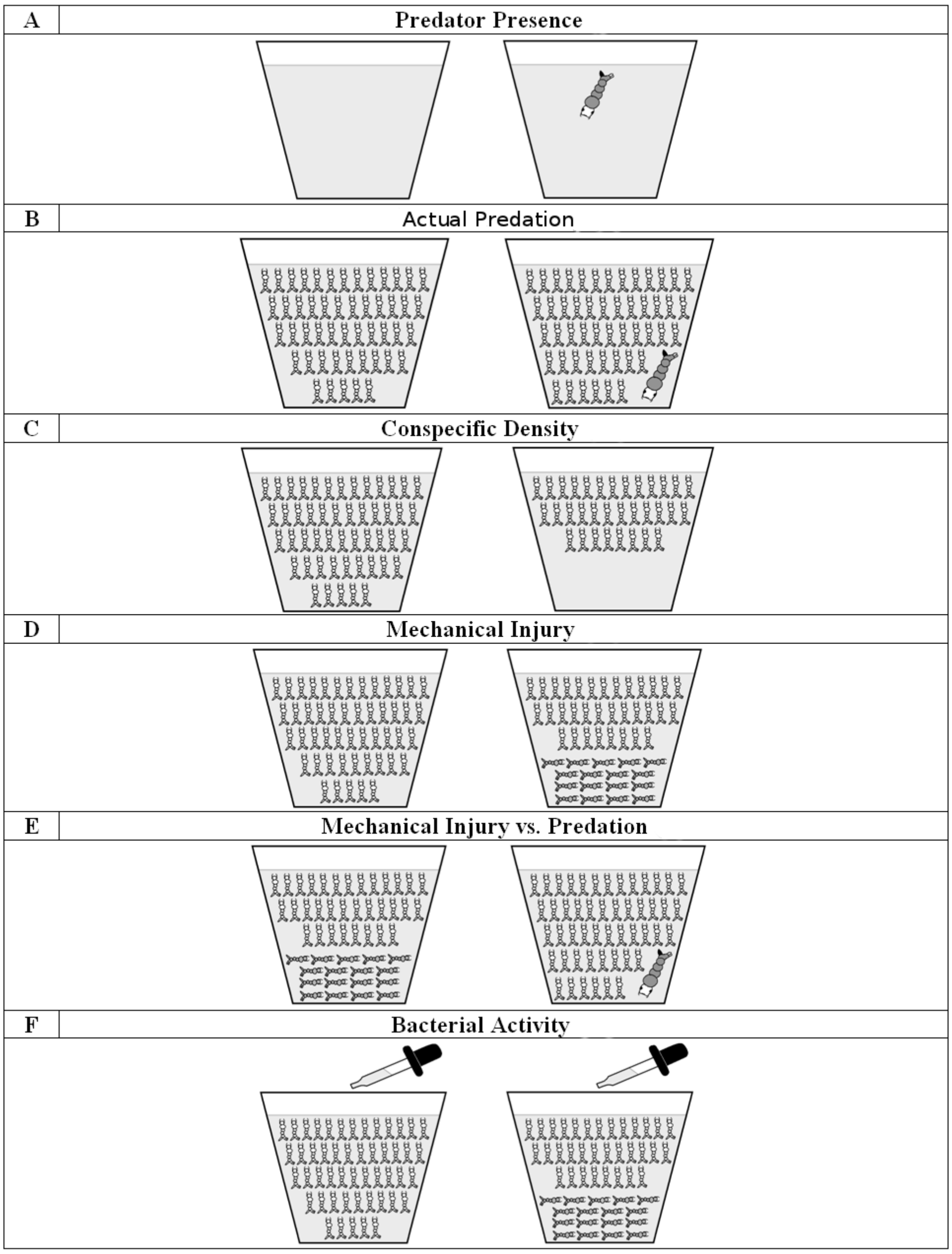

Figura 3: The six treatments presented to gravid females in this study: (A) Predator Presence, (B) Active Predator, (C) Conspecific Density, (D) Mechanical Injury, (E) Mechanical Injury vs. Predation, (F) Bacterial Activity. The control in all treatments is represented by the container on the left, while the treatment is represented by the container on the right.

7.3.1 Bacterial Productivity

Bacterial production in the treatments was assessed via measurement of bacterial protein synthesis (PS). PS was quantified by measuring incorporation of tritiated H-leucine (4,5-[H]) Kirchman (1993); refined by Kaufman *et al.* (2001). To a 1mL fluid sample, [H]-leucine was added, incubated for 30 min, and then bacterial were killed and protein precipitated by addition of trichloracetate. [H]-Leucine incorporation was quantified by liquid scintillation (Beckman LS-6500 scintillation counter).

7.3.2 Statistical analyses

The Oviposition Activity Index (OAI) (Kramer & Mulla, 1979), was used to evaluate the oviposition preferences of *A. aegypti* for test substances. The OAI standardizes the data by converting the number of eggs laid on filter paper in a test cup to a proportion after correcting for the number of eggs laid on filter paper in control cup.

$$\mathrm{OAI} = \frac{NT-NC}{NT+NC},$$

where NT is the number of eggs in the test container and NC is the number of eggs in the control container. The OAI ranges from -1 to +1, with 0 indicating no preference. Positive values indicate that greater oviposition was observed in treatment than in control cups. Conversely, more ovipositions in control than in treatment cups would result in a negative OAI. For statistical analysis we have considered the proportion of *A. aegypti* eggs deposited in treatment and control cups. All analyses (both oviposition and bacterial production experiments) were carried out using generalized linear models (Crawley, 1993) and performed using (R Development Core Team, 2006). Residual analyses were conducted to verify error distribution including checks for overdispersion. Oviposition data were analyzed using binomial family error and overdispersion corrected for quasibinomial family. Bacterial abundances were analyzed via one-way ANOVA of leucine incorporation.

8 Results

The presence of *T. theobaldi* larvae alone had no significant effect on the oviposition behavior of A. aegypti (OAI: 0.11±0.83, $F_{1,28} = 2.495$, $p = 0.11$; Fig. 4-A). However, when *T. theobaldi* larvae were allowed to feed on *A. aegypti* larvae, there was a significant preference among *A. aegypti* females for oviposition cups where predation had occurred: (OAI: 0.31±0.81, $F_{1,32} = 7.481$, $p = 0.008$; Fig. 4-B). The density of live conspecifics had no effect on oviposition behavior of *A. aegypti* (OAI: 0.11±0.75, $F_{1,20} = 0.002$, $p = 0.95$; Fig. 4-C), but when *A. aegypti* larvae were killed and added to treatment cups, there was a significant preference among *A. aegypti* females for these oviposition sites over containers with living larvae (OAI: 0.37±0.79, $F_{1,34} = 17.441$, $p < 0.0001$; Fig. 4-D). When we compared this treatment with the other treatments preferred by *A. aegypti* females (actual predation, above), we found no significant preference (OAI: -0.21±0.84, $F_{1,35} = 2.487$, $p = 0.11$; Fig. 4-E). Finally, we found no significant preference for, or avoidance of, antibiotic-treated test water that held living larvae, vs. antibiotic treated test water that held living plus killed larvae (Compare Fig. 4-D to Fig. 4-F) (OAI: 0.08±0.87, $F_{1,27} = 0.04$, $p = 0.82$).

Bacterial productivity, as indicated by leucine incorporation, did not differ significantly between the Mechanical injury (prey crushed) and Predator feeding on prey (prey+predator). Both of these treatments yielded leucine incorporation that was significantly greater than that in Control (prey alone) ($F_{1,30} = 10.08$, $p < 0.001$; Fig 5).

9 Discussion

We initially predicted that the presence of the predator *Toxorhynchites theobaldi* in *Aedes aegypti* oviposition cups would makes these sites more attractive for oviposition. Our prediction strongly contrasts with previous reports that some organisms, such as the mosquitoes *Culex pipiens* (Angelon & Petranka, 2002) and *Culiseta longiareolata* (Blaustein *et al.*, 2004; Arav & Blaustein, 2006; Silberbush & Blaustein, 2008; Silberbush *et al.*, 2010), amphibians (Orizaola & Brana, 2003) and mites (Montserrat *et al.*, 2007) avoid ovipositing where predators or their cues are present.

Our results showed that when *T. theobaldi* larvae were allowed to feed on *A. aegypti* larvae, *A. aegypti* females, prefer these cups, rather than avoiding ovipositing in these cups (Fig. 4-II). This may be because *A. aegypti* do not meet Blaustein‘s (1999) criteria for the evolution of antipredator oviposition selection. (A) Eggs for each reproductive cycle are laid together as a single clutch and are not spread across multiple sites, (B) Prey have few lifetime

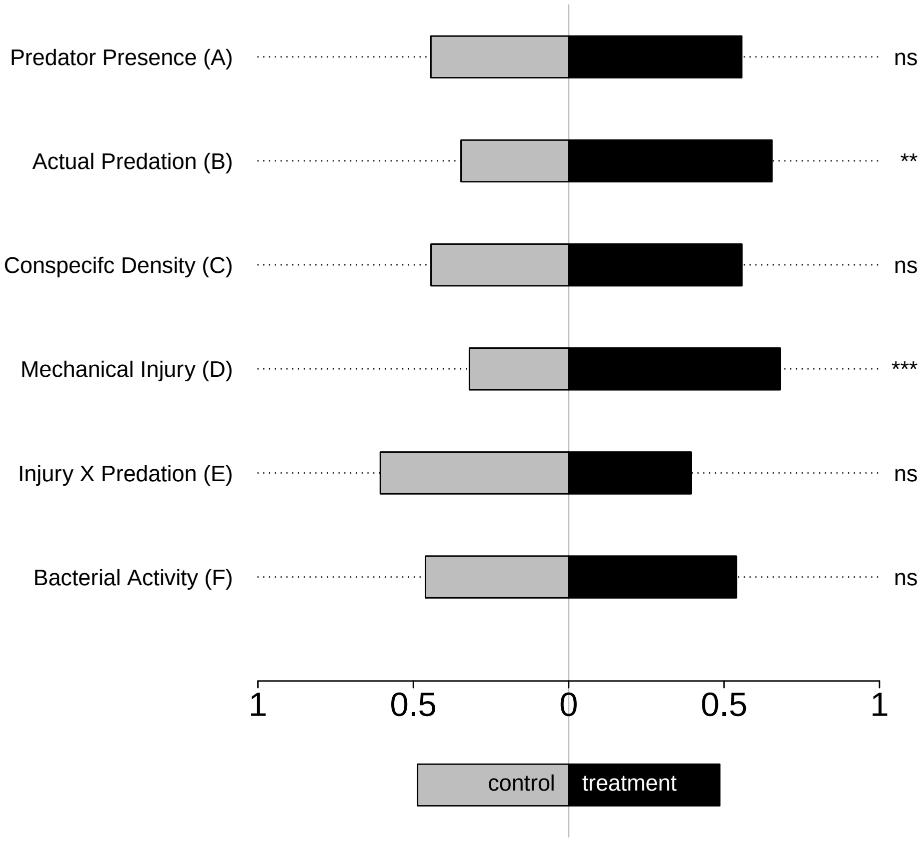

Figura 4: Oviposition response (OAI values) of *A. aegypti* gravid female to control and treatment containers within the six treatments. ***$P<0.001$, **$P<0.01$, $P>0.05$ = ns.

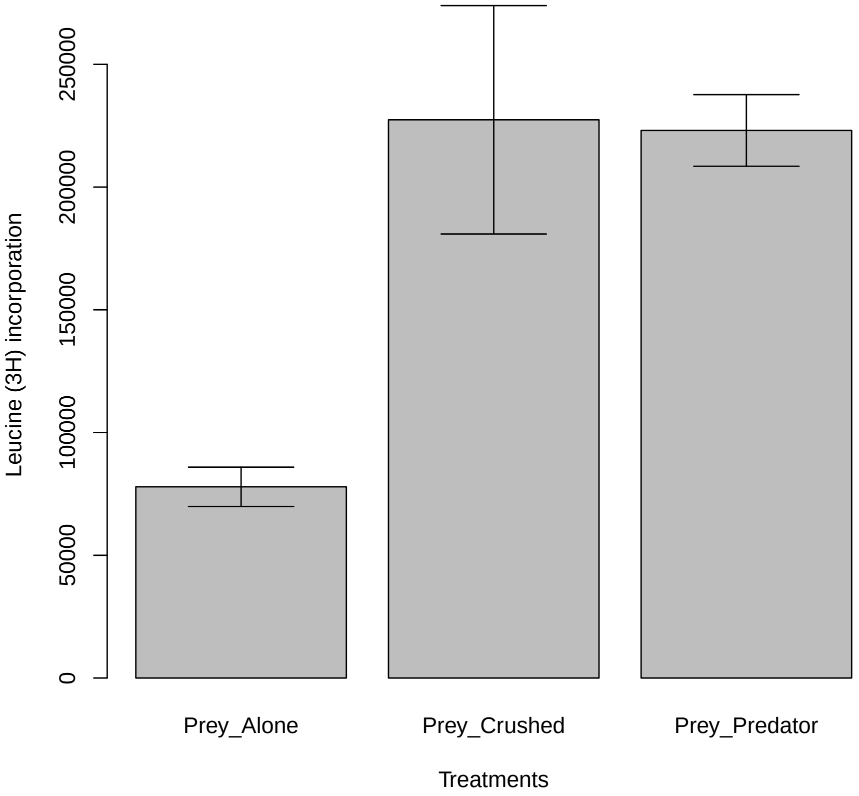

Figura 5: Production of new bacterial biomass based on measurement of protein synthesis (quantified by measuring incorporation of tritiated H-leucine (4,5-[H])) in oviposition containers with: 50 *A. aegypti* prey larvae alone (Prey alone), 33 prey alive plus 17 crushed (Prey Crushed) and 50 prey larvae plus one predator (Prey+Predator). Different letters indicate significant difference among means.

reproductive events. For species that meet both these criteria, the gain in reproductive success from predator avoidance at oviposition can be very large (Blaustein, 1999). Antipredator oviposition behavior is energetically costly Lima & Dill (1989); Blaustein (1999); thus, female mosquitoes should evolve this trait if there is a high risk of losing all of their offspring by predation during a single reproductive event. Indeed, mosquitoes that oviposit in large egg batches (e.g: *Culiseta* and *Culex*) strongly avoid ovipositing in sites with predatory cues (i.e., predator presence or cues of predation act) (Eitam *et al.*, 2002; Stav *et al.*, 2000; Blaustein & Margalit, 1995; Blaustein *et al.*, 2004). However, *A. aegypti* often distributes its eggs over multiple water-filled containers, a phenomenon called "skip oviposition behavior" [reviewed by Colton *et al.* (2003) and Reiter (2007)]. Furthermore, *A. aegypti* can have a lifespan reproduction of 95 days under laboratory conditions (Styer *et al.*, 2007), can have several lifetime reproductive events [reviewed by Reiter (2007)], and have been shown to distribute eggs to multiple sites in the field (Amador, 1995; Honório *et al.*, 2003). These behaviors patterns probably render the gain from avoidance of oviposition with predators relatively small, and may explain why *A. aegypti* shows no evidence of avoidance of oviposition in habitats with predators.

Because conspecifics often compete, females should choose oviposition sites where competition among her progeny will be low (Wilmot *et al.*, 1987; Zahiri & Rau, 1998; Sumba *et al.*, 2008). We suspect that *A. aegypti* females might be attracted to oviposit into cups with predators because conspecific density was reduced by predation. We did not, however, find this effect, perhaps because our larval densities were being 4-5 times lower than those used in a previous study where *A. aegypti* avoidance of conspecifics was reported (Zahiri & Rau, 1998). It is likely that larval response to density will occur if density is raised beyond those used here. Nevertheless, our study demonstrates that predator-induced reduction in conspecific density *per se* does not explain the observed preference of *A. aegypti* for oviposition sites where conspecifics are being subject to predation.

Organic material from detritus is highly attractive to ovipositing mosquitoes (Hazard *et al.*, 1967; Allan & Kline, 1995; Sant'ana *et al.*, 2006; Ponnusamy *et al.*, 2008a,b, 2010). In natural and artificial sites of larval development detritus often forms the base of the food web (Merritt *et al.*, 1992; Moore *et al.*, 2004).

We hypothesized that if a feeding predator leaves parts of the prey uneaten or adds substantial feces to the habitat, this may increase animal-derived organic material in oviposition places, supporting greater microbial populations, and thus making the site more attractive to ovipositing mosquitoes. The oviposition of significantly more eggs in treatment (dead larvae) than control (no dead larvae) cups (Fig. 4-IV) in our study suggests that dead larvae forms detritus that provides a substrate for bacteria, protozoa, rotifer, and fungi. These microorganisms are consumed by other larvae, either indirectly when they are exploiting detritus (Fish & Carpenter, 1982; Walker *et al.*, 1991) or directly (Yee & Juliano, 2006; Yee *et al.*, 2007b,a).

Aedes aegypti females showed no preference for cups containing predators feeding on prey versus prey killed by mechanical injury (Fig. 4-V), providing strong evidence that the predator may indirectly contribute to attractiveness of oviposition cups by increasing animal detritus. Additionally, our PS experiment results show no significant difference in bacterial abundance between these two treatments (Fig. 5).

Bacteria themselves metabolize organic detritus (Merritt *et al.*, 1992), producing volatile and nonvolatile chemical metabolites (Bentley & Day, 1989) that mediate the oviposition behavior of gravid female mosquitoes (Bentley & Day, 1989; Walker *et al.*, 1991; Allan & Kline, 1995; Navarro *et al.*, 2003; Trexler *et al.*, 2003; Ponnusamy *et al.*, 2010). The lack of *A. aegypti* preference between treatment and control antibiotic-treated cups in our study (Fig. 4-VI) suggests that bacteria or their metabolic by-products are responsible for the oviposition preferences we observed in our other treatments. It is likely that oviposition responses of *A. aegypti* gravid females to animal organic material, provide by dead conspecific larvae, were mediated by chemical substances released by bacterial activities. Unfortunately, because *A. aegypti* females were allowed to touch the water surface when ovipositing, we are unable to determine whether females detect these cues via contact or olfaction (i.e., volatile cues vs. contact cues).

Because last-instar *Toxorhynchites* larvae exhibit “pre-pupal killing behavior”, wherein prey are killed without being eaten (Steffan & Evenhuis, 1981; Albeny *et al.*, 2011), we postulate that nature *Toxorhynchites* larvae often increase the animal organic material by this behavior, making oviposition sites more attractive to *A. aegypti* (Fig. 4-II). However, if bacteria provide a profitable food source for mosquito larvae (Merritt *et al.*, 1992; Yee & Juliano, 2006; Yee *et al.*, 2007b,a; Kaufman *et al.*, 2010) it may be that mosquito females are able to recognize places with bacterial activities as excellent for larval development. Thus, we propose that bacterial presence and activity were responsible by attraction of gravid females to oviposition sites (Hazard *et al.*, 1967; Allan & Kline, 1995; Sant'ana *et al.*, 2006; Ponnusamy *et al.*, 2010). We have shown for the first time that the act of predation can increase bacterial abundance (Fig. 3) in such a way that makes these sites attractive for mosquito oviposition (Fig. 4-II).

Bacterial abundance within potential oviposition containers has a positive effect on both larval density and oviposition habitat selection, which in turn plays an important role in mosquito distribution (Rejmankova *et al.*, 1996; Nguyen *et al.*, 1999). The results from our study have implications for distribution and control of *A. aegypti* in the field, especially since bacterial biomass and species composition have been suggested as important environmental determinants of the occurrence and abundance of mosquitoes (Murrell *et al.*, 2011).

The presence of *Toxorhynchites* larvae in *A. aegypti* oviposition cups renders those cups highly attractive to ovipositing *A. aegypti* (Fig. 4-II). Therefore, if ovipositing females cannot assess the predation risk for their offspring and oviposit in places where the predator is present (Torres-Estrada *et al.*, 2001; Blaustein *et al.*, 2004; Arav & Blaustein, 2006; Pamplona *et al.*, 2009), the introduction of natural predators as a biocontrol agent for *A. aegypti* may serve a dual purpose. Not only would predators such as *Toxorhynchites* consume larvae already present in container, but they may actually render these containers more attractive for mosquito oviposition, enhancing predator-prey contact rates, and thus further reducing *A. aegypti* numbers. Additional studies will be needed to test this ideal for *A. aegypti* oviposition in the field, and also to determine the extent to which *Toxoryhnchites* could better exploited for biorational control *A. aegypti* populations.

10 Referências

Albeny, D.; Martins, G.; Andrade, M.; Krüger, R. & Vilela, E. (2011). *Aedes aegypti* survival in the presence of *Toxorhynchites violaceus* (diptera: Culicidae) fourth instar larvae. *Zoologia (Curitiba, Impresso)*, 28, 538–540.

Albeny, D. S.; Rosa, C. S.; Ataide, L. M. S. & Vilela, E. F. (2010). First record of genus *Toxorhynchites* Theobald (Diptera, Culicidae) in Mata Atlântica, Viçosa, Minas Gerais State, Brazil. *Revista Ceres*, 57, 181–184.

Allan, S. & Kline, D. (1995). Evaluation of organic infusions and synthetic compounds mediating oviposition in *Aedes albopictus* and *Aedes aegypti* (diptera: Culicidae). *Journal of chemical ecology*, 21, 1847–1860.

Allan, S. & Kline, D. (1998). Larval rearing water and preexisting eggs influence oviposition by *Aedes aegypti* and *Ae. albopictus* (diptera: Culicidae). *Journal of medical entomology*, 35, 943–947.

Alto, B. W. & Griswold, M. W. (2005). Habitat complexity and sex-dependent predation of mosquito larvae in containers. *Oecologia*, 146, 300–310.

Amador, P. (1995). Dispersal of *Aedes aegypti* in an urban area after blood feeding as demonstrated by rubidium-marked eggs. *Am. J. Twp. Med. Hyg*, 52, 177–179.

Angelon, K. & Petranka, J. (2002). Chemicals of predatory mosquitofish *Gambusia affinis* influence selection of oviposition site by *Culex mosquitoes. Journal of chemical ecology*, 28, 797–806.

Arav, D. & Blaustein, L. (2006). Effects of pool depth and risk of predation on oviposition habitat selection by temporary pool dipterans. *Journal of medical entomology*, 43, 493–7.

Azevedo-Ramos, C.; Van Sluys, M.; Hero, J. & Magnusson, W. (1992). Influence of tadpole movement on predation by odonate naiads. *Journal of Herpetology*, 26, 335–338.

Baker, R.; French, W. & Kitzmiller, J. (1962). Induced copulation in *Anopheles* mosquitoes. *Mosquito News*, 22, 16–17.

Begon, M.; Townsend, C. & Harper, J. (2006). *Ecology: from individuals to ecosystems*. Wiley-Blackwell.

Bentley, M. D. & Day, J. F. (1989). Chemical ecology and behavioral aspects of mosquito oviposition. *Annual review of entomology*, 34, 401–21.

Blaustein, L. (1999). Oviposition site selection in response to risk of predation: evidence from aquatic habitats and consequences for population dynamics and community structure. *Evolutionary theory and processes: modern perspectives, papers in honour of Eviatar Nevo*, 441–456.

Blaustein, L.; Blaustein, J. & Chase, J. (2005). Chemical detection of the predator *Notonecta irrorata* by ovipositing *Culex mosquitoes*. *Journal of vector ecology*, 30, 299.

Blaustein, L.; Kiflawi, M.; Eitam, A.; Mangel, M. & Cohen, J. (2004). Oviposition habitat selection in response to risk of predation in temporary pools: mode of detection and consistency across experimental venue. *Oecologia*, 138, 300–305.

Blaustein, L.; Kotler, B. & Ward, D. (1995). Direct and indirect effects of a predatory backswimmer *Notonecta maculata* on community structure of desert temporary pools. *Ecological Entomology*, 20, 311–318.

Blaustein, L. & Margalit, J. (1995). Spatial distributions of *Culiseta longiareolata* (Culicidae: Diptera) and *Bufo viridis* (Amphibia: Bufonidae) among and within desert pools. *Journal of Arid Environments*, 29, 199–211.

Blaustein, L. & Schwartz, S. (2001). Why study ecology in temporary pools? *Israel Journal of Zoology*, 47, 303–312.

Bonnet, D. & Hu, S. M. K. (1951). The introduction of *Toxorhynchites brevipalpis* into the territory of Hawaii. *Proceedings of the Hawaiian Entomological Society*, 14, 237–242.

Bradshaw, W. & Holzapfel, C. (1985). *The distribution and abundance of treehole mosquitoes in eastern North America: perspectives from north Florida*, Flordia Medical Entomology Laboratory.

Caro, T. (2005). *Antipredator defenses in birds and mammals*. University of Chicago Press.

Carpenter, S.; Kitchell, J. & Hodgson, J. (1985). Cascading trophic interactions and lake productivity. *BioScience*, 35, 634–639.

Chadee, D.; Corbet, P. & Greenwood, J. (1990). Egg-laying yellow fever mosquitoes avoid sites containing eggs laid by themselves or by conspecifics. *Entomologia Experimentalis et Applicata*, 57, 295–298.

Chesson, J. (1984). Effect of notonectids (Hemiptera: Notonectidae) on mosquitoes (Diptera: Culicidae): predation or selective oviposition. *Environmental Entomology*, 13, 531–538.

Chivers, D. & Smith, R. (1998). Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. *Ecoscience*, 5, 338–352.

Cochran-Stafira, D. & von Ende, C. (1998). Integrating bacteria into food webs: Studies with *Sarracenia purpurea* inquilines. *ECOLOGY*, 79, 880–898.

Collins, L. E. & Blackwell, A. (2000). The biology of *Toxorhynchites* mosquitoes and their potential as biocontrol agents. *Biocontrol News and Information*, 21, 105 – 116.

Colton, Y.; Chadee, D. & Severson, D. (2003). Natural skip oviposition of the mosquito *Aedes aegypti* indicated by codominant genetic markers. *Medical and veterinary entomology*, 17, 195–204.

Corbet, P. & Griffiths, A. (1963). Observations on the aquatic stages of two species of *Toxorhynchites* (diptera: Culicidae) in uganda. 38, 125–135.

Crawley, M. J. (1993). *GLIM for ecologists*. Oxford.

Dam, A. R. V. & Walton, W. E. (2008). The effects of predatory fish exudates on the ovipositional behavior of three mosquito species: *Culex quinquefaciatus*, *Aedes aegypti* and *Culex tarsalis*. *Medical and Veterinary Entomology*, 22, 399–404.

Darwin, C. (1859). *The origin of species*. Collier.

de Ruiter, P.; Wolters, V. & Moore, J. (2005). *Dynamic food webs: multispecies assemblages, ecosystem development, and environmental change*, vol. 3. Academic Pr.

Diehl, S.; Cooper, S.; Kratz, K.; Nisbet, R.; Roll, S.; Wiseman, S. & Jenkins Jr, T. (2000). Effects of multiple, predator-induced behaviors on short-term producer-grazer dynamics in open systems. *The American Naturalist*, 156, 293–313.

Dolan, J. & Gallegos, C. (1991). Trophic coupling of rotifers, microflagellates, and bacteria during fall months in the Rhode River Estuary. *Marine Ecology Progress Series*, 77, 147–156.

Eisenberg, J. N. S.; Washburn, J. O. & Schreiber, S. J. (2000). Generalist Feeding Behaviors of *Aedes Sierrensis* Larvae and Their Effects on Protozoan Populations. *Ecology*, 81, 921–935.

Eitam, A. & Blaustein, L. (2010). Effects of predator density and duration of predator occupancy on crustacean abundance and diversity in experimental pools. *HYDROBIOLOGIA*, 652, 269–276.

Eitam, A.; Blaustein, L. & Mangel, M. (2002). Effects of *Anisops sardea* (Hemiptera: Notonectidae) on oviposition habitat selection by mosquitoes and other dipterans and on community structure in artificial pools. *Hydrobiologia*, 485, 183–189.

Endler, J. A. (1986). *Predator-prey relationships*, Univ. of Chicago Press, chap. Defense against predators. pp. 109–134.

Engber, B.; Sone, P. & Pillai, J. (1978). The occurrence of *Toxorhynchites amboinensis* in Western Samoa. *Mosquito News*, 38, 295–296.

Fish, D. & Carpenter, S. (1982). Leaf litter and larval mosquito dynamics in tree-hole ecosystems. *Ecology*, 283–288.

Flecker, A. & Townsend, C. (1994). Community-wide consequences of trout introduction in new zealand streams. *Ecological Applications*, 798–807.

Fritz, R. (1983). Ant protection of a host plant's defoliator: consequence of an ant-membracid mutualism. *Ecology*, 789–797.

Gastreich, K. (1999). Trait-mediated indirect effects of a theridiid spider on an ant-plant mutualism. *Ecology*, 80, 1066–1070.

Gomes, A.; Sciavico, C. & Eiras, Á. (2006). Periodicidade de oviposição de fêmeas de *Aedes aegypti* (Linnaeus, 1762)(Diptera: Culicidae) em laboratório e campo. *Revista da Sociedade Brasileira de Medicina Tropical*, 39, 327–332.

Hairston, N.; Smith, F. & Slobodkin, L. (1960). Community structure, population control, and competition. *American Naturalist*, 421–425.

Hairston Jr, N. & Hairston Sr, N. (1993). Cause-effect relationships in energy flow, trophic structure, and interspecific interactions. *American Naturalist*, 379–411.

Hassell, M. & Southwood, T. (1978). Foraging strategies of insects. *Annual Review of Ecology and Systematics*, 9, 75–98.

Hazard, E.; Mayer, M. & Savage, K. (1967). Attraction and oviposition stimulation of gravid female mosquitoes by bacteria isolated from hay infusions. *Mosquito News*, 27, 133–6.

Honório, N.; Silva, W.; Leite, P.; Gonçalves, J.; Lounibos, L. & Lourenço-de Oliveira, R. (2003). Dispersal of *Aedes aegypti* and *Aedes albopictus* (diptera: Culicidae) in an urban endemic dengue area in the state of rio de janeiro, brazil. *Memórias do Instituto Oswaldo Cruz*, 98, 191–198.

Huang, C. & Sih, A. (1991). Experimental studies on direct and indirect interactions in a three trophic-level stream system. *Oecologia*, 85, 530–536.

Hurst, T.; Kay, B.; Brown, M. & Ryan, P. (2010). *Melanotaenia duboulayi* Influence Oviposition Site Selection by *Culex annulirostris* (Diptera: Culicidae) and *Aedes notoscriptus* (Diptera: Culicidae) but Not *Culex quinquefasciatus* (Diptera: Culicidae). *Environmental entomology*, 39, 545–551.

Juliano, S. (2009). Species interactions among larval mosquitoes: context dependence across habitat gradients. *Annual review of entomology*, 54, 37–56.

Juliano, S. & Lounibos, L. (2005). Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecology Letters*, 8, 558–574.

Juliano, S.; Lounibos, L.; Nishimura, N. & Greene, K. (2010). Your worst enemy could be your best friend: predator contributions to invasion resistance and persistence of natives. *Oecologia*, 162, 709–718.

Juliano, S. & Reminger, L. (1992). The relationship between vulnerability to predation and behavior of larval treehole mosquitoes: geographic and ontogenetic differences. *Oikos*, 63, 465–476.

Juliano, S. A. & Gravel, M. E. (2002). Pradation and the evolution of prey behavior: an experiment with tree role mosquitoes. *Behavioral Ecology*, 13, 301–311.

Kats, L. & Dill, L. (1998). The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience*, 5, 361–394.

Kaufman, M.; Bland, S.; Worthen, M.; Walker, E. & Klug, M. (2001). Bacterial and fungal biomass responses to feeding by larval *Aedes triseriatus* (diptera: Culicidae). *Journal of medical entomology*, 38, 711–719.

Kaufman, M.; Pelz-stelinski, K.; Yee, D.; Juliano, S.; Ostrom, P. & Walker, E. (2010). Stable isotope analysis reveals detrital resource base sources of the tree hole mosquito, *Aedes triseriatus*. *Ecological Entomology.*

Kaufman, M. & Walker, E. (2006). Indirect effects of soluble nitrogen on growth of *Ochlerotatus triseriatus* larvae in container habitats. *Journal of medical entomology*, 43, 677–688.

Kaufman, M. G.; Walker, E. D.; Smith, T. W.; Merritt, R. W. & Klug, M. J. (1999). Effects of larval mosquitoes *Aedes triseriatus* and stemflow on microbial community dynamics in container habitats. *Applied and environmental microbiology*, 65, 2661–73.

Kerfoot, W. & Sih, A. (1987). Predation: direct and indirect impacts on aquatic communities.

Kesavaraju, B.; Damal, K. & Juliano, S. a. (2007). Threat-Sensitive Behavioral Responses to Concentrations of Water-Borne Cues from Predation. *Ethology : formerly Zeitschrift fur Tierpsychologie*, 113, 199–206.

Kesavaraju, B. & Juliano, S. (2010). Nature of predation risk cues in container systems: Mosquito responses to solid residues from predation. *Annals of the Entomological Society of America*, 103, 1038–1045.

Kesavaraju, B. & Juliano, S. a. (2004). Differential Behavioral Responses to Water-Borne Cues to Predation in Two Container-Dwelling Mosquitoes. *Annals of the Entomological Society of America*, 97, 194–201.

Kesavaraju, B.; Khan, D. & Gaugler, R. (2011). Behavioral differences of invasive container-dwelling mosquitoes to a native predator. *Journal of medical entomology*, 48, 526–532.

Kirchman, D. (1993). Leucine incorporation as a measure of biomass production by heterotrophic bacteria. *Handbook of methods in aquatic microbial ecology. Lewis*, 509–512.

Kneitel, J. M. & Miller, T. E. (2002). Resource and Top-Predator Regulation in the Pitcher Plant (Sarracenia Purpurea) Inquiline Community. *Ecology*, 83, 680–688.

Kohler, S. & McPeek, M. (1989). Predation risk and the foraging behavior of competing stream insects. *Ecology*, 1811–1825.

Kramer, W. & Mulla, M. (1979). Oviposition attractants and repellents of mosquitoes: oviposition responses of *Culex* mosquitoes to organic infusions. *Environmental Entomology*, 8, 1111–1117.

Laurence, B. & Pickett, J. (1985). An oviposition attractant pheromone in *Culex quinquefasciatus* say (diptera: Culicidae). *Bulletin of entomological research*, 75, 283–290.

Lawler, S. (1989). Behavioural responses to predators and predation risk in four species of larval anurans. *Animal Behaviour*, 38, 1039–1047.

Lima, S. (1998). Nonlethal effects in the ecology of predator-prey interactions. *Bioscience*, 48, 25–34.

Lima, S. & Dill, L. (1989). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, 68, 619–640.

Lounibos, P. & Campos, R. E. (2002). Investigaciones recientes sobre *Toxorhynchites rutilus* (Diptera Culicidae) con referencia al control biologico de mosquitos habitantes em recipientes. *Entomotropica*, 17(2), 146–156.

Machado-Allison, C. (1981). Ecologia de los mosquitos (culicidae).ii. larvas y pupas. *Acta Bio. Venez*, 11, 51–59.

Mancinelli, G.; Costantini, M. & Rossi, L. (2002). Cascading effects of predatory fish exclusion on the detritus-based food web of a lake littoral zone (lake vico, central italy). *Oecologia*, 133, 402–411.

May, R. (2001). *Stability and complexity in model ecosystems*, vol. 6. Princeton Univ Pr.

Mayhew, P. (2001). Herbivore host choice and optimal bad motherhood. *Trends in Ecology & Evolution*, 16, 165–167.

McCall, P. J. (2002). *Chemoecology of insect eggs and egg deposition.*, Blackwell, Berlin., chap. Chemoecology of oviposition in insects of medical and veterinary importance. pp. 265–290.

McCrae, A. (1984). Oviposition by african malaria vector mosquitoes. ii. effects of site tone, water type and conspecific immatures on target selection by freshwater *Anopheles gambiae* giles, sensu lato. *Annals of tropical medicine and parasitology*, 78, 307.

McIntosh, A. & Townsend, C. (1996). Interactions between fish, grazing invertebrates and algae in a new zealand stream: a trophic cascade mediated by fish-induced changes to grazer behaviour? *Oecologia*, 108, 174–181.

McPeek, M. (1990). Behavioral differences between *Enallagma* species (Odonata) influencing differential vulnerability to predators. *Ecology*, 71, 1714–1726.

Merritt, R. W.; Dadd, R. H. & Walker, E. D. (1992). Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annual review of entomology*, 37, 349–76.

Messina, F. (1981). Plant protection as a consequence of an ant-membracid mutualism: interactions on goldenrod (solidago sp.). *Ecology*, 1433–1440.

Miyagi, I.; Toma, T. & Mogi, M. (1992). Biological control of container-breeding mosquitoes, *Aedes albopictus* and *Culex quinquefasciatus*, in a japanese island by release of *Toxorhynchites splendens* adults. *Medical and Veterinary Entomology.*, 6, 290–300.

Montserrat, M.; Bas, C.; Magalhaes, S.; Sabelis, M.; de Roos, A. & Janssen, A. (2007). Predators induce egg retention in prey. *Oecologia*, 150, 699–705.

Moore, J. C.; Berlow, E. L.; Coleman, D. C.; Ruiter, P. C.; Dong, Q.; Hastings, A.; Johnson, N. C.; McCann, K. S.; Melville, K.; Morin, P. J.; Nadelhoffer, K.; Rosemond, A. D.; Post, D. M.; Sabo, J. L.; Scow, K. M.; Vanni, M. J. & Wall, D. H. (2004). Detritus, trophic dynamics and biodiversity. *Ecology Letters*, 7, 584–600.

Müller, C. & Arand, K. (2007). Trade-offs in oviposition choice? food-dependent performance and defence against predators of a herbivorous sawfly. *Entomologia experimentalis et applicata*, 124, 153–159.

Murrell, E.; Damal, K.; Lounibos, L. & Juliano, S. (2011). Distributions of competing container mosquitoes depend on detritus types, nutrient ratios, and food availability. *Annals of the Entomological Society of America*, 104, 688–698.

Murrell, E. & Juliano, S. (2008). Detritus type alters the outcome of interspecific competition between *Aedes aegypti* and *Aedes albopictus* (diptera: Culicidae). *Journal of medical entomology*, 45, 375.

Navarro, D. M. a. F.; de Oliveira, P. E. S.; Potting, R. P. J.; Brito, a. C.; Fital, S. J. F. & Sant'Ana, a. E. G. (2003). The potential attractant or repellent effects of different water types on oviposition in *Aedes aegypti* L. (Dipt., Culicidae). *Journal of Applied Entomology*, 127, 46–50.

Nguyen, T.; Su, T.; Mulla, M. *et al.* (1999). Bacteria and mosquito abundance in microcosms enriched with organic matter and treated with a bacillus thuringiensis subsp. israelensis formulation. *Journal of vector ecology: journal of the Society for Vector Ecology*, 24, 191.

Oksanen, L.; Fretwell, S.; Arruda, J. & Niemela, P. (1981). Exploitation ecosystems in gradients of primary productivity. *American Naturalist*, 240–261.

Orizaola, G. & Brana, F. (2003). Do predator chemical cues affect oviposition site selection in newts. *Herpetological Journal*, 13, 189–194.

Paine, R. (1980). Food webs: linkage, interaction strength and community infrastructure. *Journal of Animal Ecology*, 49, 667–685.

Paine, R. T. (1966). Food Web Complexity and Species Diversity. *American Naturalist*, 100, 65–75.

Pamplona, L. D. G. C.; Alencar, C. H.; Lima, J. W. O. & Heukelbach, J. (2009). Reduced oviposition of *Aedes aegypti* gravid females in domestic containers with predatory fish. *Tropical medicine & international health : TM & IH*, 14, 1347–50.

Peckarsky, B. & McIntosh, A. (1998). Fitness and community consequences of avoiding multiple predators. *Oecologia*, 113, 565–576.

Persson, L. (1999). Trophic cascades: adding heterogeneity and the trophic level concept at the end of the road. *Oikos*, 85, 385–397.

Peterson, G. (1956). The introduction of megarhinus mosquitoes of the genus *Toxorhynchites* into american samoa. *Journal of Economical Entomology*, 49, 786–789.

Ponnusamy, L.; Wesson, D. M.; Arellano, C.; Schal, C. & Apperson, C. S. (2010). Species composition of bacterial communities influences attraction of mosquitoes to experimental plant infusions. *Microbial ecology*, 59, 158–73.

Ponnusamy, L.; Xu, N.; Nojima, S.; Wesson, D. M.; Schal, C. & Apperson, C. S. (2008a). Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 9262–7.

Ponnusamy, L.; Xu, N.; Stav, G.; Wesson, D. M.; Schal, C. & Apperson, C. S. (2008b). Diversity of bacterial communities in container habitats of mosquitoes. *Microbial ecology*, 56, 593–603.

Preisser, E. (2003). Field evidence for a rapidly cascading underground food web. *Ecology*, 84, 869–874.

Preisser, E.; Bolnick, D. & Benard, M. (2005). Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, 86, 501–509.

R Development Core Team (2006). *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.
URL `http://www.R-project.org`

Reiter, P. (2007). Oviposition, dispersal, and survival in *Aedes aegypti*: implications for the efficacy of control strategies. *Vector-Borne and Zoonotic Diseases*, 7, 261–273.

Rejmankova, E.; Roberts, D.; Manguin, S.; Pope, K.; Komarek, J. & Post, R. (1996). Anopheles albimanus (diptera: Culicidae) and cyanobacteria: an example of larval habitat selection. *Environmental entomology*, 25, 1058–1067.

Relyea, R. (2001). Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology*, 82, 523–540.

Relyea, R. & Werner, E. (1998). Quantifying the relation between predator-induced behavior and growth performance in larval anurans. *Ecology*, 80, 2117–2124.

Resetarits, W. (2001). Colonization under threat of predation: avoidance of fish by an aquatic beetle, *Tropisternus lateralis* (coleoptera: Hydrophilidae). *Oecologia*, 129, 155–160.

Rieger, J.; Binckley, C. & Resetarits Jr, W. (2004). Larval performance and oviposition site preference along a predation gradient. *Ecology*, 85, 2094–2099.

Riemann, B. & Azam, F. (1992). Measurements of bacterial protein synthesis in aquatic environments by means of leucine incorporation. *Aquatic Microbial Ecology*, 6, 91–105.

Rosa, C. & DeSouza, O. (2011). Spiders, ants and an amazonian myrmecophyte: A tale of trophic cascades. *Sociobiology*, 58, 403.

Rubio, Y.; Rodriguez, D.; Machado-Allison, C. E. & Lon, J. (1980). Algunos aspectos de lo comportemento de *Toxorhynchites theobaldi* (diptera: Culicidae). *Acta Cien. Venez.*, 31, 345–351.

Sant'ana, A. L.; Roque, R. A. & Eiras, A. E. (2006). Characteristics of grass infusions as oviposition attractants to *Aedes (Stegomyia)* (Diptera: Culicidae). *Journal of Medical Entomology*, 43, 214–220.

Santos, E.; Correia, J.; Muniz, L.; Meiado, M. & Albuquerque, C. (2010). Oviposition activity of *Aedes aegypti* L. (Diptera: Culicidae) in response to different organic infusions. *Neotropical entomology*, 39, 299–302.

Scheiner, S. (1993). Manova: multiple response variables and multispecies interactions. *Design and analysis of ecological experiments. Chapman and Hall, New York, New York, USA*, 94–112.

Schmitz, O. (1998). Direct and indirect effects of predation and predation risk in old-field interaction webs. *The American Naturalist*, 151, 327–342.

Schmitz, O.; Beckerman, A. & O'Brien, K. (1997). Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. *Ecology*, 78, 1388–1399.

Schreiber, E. T. (2007). *Toxorhynchites. Journal of the American Mosquito Control Association*, 23, 129–132.

Schwenk, W. S.; Strong, A. M. & Sillett, T. S. (2010). Effects of bird predation on arthropod abundance and tree growth across an elevational gradient. *Journal of Avian Biology*, 41, 367–377.

Sempala, S. D. K. (1983). Interactions between immature *Aedes africanus* (Theobald) and larvae of 2 predatory species of *Toxorhynchites* (Diptera, Culicidae) in Zika Forest, Uganda. *Bulletin of Entomological Research*, 73, 19–24.

Silberbush, A. & Blaustein, L. (2008). Scientific Note Oviposition habitat selection by a mosquito in response to a predator: Are predator-released kairomones air-borne cues? *Journal of Vector Ecology*, 33, 208–211.

Silberbush, A.; Markman, S.; Lewinsohn, E.; Bar, E.; Cohen, J. E. & Blaustein, L. (2010). Predator-released hydrocarbons repel oviposition by a mosquito. *Ecology letters*, 1–10.

Sinsabaugh, R. & Linkins, A. (1990). Enzymic and chemical analysis of particulate organic matter from a boreal river. *Freshwater Biology*, 23, 301–309.

Skelly, D. & Werner, E. (1990). Behavioral and life-historical responses of larval american toads to an odonate predator. *Ecology*, 71, 2313–2322.

Srivastava, D.; Kolasa, J.; Bengtsson, J.; Gonzalez, A.; Lawler, S.; Miller, T.; Munguia, P.; Romanuk, T.; Schneider, D. & Trzcinski, M. (2004). Are natural microcosms useful model systems for ecology? *Trends in ecology & evolution*, 19, 379–384.

Stav, G.; Blaustein, L. & Margalit, Y. (2000). Influence of nymphal *Anax imperator* (odonata: Aeshnidae) on oviposition by the mosquito *Culiseta longiareolata* (diptera: Culicidae) and community structure in temporary pools. *Journal of Vector Ecology*, 25, 190–202.

Steffan, W. A. & Evenhuis, N. L. (1981). Biology of *Toxorhynchites. Annual Reviews Entomology*, 26, 159–181.

Stoks, R.; Block, M.; Van De Meutter, F. & Johansson, F. (2005). Predation cost of rapid growth: behavioural coupling and physiological decoupling. *Journal of Animal Ecology*, 74, 708–715.

Strong, D. (1992). Are trophic cascades all wet? differentiation and donor-control in speciose ecosystems. *Ecology*, 73, 747–754.

Styer, L.; Minnick, S.; Sun, A. & Scott, T. (2007). Mortality and reproductive dynamics of *Aedes aegypti* (diptera: Culicidae) fed human blood. *Vector-Borne And Zoonotic Diseases*, 7, 86–98.

Suleman, M. & Shirin, M. (1981). Laboratory studies on oviposition behaviour of *Culex quinquefasciatus* say (diptera: Culicidae): choice of oviposition medium and oviposition cycle. *Bulletin of Entomological Research*, 71, 361–369.

Sumba, L. A.; Ogbunugafor, C. B.; Deng, A. L. & Hassanali, A. (2008). Regulation of oviposition in Anopheles gambiae s.s.: role of inter- and intra-specific signals. *Journal of chemical ecology*, 34, 1430–6.

Tabachnick, W.; Powell, J. *et al.* (1979). A world-wide survey of genetic variation in the yellow fever mosquito, *Aedes aegypti*. *Genet Res*, 34, 215–29.

Torres-Estrada, J.; Rodriguez, M.; Cruz-Lopez, L. & Arredondo-Jimenez, J. (2001). Selective oviposition by *Aedes aegypti* (Diptera: Culicidae) in response to *Mesocyclops longisetus* (Copepoda: Cyclopoidea) under laboratory and field conditions. *Journal of medical entomology*, 38, 188–192.

Trexler, J. D.; Apperson, C. S.; Zurek, L.; Gemeno, C.; Schal, C.; Kaufman, M.; Walker, E.; Watson, D. W. & Wallace, L. (2003). Role of bacteria in mediating the oviposition responses of *Aedes albopictus* (Diptera: Culicidae). *Journal of medical entomology*, 40, 841–8.

Trzcinski, M. K.; Walde, S. J. & Taylor, P. D. (2005). Local Interactions in Pitcher Plant Communities Scale-Up to Regional Patterns in Distribution and Abundance. *Environmental Entomology*, 34, 1464–1470.

Van Buskirk, J. (2000). The costs of an inducible defense in anuran larvae. *Ecology*, 81, 2813–2821.

van Rijn, P.; van Houten, Y. & Sabelis, M. (2002). How plants benefit from providing food to predators even when it is also edible to herbivores. *Ecology*, 83, 2664–2679.

Vanni, M. & Layne, C. (1997). Nutrient recycling and herbivory as mechanisms in the ”top-down”effect of fish on algae in lakes. *Ecology*, 78, 21–40.

Vanni, M.; Layne, C. & Arnott, S. (1997). ”top-down”trophic interactions in lakes: effects of fish on nutrient dynamics. *Ecology*, 78, 1–20.

Volterra, V. (1928). Variations and fluctuations of the number of individuals in animal species living together. *ICES Journal of Marine Science*, 3, 3.

Walker, E. D.; Lawson, D. L.; Merritt, R. W.; Morgan, W. T. & Klug, M. J. (1991). Nutrient dynamics, bacterial populations, and mosquito productivity In tree hole ecosystems and microcosms. *Ecology*, 72, 1529–1546.

Washburn, J. (1995). Regulatory factors affecting larval mosquito populations in container and pool habitats: implications for biological control. *Journal of the american mosquito Control association*, 11, 279–283.

Werner, E. (1992). Individual behavior and higher-order species interactions. *American Naturalist*, 140, 5–32.

Werner, E. & McPeek, M. (1994). Direct and indirect effects of predators on two anuran species along an environmental gradient. *Ecology*, 1368–1382.

Werner, E. & Peacor, S. (2003). A review of trait-mediated indirect interactions in ecological communities. *Ecology*, 84, 1083–1100.

Wetzel, R. (1995). Death, detritus, and energy flow in aquatic ecosystems. *Freshwater Biology*, 33, 83–89.

Wilmot, T. R.; Cope, S. E. & Ban, A. R. (1987). The Effect Of Immature Mosquitoes On Oviposition By *Culex pipiens quinquefasciatus* And *Culiseta incidens* (diftera: Culicidae) in the Field. *Bulletin Society Vector Ecol.*, 12, 512–516.

Wisenden, B. (2000). Olfactory assessment of predation risk in the aquatic environment. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 355, 1205.

WRBU (2001). Systematic catalog of culicidae.

Yanoviak, S. (2001). Predation, resource availability, and community structure in neotropical water-filled tree holes. *Oecologia*, 126, 125–133.

Yee, D. & Juliano, S. (2006). Consequences of detritus type in an aquatic microsystem: effects on water quality, micro-organisms and performance of the dominant consumer. *Freshwater biology*, 51, 448–459.

Yee, D. & Juliano, S. (2007). Abundance matters: a field experiment testing the more individuals hypothesis for richness–productivity relationships. *Oecologia*, 153, 153–162.

Yee, D. a.; Kaufman, M. G. & Juliano, S. a. (2007a). The significance of ratios of detritus types and micro-organism productivity to competitive interactions between aquatic insect detritivores. *The Journal of animal ecology*, 76, 1105–15.

Yee, D. a.; Kesavaraju, B. & Juliano, S. a. (2007b). Direct and indirect effects of animal detritus on growth, survival, and mass of invasive container mosquito *Aedes albopictus* (Diptera: Culicidae). *Journal of medical entomology*, 44, 580–8.

Zahiri, N. & Rau, M. (1998). Oviposition attraction and repellency of *Aedes aegypti* (Diptera: Culicidae) to waters from conspecific larvae subjected to crowding, confinement, starvation, or infection. *Journal of medical entomology*, 35, 782–787.