



## Predation rates of mixed instar Odonata naiads feeding on *Aedes aegypti* and *Armigeres moulti* (Diptera: Culicidae) larvae



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### ABSTRACT

In Thailand, several important diseases are transmitted by mosquitoes. Many vector control programs focus on the reduction of these medically important mosquitoes through the application of pesticides, bed-nets and the introduction of biological control agents. Odonates naiads are important, naturally occurring predators of vector mosquitoes. To estimate the predation rates of odonate species in Thailand, we conducted an experiment in which the predation rates were compared across a range of predator and prey densities. We used seven different predator species from different instars that represented the composition of naiads in our study area. Body sizes ranged between 2.6 mm and 15.9 mm. Two different prey species were used, larvae of the mosquito *Armigeres moulti* Edwards, 1914 and *Aedes aegypti* L. 1762. Predation rates showed a positive non-linear relationship with prey densities and a negative non-linear relationship with predator densities. The mean  $\pm$  SE predation rates per predator were  $6.2 \pm 0.8$  individuals per 24 h for dragonfly naiads and  $5.1 \pm 0.7$  for damselfly naiads. Predation rates were very low compared to previously recorded rates. However, unlike previous research, we did not focus on single species in a late stage of development but on multiple species in all stages that resembled the natural odonata community composition.

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### Introduction

Mosquito-borne diseases such as malaria and dengue fever are a tremendous burden to people worldwide. The prevalence of these diseases has increased over recent decades and the need for efficient vector control strategies is becoming more important (Lemon et al., 2008). Predation and biological control are important facets of vector population control, either as an applied strategy or as an existing ecosystem service. Mosquito predators such as frogs (Anura) (Raghavendra et al., 2008), bats (Chiroptera) (Reiskind and Wund, 2009), and bony fish (Osteichthyes) (Chandra et al., 2008) feed on adult mosquitoes or their larvae and control their populations. Some predator species are used as biological control agents in vector control programs. Mosquito fish (*Gambusia affinis*), for example, are commonly used to reduce the number of mosquito larvae in water tanks (Kumar and Hwang, 2006). Odonata naiads are another group of mosquito larvae predators. They have not been extensively studied but show great potential in biological

control (Mandal et al., 2008). Individuals of some odonate species have been shown to prey on numbers as high as 66 mosquito larvae per day (Chatterjee et al., 2007). Several studies have tried to quantify the effects of odonate predation on mosquito populations (Hirvonen and Ranta, 1996; Fincke et al., 1997; Stav et al., 2000; Mandal et al., 2008; Saha et al., 2012). Stav et al. (2000) showed that odonate naiads caused a significant reduction in mosquito oviposition as well as a 100% reduction in mosquito emergence. Other studies have also reported very high reductions in mosquito larvae populations, ranging from 66% to 89% (Fincke et al., 1997; Chatterjee et al., 2007; Kweka et al., 2011). Nevertheless, most studies of mosquito larvae predation by odonates focused on food deprived naiads in a late developmental stage. These conditions are likely to cause an overestimate in predation rates as well as mosquito reduction.

The order Odonata can be divided into two suborders: the Anisoptera, commonly known as the dragonflies, and the Zygoptera, commonly known as the damselflies. Dragonflies are generally larger than damselflies and can be easily distinguished by the dissimilar shape and open position of the wings, as well as the eyes, either touch or are nearly touching (Silsby, 2001). Many species from both suborders live most of their lives under water as naiads where they feed on mosquito larvae and other small invertebrates and vertebrates. There are many factors that affect the rate of mosquito larvae predation by

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odonates, such as habitat type, prey density, prey type, predator density, predator size, and water quality (Fincke et al., 1997; Kweka et al., 2011; Saha et al., 2012). Predation rates can also differ among habitat types. In more complex habitats, predation is generally lower; thus, the mosquito larvae can find refuges and it requires more effort for the odonate naiads to locate their prey (Saha et al., 2012).

The aim of the current study was to experimentally estimate the predation rates of Thai Odonata on mosquito larvae in response to varying predator and prey densities using Odonata naiads from varying instars and different species that represent a compositionally and demographically realistic predator community. Several mosquito-borne diseases are prevalent in Thailand, including malaria, Japanese encephalitis, dengue fever, chikungunya, and filariasis (Rattanarithikul et al., 2005); however, few studies have focused on the biological control potential of local predators of mosquito larvae. We used odonate community compositions in our experimental treatments that would result in predation rate estimates resembling those expected when using all Odonata instars available for use in biocontrol projects. Biological control using Odonates has previously been reported in Myanmar (Sebastian et al., 1990). Here, local communities were involved in catching wild odonates and adding them to water storage containers (Sebastian et al., 1990). This approach has been reported to be one of the very few successful methods for using odonates as biological control agents of mosquitoes (Corbet, 2004). In Thailand, and other tropical countries, wild odonate communities generally consist of several different species at a range of developmental stages (Kalkman et al., 2008). In the context of biological control, it is thus of great interest to assess the predation rates of odonates based on a community of different species and instar levels. Therefore, treatments consisted of species mixtures using a variety of species and developmental stages that represented the naiad community composition in our study area. We developed several *a priori* models of predation rates that were based on the following series of non-mutually exclusive hypotheses. (1) Higher predator densities result in lower predation rates. Mosquito larvae will adapt their behaviour to an increased perception of predation risk (Juliano and Gravel, 2002). This will result in lower capture rates by odonate predators. (2) Predation rates are higher for larger, older individuals (Fincke et al., 1997). Larger individuals have a higher energy requirement and thus need to feed on more prey. (3) Predation rates are higher when prey densities increase. With increased prey density, encounters between prey and predator become more likely (Saha et al., 2012). Moreover, odonates might reduce their handling time per prey item, and only feed on the most nutritional parts as observed in *Notonecta* (Chesson, 1989). Satiation is likely to occur after a certain threshold; therefore, we expect this relationship to be non-linear (Mathavan, 1976). (4) Predation rates differ between different prey types. To gain the same nutritional value, a predator needs fewer prey when the prey are larger. Moreover, some prey species can behave differently from others, certain species can adapt to predation risk while others might not be able to do so (Juliano and Gravel, 2002). By testing these hypotheses, we sought to characterise mosquito–Odonata predator–prey relationships and evaluate the relative roles of predator densities, prey densities and prey type.

## Methods

### Sample preparation

Odonate naiads were acquired using two methods. The majority of dragonfly and damselfly naiads were caught with a handheld net that was scraped through the substrate of an odonate-rich canal with stagnant water in Mueang Kamphaeng Phet, Thailand (latitude: 16°29' 27.3474" N, longitude: 99°31' 19.923" E). The content of the net was sieved to remove sand from the larger particles, after which we collected all odonate naiads with tweezers, regardless of size, to get a representative odonate community. In the field, naiads were

stored in 1.5 L plastic containers with a water-plant, *Pistia stratiotes* L and water from the canal. Naiads were subsequently transferred to 25, 1.5 L, plastic containers with *P. stratiotes* and water from the canal; each container held no more than five naiads to reduce potential for cannibalism. After 5 h, the naiads were fed with two mosquito larvae per naiad to familiarize them with their experimental prey and also to further reduce the potential for cannibalistic behaviour. The naiads were kept in this new habitat for two days to acclimatize. Containers were provided with two mosquito larvae per day during this acclimatization period to avoid producing artificially high predation rates in the experiments due to food deprivation.

A smaller number of additional dragonfly naiads were reared from eggs. A mating pair of *Brachythemis contaminata* (Fabricius, 1793) was located near the canal. After mating, the female deposited her eggs onto a floating branch. This branch with eggs was collected and kept in a large cylindrical plastic container (9.5 L). The container was filled with rainwater and covered with mosquito netting. The container was stored outside in a shady area to avoid heat stress. The first naiads were observed after approximately 6 to 8 weeks, and the young naiads were fed with chironomid larvae, which were readily available at that time.

A sufficient identification key for Odonata naiads from Thailand does not currently exist. Therefore, we first identified adult odonates in the study area over a four month period. Adult odonates were caught on 18 different days with a handheld net and close-up photographs were made of all specimen after which the odonates were released. The photographs were used for identification of the species with help of Subramanian (2008) and additional Internet resources (<http://thaiondonata.blogspot.com/>). We then identified Odonata naiads to a family level with the keys provided by Hartmann (2006) and additional Internet resources (<http://odonata-malaysia.blogspot.com/>). The families were then compared with the set of present species to positively identify naiads at a species level. The naiads that belonged to families for which more than one adult was recorded were reared to adulthood after the experiment after which they were positively identified to the species level. Each naiad was photographed alongside a ruler. Body size of the predators was measured from these digital images with the ImageJ software, version 1.46a (Ferreira and Rasband, 2012).

Mosquito larvae were collected from two locations; a roof drain in Mueang Kamphaeng Phet and water storage containers in Ban Nong Pling. Mosquito larvae were stored in a 5 L plastic container. Mosquito larvae were identified using the keys provided by Rattanarithikul et al. (2010). The mosquito larvae from the roof drain were all identified as *Armigeres moultoni*, a medically unimportant species from the tribe Aedini. The larvae from the water storage containers were all identified as *Aedes aegypti*, an important vector of dengue fever and other diseases.

### Experimental design

We used different densities of both predators and prey in experimental treatments to assess what the effects of these variables were on the predation rate. During the experiment, we counted the number of mosquitoes that were eaten by the naiads over a 24 h period. The experiment was conducted twice, each trial being 24 h in duration. During the first trial, we used mosquito larvae of the species *A. moultoni*, whereas *A. aegypti* larvae were used for the second trial. Each experimental replicate consisted of a 1.5 L cylindrical plastic container filled with 1 L of water from the habitat where we collected the odonate naiads. A specimen of *Pistia stratiotes* was added to create a more natural, complex habitat (Fig. 1). We used a total of 70 containers: 43 for the dragonfly naiads (25 with *A. moultoni* and 18 with *A. aegypti*) and 27 for the damselfly naiads (14 with *A. moultoni* and 13 with *A. aegypti*). Odonate naiads were added to the containers in different densities. Dragonfly naiad densities ranged from 1 to 5 individuals per litre (container) and damselfly naiad densities ranged from 1 to 4 individuals per litre (container). During the first experiment, the naiads

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