

Factors influencing the predation rates of *Anisops breddini* (Hemiptera: Notonectidae) feeding on mosquito larvae

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Abstract

Notonectidae are a family of water bugs that are known to be important predators of mosquito larvae and have great potential in the biological control of vector mosquitoes. An experiment was conducted to assess mosquito larvae predation by *Anisops breddini*, a species common to Southeast Asia. The predation rates were recorded in context of prey density, predator density, predator size and prey type. Predation rates were strongly affected by prey type and less by prey density and predator density. They ranged between 1.2 prey items per day for pupae of *Aedes aegypti* and *Armigeres moultoni* to 5.9 for *Ae. aegypti* larvae. Compared with studies on other Notonectidae species, the predation

rates appear low, which is probably caused by the relative small size of the specimens used in this study. *An. breddini* is very common in the region and often found in urban areas; therefore, the species has potential as a biological control agent.

Introduction

Mosquitoes are the world's number one arthropod vector of diseases such as malaria, dengue fever, filariasis or West Nile fever (Becker *et al.*, 2010). There are many predatory species that feed on mosquito larvae such as dragonfly naiads, aquatic beetles and fish (Shaalán & Canyon, 2009). Notonectidae (Hemiptera) are a family of mosquito larvae predators that have often been the focus of biological control studies (Shaalán & Canyon, 2009). These aquatic predators are commonly known as backswimmers (Gillot, 2005). Predation of mosquito larvae by backswimmer has been investigated throughout the world (Chesson, 1989; Martín & López, 2004; Saha *et al.*, 2007; Shaalan *et al.*, 2007; Zuhara & Lester, 2010; Silberbush & Blaustein, 2011; Fischer *et al.*, 2012). Many species of backswimmers are considered to have a strong feeding preference towards mosquito larvae.

Several studies have reported predation rates of backswimmers. Saha *et al.* (2007) showed that food deprived *Anisops bouvieri* fed on two to 34 mosquito larvae per day. Another study noted rates of eight to 30 mosquito larvae per day for *Notonecta sellata* (Fischer *et al.*, 2012). In that study, 30 was the maximum number of mosquito larvae in each experimental trial, thus predation rates could possibly have been higher. *Notonecta sellata* fed on early mosquito instars at significantly higher rates than on late instars. A mean predation rate of 16 mosquito larvae per day was reported from Australia for an *Anisops* species (Shaalán *et al.*, 2007). Here the predation rate for first instar mosquito larvae was 25 larvae per day, while fourth instars were preyed upon at rates of 13 larvae per day.

The current study provides insights into the mosquito predation rates of *Anisops breddini*, Kirkaldy 1901, a backswimmer species common to Southeast Asia. This species is often found in ponds and canals (Leong, 1962), but it was also frequently noticed in water storage tanks and other container-like ornaments. Water-filled containers are a major source of vector mosquitoes such as *Aedes aegypti* (Clements, 1999). Therefore, mosquito predators that inhabit these habitats are of particular interest for vector control. The effects of predator density, prey density and prey type on the predation rates were experimentally studied. It was hypothesized that with increasing predator densities the predation rates decrease. Interfering behaviour such as social behaviour is known to reduce predation rates (Beddington, 1975; Crowley & Martin, 1989). In backswimmers, cannibalism is not uncommon and can cause a decrease in active hunting behaviour (Martín & López, 2004). Prey density was expected to positively affect

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predation rates, subsequently showing a typical Holling type II functional response (Holling, 1959). The predation rates decelerate when the rates reach a food saturation point and handling time increases with lower prey densities (Holling, 1959; Giller, 1980; Gergs *et al.*, 2010). Also, prey size and type are known to affect the predation rates of backswimmers (Murdoch *et al.*, 1984; Chesson, 1989). A backswimmer needs to consume smaller prey to gain the same amount of energy in comparison with a larger prey (Gergs *et al.*, 2010). Therefore predation rates were expected to differ among different prey types.

Materials and methods

An. breddini specimens were collected with a hand-held net in a temporary pond on October 22nd, 2012 in Kamphaeng Phet, Thailand (Latitude: 16° 29' 34.551" N, Longitude: 99° 30' 53.0778" E). The specimens were kept in a bucket filled with water from the same pond. The specimens were collected one day prior to the experiment. Mosquito larvae were collected from a roof drain in the same area and from water storage containers in the nearby village, Nong Pling. The specimens were then identified using pictures taken with a digital microscope and the keys provided by Nieser (2004).

On the day prior to the experiment, *An. breddini* specimens were divided into 25 1.5-L transparent plastic containers, each filled with 1 L of water from the pond where the backswimmers were collected. This water was filtered to remove other aquatic organisms and debris. Backswimmers were then added to the containers in different densities ranging from one to five individuals per container. In total, there were five containers for every density treatment. However, a single backswimmer in one of the treatments with five backswimmers was found dead and was excluded from the study. A total of 70 backswimmers were used, which included both nymphs and adults with an average size of 3.7 mm (\pm 0.13). The smallest specimen was 1.57 mm and the largest was 6.67 mm. The mean body size for each predator density treatment is displayed in Table 1. This study did not focus on adult specimens only, because this would inflate the predation rates. Natural populations consist of a mix of different age classes; therefore, a range of developmental stages was used that was representative for the habitat from which we collected the backswimmers (Table 2). A water plant (*Pistia stratiotes* L.) was also added to each of the containers to simulate a more natural habitat and to provide a substrate for the backswimmers and refuge for the mosquito larvae. The backswimmers were kept in their experimental habitat for one day to acclimatise.

The mosquito larvae were grouped per species (*Ar. moultoni* and *Ae. aegypti*) and were added to the containers in different densities, ranging from 5 to 30 mosquito larvae per container. Mosquitoes were added one day after the backswimmers were added, which marked the start of the experiment. Only 3rd and 4th instar mosquito larvae and pupae were used for this experiment. Prey treatments consisted of *Ar. moultoni* larvae, *Ae. aegypti* larvae or pupae of both species. Three containers were supplied

with five prey items, five with ten prey items, five with 15 prey items, five with 20 prey items, four with 25 prey items, and two with 30 prey items. The mosquito larvae were added to the container in a way that maximized the number of unique treatment combinations (prey and predator densities), which increased the variation in the data. After 24 h, the number of remaining mosquitoes were counted. This number was then subtracted from the starting density to estimate the number of mosquito larvae preyed upon. After the experimental trials, the backswimmers were placed onto a ruler and photographed. The photographs were analysed on the computer with ImageJ 1.46a software (Ferreira & Rasband, 2012) to measure the length of every backswimmer.

Finally, the number of mosquitoes preyed upon were divided by the number of backswimmers in each container to gain an estimated predation rate (larvae/day/backswimmer). These predation rates were then analysed using linear regression models. *A priori* hypothesized models were developed following the model inference approach described by Anderson (2008). The predation rates were (log+1)-transformed to achieve normality. Nonlinear relationships were linearised using log transformations on the specific explanatory variables. All explanatory variables were standardised to a mean of zero and a standard deviation of one (Zuur *et al.*, 2009). The Levene's test for equal variances was used to check for any violation of the assumption of equal variances among the different prey types.

The Akaike information criterion (AIC) scores were used to select the best model, the model with the lowest AICc score being the best model. AICc scores are similar to AIC scores but contain an extra penalty for additional variables; therefore, the AICc score is more conservative (Anderson, 2008). The model with the lowest AICc score was visualised using the *visreg* package (Breheny & Burchett, 2012) in RStudio, version 0.97.551 (Rstudio, 2012), built on R version 3.0.2 (R Development Core Team, 2013). Eventually, model selection probabilities were calculated to indicate the likeliness of a specific model to be the best model. These model probabilities were used to calculate an average model based on all the tested models (Anderson, 2008).

Results

The regression models displayed a typical non-linear functional response curve for predator density but not for prey density (Figure 1). Prey type was the most important variable in our models, followed by predator density and prey density, respectively (Table 3). The model with the lowest AICc score differed more than three points, which made calculation of an average model unnecessary (Table 4). This is reflected in the small differences of the parameter estimates between the best and averaged model (Table 3).

The log-transformed predation rates were significantly different for the three different prey types ($F=6.14$, degree of freedom=21, $P=0.008$). Tukey's *post hoc* test showed that the transformed predation rates were different for *Ae. aegypti* and pupae. There was no significant difference between *Ar. moultoni* and *Ae. aegypti* or *Ar. moultoni* and

Table 1. Mean size of *An. breddini* for treatments with different predator densities.

Treatment	Mean predator size (mm)
1 predator	3.8 (\pm 0.6)
2 predators	3.9 (\pm 0.6)
3 predators	3.9 (\pm 0.3)
4 predators	3.6 (\pm 0.6)
5 predators	3.5 (\pm 0.5)

Table 2. Number of *An. breddini* in different size classes.

Size class (mm)	Number of individuals
0-2	4
2.1-3	15
3.1-4	20
4.1-5	19
Greater than 5	7

pupae. The mean (\pm standard error) predation rate for *Ae. aegypti* was 5.94 (\pm 0.79) mosquito larvae per day for *Ar. moultoni*, and for the pupae this was 3.78 (\pm 2.2) and 1.23 (\pm 0.42), respectively. The mean predator size was 3.71 (\pm 0.14).

Discussion

The results of this study suggest that *An. breddini* feeds on a moderate number of mosquito larvae. The predation rates are highly dependent on the type of prey, the predator density and the prey density. Previous studies of other Notonectidae species have shown similar

results. The predation rates were generally lower than that of other Notonectidae species. Saha *et al.* (2007) found predation rates between 2-32 mosquito larvae per day, with a mean of 15 for *An. bouvieri*. These predators, with a mean size of 6.22 mm, were fed with *Culex quinquefasciatus*, a slightly smaller species than those from the genus *Aedes* (Saha *et al.*, 2007). Moreover, their study did not include different predator densities (Saha *et al.*, 2007). When considering only the treatment with a single predator and the smallest prey item, the mean predation rate was 8.7. Adult *An. breddini* and *An. bouvieri* are generally equal in size, with an approximate body length of 5.7-6.8 mm and 5.7-6.3 mm, respectively (Nieser, 2004). Nevertheless, the *An. breddini* specimens used in the current study were generally much smaller than *An. bouvieri*, which might explain the difference in predation rates. Predator

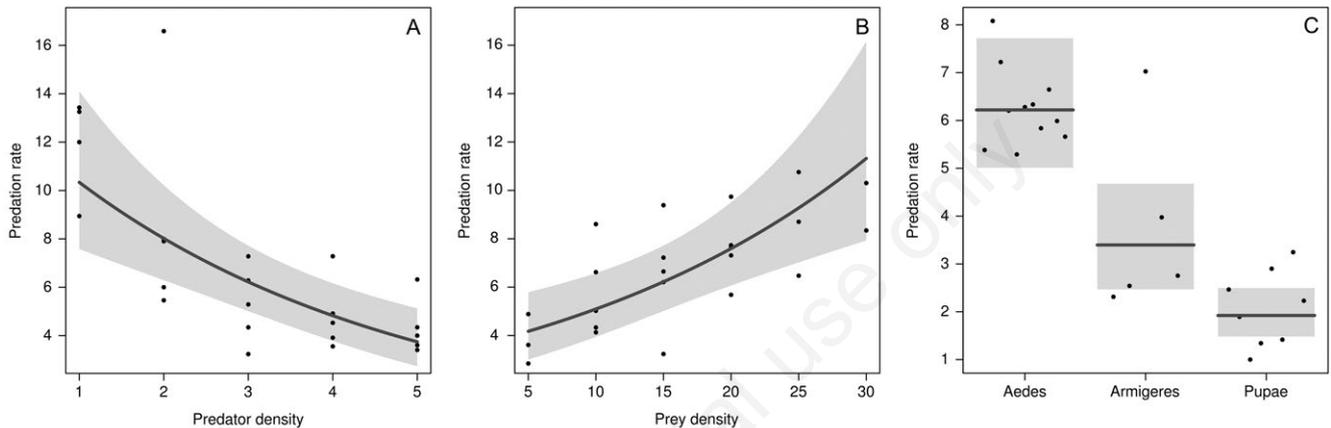


Figure 1. Visualisation of the best model based on back-transformed predation rates (Larvae per day). The grey areas display confidence bands. The dots in the graphs are kept constant for the variables that are not displayed in each specific graph. A) predator density versus predation rates, B) prey density versus predation rates and C) prey type versus predation rates.

Table 3. Model estimates of the best model and the averaged model for the regression models in which the predation rates were log+1 transformed.

Model parameter	Best model		Averaged model	
	Estimate	Standard error	Estimate	Standard error
log(pre y density)	0.33	0.07	0.33	0.07
log(predator density)	0.38	0.07	0.38	0.07
Type=Aedes	1.83	0.09	1.83	0.09
Type=Armigeres	0.43	0.17	0.43	0.17
Type=pupae	1.15	0.14	1.14	0.14

Table 4. Comparison of all linear regression models.

Model*	K	AICc	Δ_i	W_i	Adjusted R ²
M+P+T	6	22.55	0.00	1.00	0.82
P+T	5	36.75	14.20	0.00	0.64
M+T	5	41.75	19.20	0.00	0.55
T	4	42.33	19.78	0.00	0.50
M+P	4	50.98	28.43	0.00	0.28
P	3	52.66	30.11	0.00	0.17
Null	2	55.63	33.08	0.00	NA
M	3	57.39	34.84	0.00	-0.01

*The variables for each model are given in the first column. M, prey density; P, predator density; T, prey type; K, number of parameters; AICc, Akaike information criterion; Δ_i , difference in AICc score in comparison with the best model; W_i , the model weights.

and prey size are important factors that strongly influence handling time and capture rates (Thompson, 1975; Hewett, 1980; Hirvonen & Ranta, 1996). Fischer *et al.* (2012) also found higher predation rates for *Notonecta sellata* when feeding on *Culex pipiens*. They found a mean predation rate of 22 for 4th instar mosquito larvae. These rates were slightly higher for 2nd and 3rd instar mosquito larvae (Fischer *et al.*, 2012). *Notonecta sellata* is a relatively large species with a mean size of 8.7-9.6 mm (Heckman, 2011).

Predator densities strongly affected the predation rates of *An. breddini*. Predator density was also shown to affect predation rates in other studies on Notonectidae (Sih, 1981). In general, the mechanism behind this effect is that, with higher predator densities, the number of interactions between the predators increases (Sih, 1982; Crowley & Martin, 1989). Also, cannibalism is more likely to occur with increased predator densities; this specifically affects predation rates of the smaller specimen when encountering larger conspecifics (Sih, 1982). Our data did not show a typical functional response curve for prey densities. We predicted that increased prey densities would positively affect predation rates in a decelerating manner until it reaches a maximum. This effect is often caused by satiation (Holling, 1959). If prey densities would have been further increased, this satiation level might have been reached, which also indicates that predation rates could potentially be higher with higher prey densities.

Predation rates differed significantly among different prey types. Predation rates were much higher for *Ae. aegypti* larvae in comparison to *Ar. moultoni* larvae or pupae. Although larval size was not measured, *Ae. aegypti* are generally smaller than *Ar. moultoni* larvae (Clements, 1999). Therefore, more prey items are needed to meet the same energy demands (Stephens & Krebs, 1986). Prey size is an important driver in the predation rates of Notonectidae, which affects both the handling time as well as the capture rates (Murdoch *et al.*, 1984). Several studies have investigated the prey preference of different Notonectidae species. When Notonectidae are exposed to different prey items, they generally show a preference for mosquito larvae over three species of water fleas (*Cladocera*) (Murdoch *et al.*, 1984). Other studies, however, do not identify a preference for mosquito larvae (Chesson, 1989). Sih (1986) conducted an experiment on *Notonecta undulata* in which he compared the predation on *Culex* versus *Aedes* larvae. This study showed that the behaviour of the mosquito larvae was an important factor in prey selection. *Aedes* larvae generally did not display predator avoiding behaviour, while the *Culex* larvae did. As a result, *Aedes* larvae were preyed upon at much higher rates. This might partially explain the difference in predation rates, in particular the low predation rates for the pupae (pupae are generally less active). Nevertheless, in the current study, prey behaviour was not observed, nor were the predators exposed to different prey types simultaneously. Therefore, conclusions with regard to prey behaviour and predation rates cannot be drawn.

The current study showed that *An. breddini* feeds on only a small number of mosquito larvae compared to other backswimmer species (Saha *et al.*, 2007; Fischer *et al.*, 2012). These relative low predation rates can partly be ascribed to the use of mixed predator instars. Natural communities do not only consist of adult specimens, and these lower predation rates are therefore more realistic in comparison with predation rates based on adult specimens. In the current experiment, only third and fourth instar mosquito larvae were used. Early instar mosquito larvae are generally preyed upon in much higher rates than third and fourth instar mosquito larvae (Saha *et al.*, 2007; Fischer *et al.*, 2012). Predation rates are thus likely to be higher when *An. breddini* is exposed to first or second instar mosquito larvae.

An. breddini is very common in Thailand and other Southeast Asian countries (Leong, 1962), which is beneficial for the control of vector mosquitoes. Although other mosquito larvae predators might be more efficient in vector control, their wide distribution and abundance could potentially make *An. breddini* a valuable addition to existing biological

vector control agents. Not only does *An. breddini* feed on mosquito larvae, there might also be other mechanisms that can benefit mosquito control. Other Notonectidae species are known to affect the development of mosquito larvae into adults (Fischer *et al.*, 2012). In the presence of Notonectidae, development of mosquito larvae can take longer and adults tend to be smaller (Fischer *et al.*, 2012). Other species are known to repel certain mosquito species from ovipositing (Blaustein *et al.*, 2005; Silberbush & Blaustein, 2011). More research is needed that focus on these last two aspects for *An. breddini*, which might reveal all the vector control benefits of this common species.

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