A mosquito predator survey in Townsville, Australia, and an assessment of *Diplonychus* sp. and *Anisops* sp. predatorial capacity against *Culex annulirostris* mosquito immatures

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ABSTRACT: A twelve-month survey for mosquito predators was conducted in Townsville, Queensland, Australia, which is located in the arid tropics. The survey revealed the presence of five predaceous insects but only *Anisops* sp. (backswimmers) and *Diplonychus* sp. were common. Predatorial capacity and factors influencing this capacity were then assessed for adult *Anisops* sp. and adult and nymph stages of *Diplonychus* sp. against *Culex annulirostris* mosquito immatures under laboratory conditions. Predatorial capacity bioassays showed that adult *Diplonychus* sp. preyed upon both larval and pupal stages of *Cx. annulirostris* quite successfully. Nymphs of *Diplonychus* sp. proved to be more successful with smaller prey immatures, and *Anisops* sp adults did not prey successfully on any prey pupae. Increasing the foraging area and introducing aquatic vegetation significantly reduced the predatorial capacity of *Diplonychus* sp. nymphs, while only vegetation and not foraging area had a significant effect on adult *Diplonychus* sp. predation capacity. Overall, adult *Diplonychus* sp. proved to be a more efficient predator than *Anisops* sp., and field trials are now recommended to further assess the potential of *Diplonychus* sp. as a biocontrol agent. ***Journal of Vector Ecology* 32 (1): 16-21. 2007.**

Keyword Index: Biological control, predator, mosquito, Hemiptera.

INTRODUCTION

A variety of aquatic insects in the orders Odonata, Hemiptera, Coleoptera, and Diptera are known to prey upon mosquito larvae. Generalist predators that feed on a broad range of prey species are polyphagous, while specialist predators with a restricted range of prey can be oligophagous or monophagous with a limited range or single species of prey. Although most predators of mosquitoes tend to be generalists (Collins and Washino 1985), there are exceptions. For instance, Washino (1969) found that corixids fed less upon mosquito larvae than any other hemipteran predators (*Corisella* sp., *Belostoma flumineum*, and the giant waterbug *Abedus indentatus*) when tested experimentally. On the other hand, *Notonecta undulata* was found to prefer mosquito larvae over other prey like corixids, ephemedrids, chironomids, and chaoborids when given a choice (Ellis and Borden 1970), indicating a degree of predatory specialization.

Although predation may occur during any prey life stage, research has focused on the immature larval and pupal stages. Egg predation appears to be a minor component of mosquito mortality and predation on the adult stage is unlikely to provide reliable levels of control (Collins and Washino 1985). Few predators, particularly *Toxorhynchites* larvae, kill mosquito pupae without ingesting them afterwards. This killing activity is fortunate in the context of control, because pupal production is most highly correlated with subsequent adult densities (Padgett and Focks 1981).

Laboratory research into aquatic insect predation is fairly common and studies of aquatic bugs have shown that they are quite effective predators of mosquito larvae. For instance, Miura and Takahashi (1988) demonstrated that *Microvelia pulchella* (Hemiptera: Vellidae) is able to derive nutrients from mosquito larvae to survive, grow, and reproduce. Venkatesan and Sivaraman (1984) investigated the predation potential of the water bug *Diplonychus indicus* (Hemiptera: Belostomatidae) against larval instars of two different mosquito prey species, *Aedes aegypti* and *Cx. fatigans*, at varying densities. They showed that the largest predator (5th instar) was more effective than smaller instars at killing the smallest prey (1st instar). This was due to larger predator instars exhibiting more successful attacks and a shorter handling time than smaller predator instars. Similarly, Scott and Murdoch (1983) found that when the backswimmer *Notonecta huffmani* was fed on mosquito larvae under laboratory conditions, its appetite decreased with increasing prey size. The same behavior was observed in the notonectid bug *Enithares indica* (Wattal et al. 1996). The feeding rates of *E. indica* on the immature stages of *Anopheles stephensi* and *Cx. quinquefasciatus* decreased with increasing mosquito larval stage. Maximum predation was observed on 1st instar larvae while minimum predation was observed for the pupal stage.

The aforementioned studies investigated the predation potential of some hemipteran predators and their prey preference against immature stages of different mosquito species. Hence the present work, located in Townsville, North Queensland, Australia, advanced this research by investigating the predatorial capacity of *Anisops* sp. and *Diplonychus* sp. using *Cx. annulirostris* (Skuse) as prey. We addressed how two factors, the size of foraging area and the presence of vegetation, might affect predatorial capacity.

MATERIALS AND METHODS

Field survey

Local predators were surveyed and collected to identify the most prevalent species and to subsequently investigate their efficacy against mosquito immatures. Being located in the arid tropics, almost no suitable breeding sites were found that contained water throughout the year, so near-permanent flood areas were selected. A fresh water runoff marsh with occasional brackish water was selected in Oonoonba (a peri-urban suburb adjacent to extensive mangrove habitat) and a receding marshy brackish lake often frequented by wild pigs and crocodiles was selected in the city's Town Common (a conservation park). These sites were surveyed monthly using long-handled nets with 15 cm diameter x 30 cm long muslin sleeves. Predators were transported alive from the field to the laboratory in plastic boxes half-filled with water and debris from the breeding sites. In the laboratory, predators were washed with clean water and sorted into small plastic trays (15 x 11 x 5 cm) half-filled with de-ionized water. They were identified according to keys of Gooderham and Tsyrlin (2002) and left to acclimatize to laboratory conditions. All predators were starved for two days before tests were conducted.

Experiment 1

The predatorial efficacy assessment of adult *Anisops* sp. and *Diplonychus* sp. on *Cx. annulirostris* immature prey was conducted in glass beakers (500 ml) using five different life stages of the prey (1st, 2nd, 3rd, or 4th larval instars or pupae) and in 250ml of de-ionized water. All settings were controlled and replicated four times. Each experimental beaker contained 25 immature mosquitoes of the same life stage and one predator. Identical settings were used as controls but contained no predators. The outcome was assessed after 24 h by counting the number of immatures consumed or killed by the predators. Killed immature stages were distinguished by lack of movement, darker coloring and distorted shape. Since the initial tests showed that adult *Anisops* sp. were less effective, additional experiments were conducted using *Diplonychus* sp. nymphs.

Experiment 2

The effect of foraging area size and the presence of vegetation was assessed by replicating the above described settings (Experiment 1) twice in larger plastic containers (15 x 11 x 5 cm with 700 ml of de-ionized water) with and without the addition of seven branches of aquatic weed of the same length, which were collected from the breeding sites. *Anisops* sp. were dropped from these series of experiments since the inability of *Anisops* sp. to predate on pupae (Experiment 1) is a predatorial flaw that renders the species ineffective as a mosquito predator.

All data analysis was performed using factorial ANOVA models (SPSS ver. 12.0.1 for Windows).

RESULTS

Field survey

Five different species of predators (Table 1) representing two Orders (Hemiptera and Odonata), were collected from the two survey sites over a 12-month period. All predators were found in the Oonoonba site, but only two of them were collected from the Town Common. The hemipteran

Table 1. Survey of aquatic predaceous insects in the peri-urban suburb of Oonoonba and the Town Common conservation park in Townsville Qld Australia (+ predators were present; - predators were absent).

Date	Oonoonba					Town Common	
	Order Hemiptera			Order Odonata		Order Hemiptera	
	Anisops sp.	*Diplonychus*sp.	Corixids	Damselflies	Dragonflies	*Anisops* sp.	*Diplonychus* sp.
04/03	+	+	-	+	+	+	+
05/03	+	+	-	+	+	+	+
06/03	-	-	-	-	-	-	+
07/03	-	-	-	-	-	-	-
08/03	-	-	-	-	-	-	-
09/03	+	-	+	-	-	-	-
10/03	-	-	-	-	-	-	-
11/03	-	-	-	-	-	-	-
12/03	-	-	-	-	-	-	-
01/04	-	+	-	-	+	-	-
02/04	-	+	-	-	-	+	+
03/04	-	+	-	+	+	-	+

Table 2. Factorial one-way ANOVA models for *Anisops* sp. adults, *Diplonychus* sp. adults and *Diplonychus* sp. nymphs and a summary.

***Anisops* sp. adults**					
Source	DF	MS	F Ratio	F Prob	
Between Groups	4	6093.2000	42.7893	0.0000	
Group (Prey stage)	Count	Mean	SD	SE	95% CI
1st Instar	4	100.00	0.00	0.00	100.00 – 100.00
2nd Instar	4	87.00	15.79	7.90	61.87 – 112.13
3rd Instar	4	72.00	11.31	5.66	54.00 – 90.00
4th Instar	4	53.00	18.29	9.15	23.89 – 82.11
Pupae	4	0.00	0.00	0.00	0.00 – 0.00
Total	20	62.40	37.35	8.35	44.92 – 79.88
***Diplonychus* sp. adults**					
Source	DF	MS	F Ratio	F Prob	
Between Groups	4	11.2500	1.000	0.4380	
Group (Prey stage)	Count	Mean	SD	SE	95% CI
1st Instar	4	100.00	0.00	0.00	100.00 – 100.00
2nd Instar	4	100.00	0.00	0.00	100.00 – 100.00
3rd Instar	4	100.00	0.00	0.00	100.00 – 100.00
4th Instar	4	100.00	0.00	0.00	100.00 – 100.00
Pupae	4	96.25	7.50	3.75	84.32 – 108.18
Total	20	99.25	3.35	0.75	97.68 – 100.82
***Diplonychus* sp. nymphs**					
Source	DF	MS	F Ratio	F Prob	
Between Groups	4	2519.2000	55.2456	0.0000	
Group (Prey stage)	Count	Mean	SD	SE	95% CI
1st Instar	4	100.00	0.00	0.00	100.00 – 100.00
2nd Instar	4	100.00	0.00	0.00	100.00 – 100.00
3rd Instar	4	96.00	8.00	4.00	83.27 – 108.73
4th Instar	4	80.00	8.64	4.32	66.25 – 93.75
Pupae	4	41.00	9.45	4.73	25.96 – 56.04
Total	20	83.40	23.80	5.32	72.26 – 94.54
Summary					
Source	DF	MS	F Ratio	F Prob	
Between Groups	2	6833.8167	10.3920	0.0001	
Group	Count	Mean	SD	SE	95% CI
Anisops sp. adults	20	62.40	37.35	8.35	77.92 – 79.88
Diplonychus sp. adults	20	99.25	3.35	0.75	97.68 – 100.82
Diplonychus sp. nymphs	20	83.40	23.80	5.32	72.26 – 94.54
Total	60	81.68	29.44	3.80	74.08 – 89.29

bugs *Anisops* sp. (Notonectidae) and *Diplonychus* sp. (Belostomatidae) were encountered at both sites while damselflies, dragonflies (Odonata), and corixid bugs (Corixidae: Hemiptera) were collected only from Oonoonba. Both *Anisops* sp. (backswimmers) and *Diplonychus* sp. were the most common predators followed by damselflies and dragonflies. The five corixid bugs were found in September.

Experiment 1

Predatorial efficacy in relation to prey life stage differed significantly in *Anisops* sp. adults and *Diplonychus* sp. nymphs, but not in *Diplonychus* sp. adults (Table 2). There was a significant difference between the overall sum eaten between the different types of predators (Tables 2 and 3) ($p<0.001$).

Experiment 2

Table 4 details the results for Experiment 2. Increasing the foraging area and including vegetation made it significantly more difficult for predators to catch prey (Figures 1 and 2). *Diplonychus* sp. adults were significantly better than *Diplonychus* sp. nymphs at predating on *Cx. annulirostris* immatures. There was also a significant overall difference with respect to prey stage in the sense that the more developed the immatures, the fewer were caught. One significant interaction (all possible 2-way and 3-way interactions were assessed) was found between prey stage and predator "age": *Diplonychus* sp. nymphs, while being quite successful with small prey, experience significantly more difficulty when preying on older prey than do *Diplonychus* sp. adults that prey on all *Cx. annulirostris* immature stages without difficulty. These results seem quite stable since all these factors together explain close to two-thirds of the variability in the data.

DISCUSSION

It is important to correlate predator and prey seasonality and habitats if mosquito control is to be considered for a particular predator that has proven to be effective in experimental conditions. An ecological and biological study by Rae (1990) at the Ross River Dam, North Queensland, Australia, located in the same geographical region as this study, indicated the prevalence of several predacious

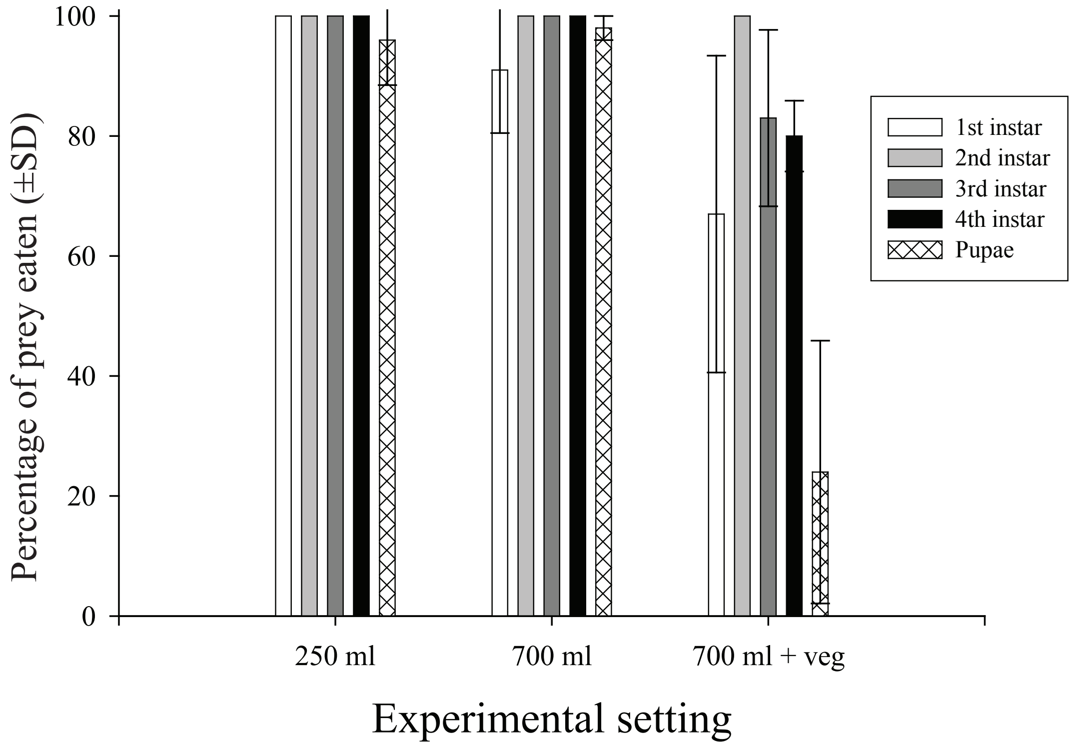

Figure 1. Predatorial capacity of *Diplonychus* sp. adults against different *Culex annulirostris* immatures in different settings (250 ml, 700 ml, and 700ml + vegetation).

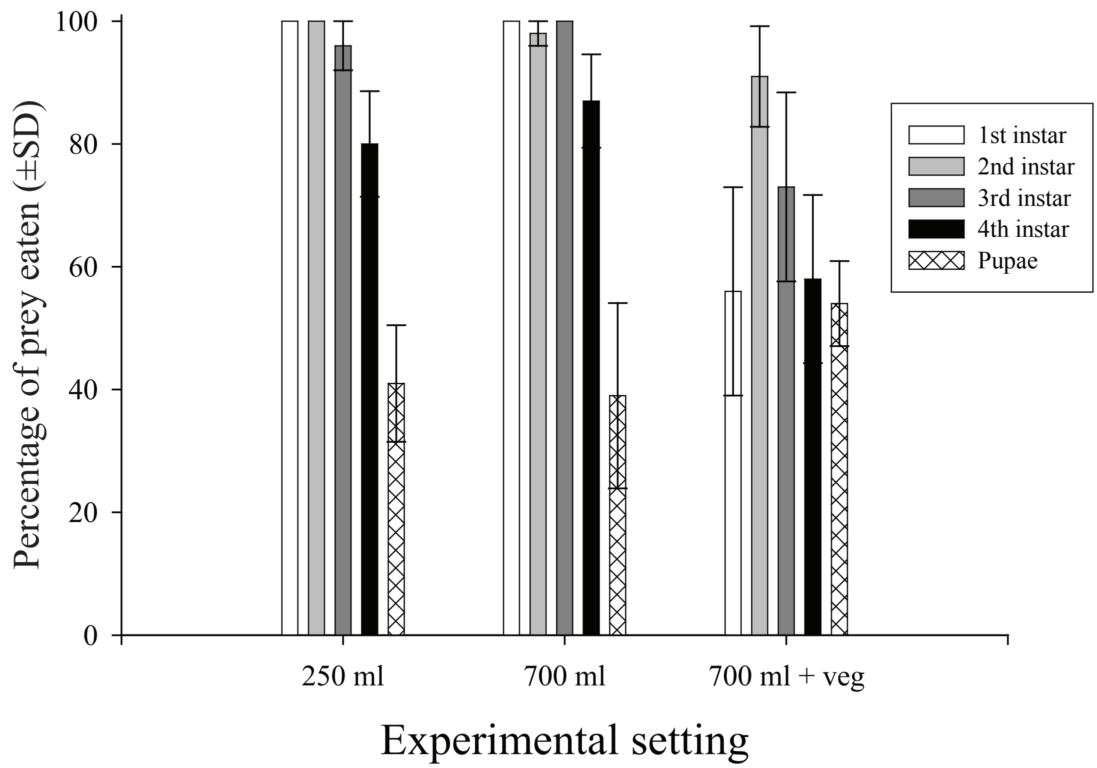

Figure 2. Predatorial capacity of *Diplonychus* sp. nymphs against different *Culex annulirostris* immatures in different settings (250 ml, 700 ml, and 700ml + vegetation).

Table 3: Summary of predatorial efficacy on different prey stages by different predator groups in beakers. P-values refer to one-way ANOVA models over prey stages within each predator group.

Prey Stage	*Anisops* sp. adults (SD) p<0.0001	*Diplonychus* sp. adults (SD) p=0.44	*Diplonychus* sp. nymphs (SD) p<0.0001
1st instar	100 (0)	100 (0)	100 (0)
2nd instar	87 (15.8)	100 (0)	100 (0)
3rd instar	77 (11.3)	100 (0)	96 (8.0)
4th instar	54 (18.3)	100 (0)	80 (8.6)
Pupa	0 (0)	96.3 (7.5)	41 (9.5)
Total	62.4 (37.5)	99.25 (3.4)	83.4 (23.8)

insects that impacted the development and survival of *Cx. annulirostris* immatures. These predators were dominated by Coleoptera (Dytiscidae and Hydrophilidae), Odonata ,and Hemiptera (*Diplonychus* sp., *Plea* sp. and Mesovlidae). Our predator survey revealed the presence of well-known predator species in Hemiptera and Odonata orders but did not find Coleoptera. During the monsoon period, both *Diplonychus* sp. and dragonflies prevailed, while only a limited number of *Anisops* sp. and damselflies were present. In a less tropical location in Southeast Queensland, Mottram and Kettle (1997) found a blend of coleopteran larvae, nymphs of Hemiptera (*Anisops* sp. and *Diplonychus rusticus*), and Odonata. These predators were significantly more common in flooded grassland than semi-permanent and temporary pools. In temperate Victoria, Australia, the commonest predators, from most prevalent to least, were beetle larvae, damsel fly naiads, and backswimmers (McDonald and Buchanan 1981). Insect predators may be more common in the wet tropics in which prey and habitat are available throughout the year.

In this study, predatorial capacity varied significantly with *Diplonychus* sp. emerging as superior. These results compare well to those obtained in southeast Queensland by Mottram and Kettle (1997). They tested seven predators in the laboratory and found that only *D. rusticus* and Coenagrionidae (Odonata) killed *Cx. annulirostris* pupae. *Notonecta undulata*, another common predator, was also found to avoid *Ae. aegypti* pupae (Ellis and Borden 1970). All predators consumed a greater quantity of smaller instars than larger ones. This has been observed in the notonectids, *N. undulata* (Ellis and Borden 1970) and *Enithares indica* (Wattal et al. 1996), and *Toxorhynchites splendens* (Amalraj and Das 1998). The reason is thought to be that 1st instars are easier to catch but provide less nutrition and must therefore be consumed in greater quantities than larger instars.

It was expected that an increase in forage area would decrease the attack rate of a predator which has to spend more time searching for the prey. However, the effect of an increased foraging area size, while significant overall, did not affect the predatorial capacity of adult *Diplonychus* sp. *Diplonychus* sp. adults are highly active predators that excel in alternating between stalking and fast attacks. Amalraj and Das (1998) also found that the attack rate of *Tx. splendens* larvae against immature *Ae. aegypti* did not differ significantly among containers of different sizes. Similarly, capacity of the predaceous mosquito larvae *Lutzia* (= *Culex*) *raptor* was not influenced by difference in the volume of aquarium water ranging from 150 to 700 ml (Prakash and Ponniah 1978). Thus, foraging area will affect predatorial capacity of some, but not all, predators.

The presence of vegetation significantly influenced predation activity in both nymph and adult *Diplonychus* sp. The influence was highly significant and more pronounced against 1st, 3rd, and 4th larval instar and pupal stage (adult stage only). This could be due to *Cx. annulirostris* immatures having an affinity for vegetation in larval habitats. Likewise, *N. maculata* was generally most efficient at preying on the pelagic species *Culiseta longiareolata* than the vegetation-dwelling *Culex* and *Anopheles* mosquitoes (Blaustein et al. 1995). Although this affinity is protective, it is not known if it is due to vegetation acting as a refuge or an anchoring point.

To summarize, surveyed predators were heavily reliant on seasonal rain and, in the arid tropics, were mostly found over the three-month long monsoon season. *Diplonychus* sp. was more efficient than *Anisops* sp. and also preyed well on pupae. This is important from a control point of view since pupal production is highly correlated with subsequent adult densities (Padgett and Focks 1981). *Diplonychus* sp. is thus recommended for further investigation as a potential biological control agent for mosquito immatures.

Table 4. Factorial ANOVA model for *Diplonychus* sp. adults and nymphs. 120 cases were processed with no missing cases.

Source	D.F.	Mean Squares	F Sig.	F Prob.
Within+Residual	108	210.54		
Predator (Life Stage)	1	3586.13	17.03	<0.001
Experimental Setting (forage area size and vegetation)	2	7015.06	33.32	<0.001
Prey Life Stage	4	5383.60	25.57	<0.001
Predator * Prey Life Stage	4	832.9	3.96	<0.01
(Model)	11	3862.03	18.34	<0.001
(Total)	119	548.07		
R-Squared = 0.651				

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