APPLIED ISSUES

Selective predation by larval *Agabus* (Coleoptera: Dytiscidae) on mosquitoes: support for conservation-based mosquito suppression in constructed wetlands

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SUMMARY

1. Wetland insect predators can structure aquatic prey communities via selective predation, but receive considerably less attention than vertebrate predators. We conducted laboratory experiments to test selective predation by two species of larval dytiscid beetles (*Agabus*; Coleoptera: Dytiscidae) and the potential contribution of these beetles to suppression of mosquito populations in constructed wetlands.

2. *Agabus* consumed copepods, ostracods and mosquito larvae in no-choice tests. When offered a choice, 76% of all prey consumed were mosquito larvae, indicating selective predation. Subsequent experiments revealed this preference was due to ease of capture of mosquito larvae over alternative prey.

3. Cannibalism and intraguild predation were common within and between species of *Agabus*, which may reduce the overall impact of the observed selective predation.

4. *Agabus* larvae selectively preyed on mosquito larvae over alternative prey, which is not characteristic of some fish used as biological control agents for mosquitoes. Predator exclusion or similar experiments in the field could document how these results translate into a natural setting.

5. The findings of this study suggest developing mosquito suppression strategies focused on conservation of native wetland predators. These strategies are preferable to introducing non-native generalist predators, or applying pesticides.

Keywords: constructed wetlands, Dytiscidae, mosquitoes, predation, prey selection

Introduction

Predator impacts on prey communities are variable (Sih *et al.*, 1985; Shurin, 2001), but most ecologists consider predation an important force regulating prey populations in natural systems (Kerfoot & Sih, 1987; Sih, Englund & Wooster, 1998). In freshwater wetlands, predators can regulate insect populations and shape the overall aquatic animal community (Batzer &

Wissinger, 1996; Batzer & Sharitz, 2006). Often, top predators in wetlands are fish, which can impact macroinvertebrate communities in terms of species richness, density and biomass (e.g. Batzer, Pusateri & Vetter, 2000; Tangen, Butler & Ell, 2003; Lehtiniemi *et al.*, 2007; Dorn, 2008). In temporary wetlands that are unable to support fish populations, predaceous insects such as beetle larvae, odonate larvae and hemipterans are the top predators (Batzer & Wissinger, 1996). Insect predators have the potential to structure prey communities via selective predation (Peckarsky *et al.*, 1993; Eubanks & Denno, 2001) and could have important consequences for specific pest populations,

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but considerably less attention is given to insect predators in wetlands than vertebrate predators such as fish and amphibians (Blaustein, 1998).

Prey size and availability, ease of capture and ingestion or nutritional composition of a specific prey species are often cited as important factors driving selective predation (Solomon, 1949; Dicke, Sabelis & van den Berg, 1989; Wilbur, 1997). Selective predation on profitable prey species can ensure efficient exploitation of resources. This is critical for the success and survival of predaceous animals (Emlen, 1966), especially for those occurring in temporary wetlands that must complete the aquatic stage of their life cycle before the habitat dries. Strong selective predation in temporary wetlands could therefore alter community structure by changing the relative proportions of prey species in the environment (Peckarsky *et al.*, 1993).

Cannibalism and intraguild predation are also common among predaceous aquatic insects (Wissinger & McGrady, 1993; Fincke, 1994; Wissinger *et al.*, 1996; Ilmonen & Suhonen, 2006) and may be important in supporting predator populations if predaceous larvae hatch before preferred prey are present (Batzer & Wissinger, 1996). Consumption of controphics, i.e. species sharing the same trophic level, may also release the prey community from predation pressure (Claessen, de Roos & Persson, 2004), rendering selective predation insignificant to prey communities. Consideration of cannibalism and intraguild predation is therefore necessary when examining the potential for predators to structure prey communities.

Previous studies have documented community impacts via selective predation by invertebrate predators such as notonectids (e.g. Murdoch, Scott & Ebsworth, 1984), belostomatids (e.g. Runck & Blinn, 1994) and dytiscids (e.g. Tate & Hershey, 2003). Additionally, invertebrate predators may be important in suppressing mosquito populations (Lundkvist *et al.*, 2003; Quiroz-Martínez & Rodríguez-Castro, 2007; Saha *et al.*, 2007). Lundkvist *et al.* (2003) showed that species within the family Dytiscidae exhibited selective predation of mosquito larvae, and reduced larval mosquito populations in artificial ponds. This study focused on adult dytiscids that often scavenge, and did not address the predation potential of exclusively predaceous dytiscid larvae (Larson, Alarie & Roughley, 2000). Selective predation of mosquito larvae by larval dytiscids could contribute to suppression of mosquito populations, and help explain the low levels of mosquitoes found in many constructed wetlands (e.g. Culler, 2008). Dytiscid suppression of mosquitoes would lend support to wetland construction projects, since one major objection results from the public's fear of mosquito annoyance and disease transmission (Schäfer *et al.*, 2006).

The goals of this study were to test prey selection and negative predator–predator interactions for two coexisting larval dytiscid beetles, and to investigate the potential contribution of these beetles to suppression of mosquito populations in constructed wetlands. We based all hypotheses and experiments on the communities of predators and prey found in the constructed wetlands at the Jackson Lane Preserve (Caroline County, MD, U.S.A.; Culler, 2008). We used larvae of the beetle predators, *Agabus punctatus* Melsheimer and *Agabus disintegratus* Crotch (Coleoptera: Dytiscidae) and three prey taxa, ostracods (Podocopida: Notodromadidae), copepods (Cyclopoida: Cyclopidae) and mosquito larvae (*Aedes albopictus* Skuse; Diptera: Culicidae). We predicted that larvae of *A. punctatus* and *A. disintegratus* would consume all three prey taxa, but that they would exhibit preference for a certain type of prey due to availability, profitability or ease of capture. Since *A. punctatus* and *A. disintegratus* coexist (Culler, 2008), we expected to find cannibalism within and intraguild predation between these species, but did not expect levels of cannibalism and intraguild predation to differ. Laboratory experiments were conducted to assess: (i) the ability of *A. punctatus* and *A. disintegratus* to consume different prey taxa, (ii) prey selection by the two predators, (iii) performance of *Agabus* larvae when fed exclusively one prey type, (iv) behavioural interactions between *Agabus* and its prey and (v) the frequency of cannibalism and intraguild predation within and between *Agabus* species. Characterising these aspects of predation by *Agabus* will contribute to a better understanding of the importance of insect predators in wetlands, and provide insight into their potential role in mosquito suppression.

Methods

Study system

In 2003, The Nature Conservancy, U.S. Fish & Wildlife Service, Maryland Department of the Environment, and the Natural Resource Conservation Service

partnered to construct approximately 30 wetlands at the Jackson Lane Preserve (39°03′11.9″N, 75°44′50.2″W) on land that had been used for agriculture since the 1970s. Restoration activities began in August and included plugging drainage ditches, construction of 23 earthen ditch plugs and the addition of coarse woody debris to provide microhabitat. Wetlands ranged in size (0–36 000 m^2) and depth (0–2 m) throughout the year (Culler, 2008; D. Samson, personal communication). The first predators in the constructed wetlands at the end of winter were larval *A. punctatus* and *A. disintegratus* (Culler, 2008). These species overwinter as adults and lay eggs when ice is still present (Hilsenhoff, 1986; Larson *et al.*, 2000; W. Lamp & L. Culler, pers. obs), and are likely some of the few taxa capable of taking advantage of abundant prey in early spring (Higgins & Merritt, 1999). Several previous studies suggest larval *Agabus* could be effective predators of mosquito larvae, as their hatching and activity at low water temperatures corresponds to the hatch of mosquito larvae (James, 1961; Nilsson & Söderström, 1988; Higgins & Merritt, 1999). Microcrustaceans (e.g. ostracods, cladocerans and copepods) and dipteran larvae (e.g. culicids, chironomids) were available as prey when *Agabus* were present in the Jackson Lane Preserve constructed wetlands (Culler, 2008), all of which are potential prey for dytiscids (James, 1961, 1969; Nilsson & Söderström, 1988; Friis, Bauer & Betz, 2003).

General experimental set-up

We collected *A. punctatus* and *A. disintegratus* larvae from the constructed wetlands at the Jackson Lane Preserve in March and April, 2008, at least 2 days prior to the start of each experiment. Individual larvae were placed in 300 mL plastic cups containing water from the collection site that we filtered through a 250 µm sieve. We also added a variety of prey from the collection site so that feeding prior to the start of each experiment would not influence results. Beetle larvae were kept in a 13 °C walk-in environmental chamber with alternating 10 h of light and 14 h of dark. We conducted all experiments in the same environmental chamber under the same temperature and light conditions. Beetle larvae were removed from cups and placed in 16 × 100 mm glass culture tubes without prey 24 h prior to the start of each experiment to standardise hunger levels. We used only third-

instar larvae in the experiments. These were easily distinguished by the presence of spiracles on the mesonotum and abdominal segments (Barman, Nichols & Sizer, 1996; Barman, Wright & Mashke, 2000; E. H. Barman, pers. comm).

We tested prey selection using ostracods, copepods and first-instar mosquito larvae. We collected ostracods (Podocopida: Notodromadidae) and copepods (Cyclopoida: Cyclopidae) in the field, and hatched mosquito larvae (*Aedes albopictus*; Diptera: Culicidae) from eggs obtained from a colony at the Insect Transformation Facility, University of Maryland Biotechnology Institute in Rockville, Maryland.

Microcosms for all experiments were prepared by adding 175 mL of filtered wetland water (through a 250 µm sieve; pH = 5.6) and a 7 cm plastic aquarium plant (Tetra®-WaterWonders™ Decorative Plants, Melle, Germany) to a 300 mL plastic cup. We added prey to the cups before introducing predators in all experiments to allow acclimatisation.

Prey consumption

We tested for differences in prey consumption rates by *A. punctatus* and *A. disintegratus* individually and in different predator combinations. The experiment was a randomised complete block design (6 × 3 factorial) with predator combination [no dytiscid larvae (control), one *A. punctatus* larva, one *A. disintegratus* larva, two *A. punctatus* larvae, two *A. disintegratus* larvae or one larva of each species] and prey type (ostracods, copepods or mosquito larvae) as the factors. The purpose of the two predator treatments was to initially assess if intraguild predation and cannibalism occurred, and to measure how these antagonistic interactions affected prey consumption. We added 20 prey per cup in the single predator treatments and 40 prey per cup in the double predator treatments. Each treatment combination was replicated four times, and each replicate was blocked by location in the walk-in chamber.

Predators and prey were allowed to interact for 24 h, at which time we removed predators and counted the number of prey consumed. Dytiscid larvae are piercing-sucking predators with falcate mandibles (Larson *et al.*, 2000), and leave behind partially digested prey items that are easy to count. We did not replace prey as they were consumed. We used two-way ANOVA followed by Tukey's multiple

comparison test (PROC MIXED, SAS v.9.1; SAS Institute Inc., 2003) to test for effects of predator combination and prey type on prey mortality rate (*m*), calculated as:

$$m = [\ln(N_{\mathrm{o}}) - \ln(N_{\mathrm{f}})](d^{-1}),$$

where N_{o} represents initial prey abundance (20 individuals per predator), N_{f} represents final prey abundance (adjusted for non-predator induced mortality in the controls, and divided by two in the double predator treatments to estimate number of prey consumed per predator) and *d* is duration, in days, of the experiment (Dodson, 1975; Peckarsky, 2006). The units of the parameter *m* are prey killed per predator per day (prey predator^{-1} day^{-1}), hereafter termed prey mortality rate (Peckarsky, 2006).

Prey selection

We set up feeding trials to compare consumption of mosquito larvae and microcrustaceans in the presence of alternative prey, and to test which prey larval *A. punctatus* and *A. disintegratus* select. Treatments consisted of prey ratios (mosquito larvae:microcrustaceans) of 20 : 10 or 10 : 20. Microcrustaceans consisted of equal numbers of copepods and ostracods. Each beetle species was tested individually and each experiment was replicated five times. After 24 h, we removed predators and counted numbers of prey consumed.

We expected beetle larvae to consume prey in the proportions offered if they had no prey preference. For example, in the treatment with 20 mosquito larvae and 10 microcrustaceans, the expected proportion of mosquito larvae consumed was 0.67. We used chi-square analysis to test for differences between these expected proportions applied to the total number of prey consumed and the observed numbers of each prey type consumed (PROC FREQ, SAS v.9.1). We considered selective predation to have occurred if the observed numbers of each type of prey consumed differed significantly from the expected numbers.

Performance of *A. disintegratus*

We tested the effects of different prey types on the performance of *A. disintegratus* by feeding larvae one type of prey (ostracods, copepods or mosquito larvae) for nine days and measuring growth. We photographed each individual *A. disintegratus* larva and

obtained initial size measurements by measuring thorax + abdomen length using ImageJ software (Rasband, 2007). At the start of the experiment (day 0), each cup was stocked with 40 prey items. At day three and day six, we restocked prey to the original density of 40 and recorded the number of prey consumed. A final photograph of each beetle was taken at day nine to obtain a final measurement of thorax + abdomen length, and each beetle was dried and weighed on a microbalance. We replicated each prey type treatment five times. We calculated growth rates (mm growth per day) for each beetle, and compared growth rates between prey types using one-way ANOVA and Tukey's multiple comparison test (PROC MIXED, SAS v.9.1). There was no relationship between final beetle size and total number of prey consumed, therefore we did not consider the total number of prey consumed in the analysis (*r* = 0.08, *P* = 0.77). Final dry weight and length were strongly related (*r* = 0.88, *P* < 0.0001), so we used length as the measurement of final size.

Prey capture

We conducted behavioural trials to determine which components of the predator–prey interaction were responsible for the observed patterns of selective predation (Peckarsky, 2006). For each trial, 20 prey items and one predator (*A. punctatus*) were placed in a Petri dish with water and observed for 10 min. We tallied number of encounters with prey, number of attempted attacks and number of successful captures (resulting in consumption) for each predator. Eight trials were run for each prey type, and we used a new individual for each trial. We compared number of encounters and attacks per encounter between prey types using one-way ANOVA and Tukey's multiple comparison test (PROC MIXED, SAS v. 9.1). Encounter data were log transformed prior to analysis to meet the assumptions of normality and homogeneity of variances. We compared captures per attack data and total numbers of prey consumed between prey types using Kruskal–Wallis tests because the assumptions of ANOVA could not be met (Quinn & Keough, 2002).

Predator interactions

We used a randomised complete design with three predator treatments (two *A. punctatus* larvae, two

A. disintegratus larvae or one larva of each species) to measure cannibalism within *A. punctatus* and *A. disintegratus* and intraguild predation (IGP) between these species. Each predator treatment was replicated eighteen times and approximately 40 prey (combination of ostracods, copepods and mosquito larvae) were added to each cup so that cannibalism and IGP were not the only means of obtaining food. Beetle larvae paired in each cup were third-instars of the same size. We documented the occurrence of cannibalism or IGP every 24 h for 18 days.

We tested the hypothesis that occurrence of cannibalism and IGP did not differ using a chi-square test at two time points, day five and day 18 (PROC FREQ, SAS v.9.1). We performed a second chi-square test to determine if IGP was symmetric (each species exhibited IGP on the other species equally) at these same time points (PROC FREQ, SAS v.9.1). We did not measure body size or replace prey in this experiment.

Results

Prey consumption

There was a non-significant interaction for prey mortality rate between prey type and predator combination (ANOVA; $F_{8,42} = 1.27$; $P = 0.29$), but the main effects of prey type ($F_{2,42} = 40.63$; $P < 0.0001$) and predator combination ($F_{4,42} = 2.85$; $P = 0.036$) were significant. Across all predator combinations, mean prey mortality rate (±1SE) was highest for mosquito larvae (1.21 ± 0.11 mosquito larvae beetle^{-1} day^{-1}), intermediate for copepods (0.66 ± 0.05 copepods beetle^{-1} day^{-1}) and lowest for ostracods (0.28 ± 0.06 ostracods beetle^{-1} day^{-1}; Fig. 1a). When a second beetle larva was present, prey mortality rates were lower, though Tukey's multiple comparison test revealed no significant pairwise differences (Fig. 1b).

Prey selection

When we offered beetle larvae combinations of prey, the observed numbers of mosquito larvae consumed were higher than expected numbers in all treatments (Fig. 2). When offered 10 mosquito larvae (33.3%) and 20 microcrustaceans (66.6%), 68% and 66% of prey consumed were mosquito larvae for *A. disintegratus* and *A. punctatus* respectively. A chi-square
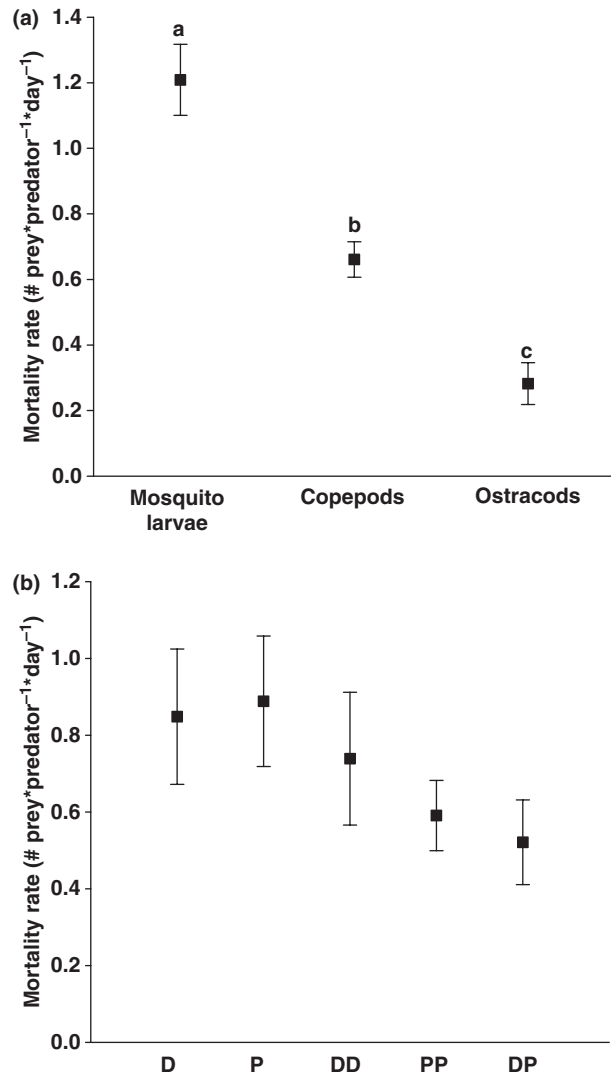

Fig. 1 Mean prey mortality rates (±1SE) of mosquito larvae, copepods and ostracods by dytiscid larvae (a), and in treatments with two predators (b). D = one *Agabus disintegratus*; P = one *A. punctatus*; DD = two *A. disintegratus*; PP = two *A. punctatus*; DP = one of each species. Comparisons with different letters indicate significant Tukey's differences at α = 0.05.

test revealed significant differences between observed and expected numbers of prey consumed ($\chi^2 = 30.0$, $P < 0.0001$; and $\chi^2 = 27.0$, $P < 0.0001$; Fig. 2). When offered 20 mosquito larvae (66.6%) and 10 microcrustaceans (33.3%), 91% and 79% of prey consumed were mosquito larvae for *A. disintegratus* and *A. punctatus* respectively. A chi-square test revealed that the observed–expected difference was significant for *A. disintegratus* only ($\chi^2 = 11.3$, $P = 0.0008$; Fig. 2).
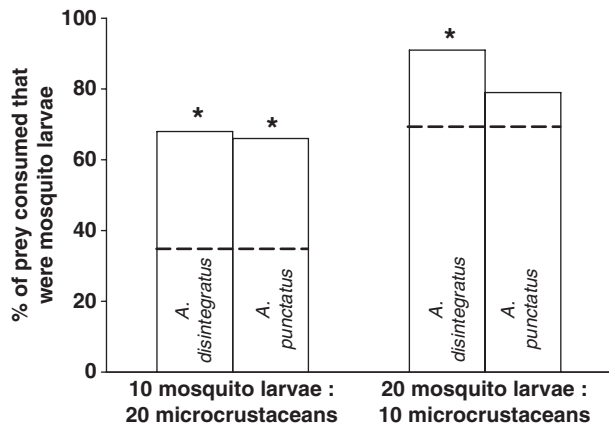
Fig. 2 Percentages of prey consumed by *Agabus disintegratus* and *A. punctatus* that were mosquito larvae (observed represented by bars) compared to prey offered (expected represented by dashed lines). Asterisks represent significant differences (chi-square: observed numbers − expected numbers).

Performance of A. disintegratus

Growth rates were significantly different between prey types ($F_{2,12} = 5.4$; $P = 0.02$; Fig. 3). Mean growth rate (±1SE) of beetles consuming ostracods was −0.01 ± 0.03 mm day^{-1}, mosquito larvae 0.08 ± 0.02 mm day^{-1} and copepods 0.11 ± 0.03 mm day^{-1}. Tukey's multiple comparison test revealed significant pairwise differences between copepods and ostracods only (Fig. 3).

Prey capture

During a ten minute period, *Agabus* encountered ostracods 2.5 times more frequently than mosquitoes
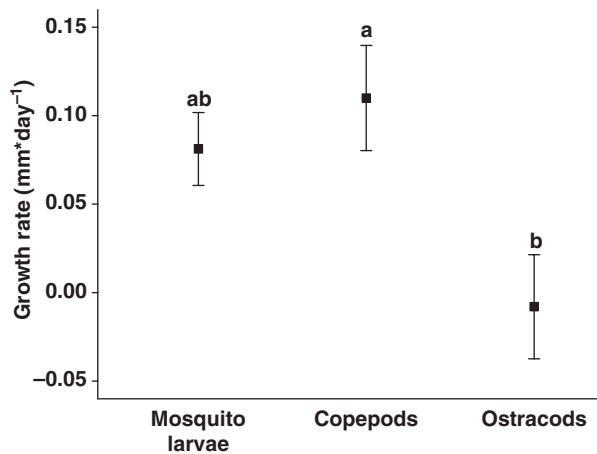

Fig. 3 Mean growth rate (±1SE) for mosquito larvae, copepods and ostracods. Comparisons with different letters indicate significant Tukey's differences at $\alpha = 0.05$.

or copepods (ANOVA; $F_{2,16} = 10.13$; $P = 0.0014$; Fig. 4). Attacks per encounter did not differ significantly between prey types (ANOVA; $F_{2,16} = 0.09$; $P = 0.92$; Fig. 4), but captures per attack were different with 30.3% of all attacks on mosquito larvae resulting in a
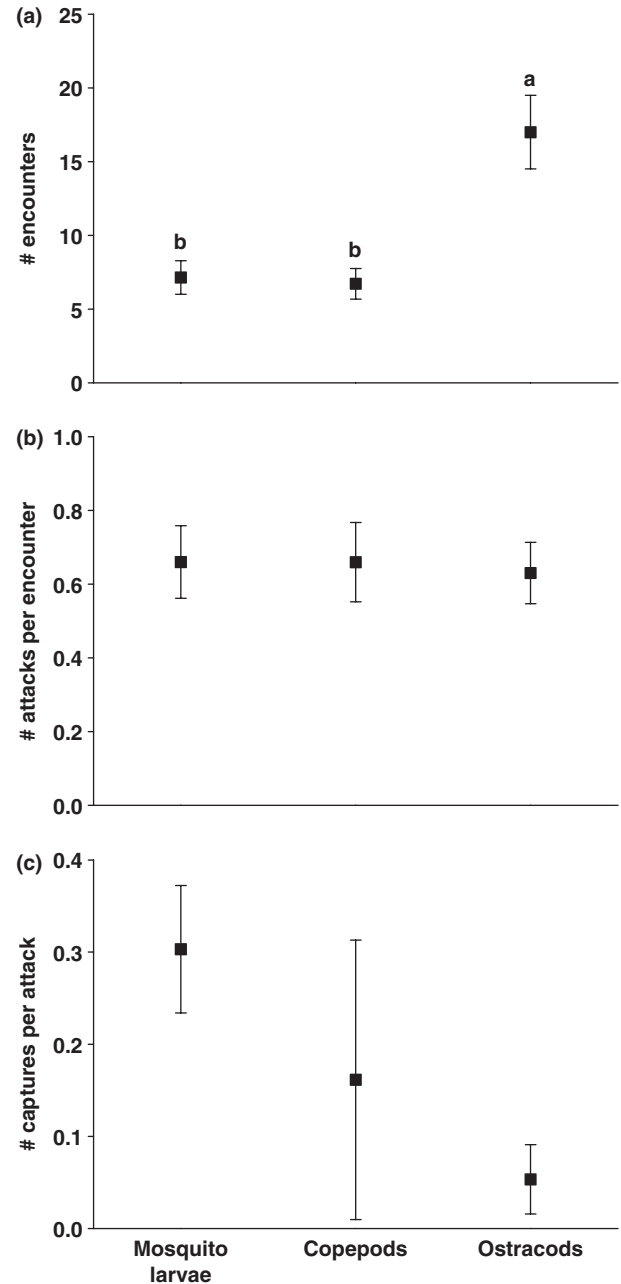

Fig. 4 Mean number of encounters between *Agabus* larvae and prey (a), mean number of attacks per encounter across prey types (b) and mean number of captures per attack for mosquito larvae, copepods and ostracods (c). Comparisons with different letters indicate significant Tukey's differences at $\alpha = 0.05$. Error bars show ±1SE.

capture, followed by copepods (16.1%) and then ostracods (5.3%) (Kruskal–Wallis, $P = 0.057$). Average numbers of prey consumed per beetle in 10 min were 1.4 mosquito larvae, 0.71 copepods or 0.75 ostracods, but these differences were not significantly different (Kruskal–Wallis, $P = 0.15$).

Predator interactions

After 5 days, cannibalism occurred in 57% and 43% of the replicates with *A. punctatus* and *A. disintegratus* respectively. Intraguild predation occurred in 67% of the treatments with both species. A chi-square test revealed no significant difference in the frequency of cannibalism compared to IGP (Table 1). After 18 days, cannibalism in *A. punctatus* and intraguild predation occurred significantly more times than cannibalism in *A. disintegratus* (Table 1).

After 5 days, *A. punctatus* IGP on *A. disintegratus* was observed five times, and *A. disintegratus* IGP on *A. punctatus* was observed seven times. A chi-square test revealed no significant difference in the number of times each species consumed the other (Table 2). After 18 days, IGP was completely symmetric between the two species (Table 2).

Discussion

Prey consumption and selective predation

Selective predation by an insect predator could have measurable effects on prey populations and prey communities (Peckarsky *et al.*, 1993; Eubanks & Denno, 2001). Through selective predation, *Agabus* larvae could suppress preferred prey populations and

Table 1 Frequency of cannibalism and intraguild predation (IGP) events in 18 replicates each of three predator treatments (cannibalism: two *Agabus disintegratus* larvae, two *A. punctatus* larvae or IGP: one larva of each species) after 5 and 18 days. No significant differences were found until after 18 days, when IGP and cannibalism in *A. punctatus* occurred significantly more times than cannibalism in *A. disintegratus*

	Cannibalism			
	A. disintegratus	*A. punctatus*	IGP	
5 days	9	12	12	χ^2; $P = 0.50$
18 days	13	18	18	Fisher's exact; **$P = 0.0027$**

Bold value indicates significance.

Table 2 Outcomes of intraguild predation (IGP) events after 5 and 18 days. Intraguild predation was symmetric, with no significant differences in the number of times each species consumed the other

	A. disintegratus IGP on *A. punctatus*	*A. punctatus* IGP on *A. disintegratus*	
5 days	7	5	χ^2; $P = 0.48$
18 days	9	9	χ^2; $P = 1.0$

change the proportions of prey in the community. We found that both species of *Agabus* consumed all three prey taxa, but mosquito larvae had a higher predator induced mortality rate than copepods or ostracods. Moreover, when offered a choice of prey, 76% of all prey consumed were mosquito larvae. Predation pressure on mosquito larvae was not reduced as the ratio of mosquito larvae : microcrustaceans decreased, demonstrating that selective predation was not driven by relative prey abundance. If these results translate into the field, when *Agabus* is present, proportions of mosquito larvae should be relatively lower compared to microcrustaceans, leading to suppression of the larval mosquito population. This could be tested with field predator exclusion experiments, which would be an important next step in determining if *Agabus* contributes to mosquito suppression. In addition to the consumptive effects we found, *Agabus* could also affect larval mosquito populations through non-consumptive effects, e.g. changes in larval mosquito behavioural traits or habitat shifts that affect emergence. Finally, diet shifts through ontogeny are expected because *Agabus* undergo changes in size and mandibular morphology from the first to third instar (Brannen, Barman & Wall, 2005).

Predator performance and behaviour

Selective predation can result from a number of differences in potential prey, including nutritional quality and ease of capture (Solomon, 1949; Dicke *et al.*, 1989; Wilbur, 1997), but few studies have experimentally addressed the prey selection process of wetland insect predators. Optimal foraging theory predicts that the most important criterion used in prey selection is nutritional quality relative to foraging costs (Stephens & Krebs, 1986). We tested how the underlying mechanisms of predator performance (nutritional quality) and behavioural interactions with

prey (foraging costs) may contribute to the selective predation we observed.

We found that *Agabus* grew larger when fed exclusively mosquito larvae or copepods than when fed exclusively ostracods. This suggests that for *Agabus*, mosquito larvae and copepods have greater nutritional value than ostracods. The lack of predator avoidance behaviour in ostracods caused *Agabus* to encounter them most frequently even though they were rarely captured. This was not because ostracods were difficult to capture. Rather, upon attack, ostracods were often immediately rejected. Ostracods are also unpalatable to predaceous mosquitoes (Campos & Lounibos, 2000) and predaceous microcrustaceans (Boix *et al.*, 2006). Mosquito larvae and copepods demonstrated predator avoidance, but *Agabus* captured and consumed them more frequently than ostracods. High variation in captures per attack of copepods resulted from the general difficulty of *Agabus* successfully attacking copepods. Often, copepods were able to rapidly move away from an attacking *Agabus*, but copepods that *Agabus* did successfully attack were not rejected. Mosquito larvae, once attacked, were easily captured and were not rejected.

In sum, despite their apparent ease of capture, ostracods were not preferred prey, likely because they lacked the nutritional composition required for growth of *Agabus* larvae. Mosquito larvae and copepods were more profitable in terms of growth. Improved growth performance when *Agabus* fed on mosquito larvae or copepods outweighed the possible foraging costs associated with successful encounter, attack and capture of these prey. This indicates a potential mechanism for selective predation of mosquitoes.

Cannibalism and intraguild predation

Cannibalism, defined as predation of conspecifics, and intraguild predation, defined as predation of interspecific competitors, are likely to reduce the impact of predation on prey species in the community (Claessen *et al.*, 2004). Dytiscid beetle larvae can be cannibalistic (Juliano & Lawton, 1990), and because *A. disintegratus* and *A. punctatus* occur in the same habitat at the same time of year, intraguild predation is also likely (Nilsson & Söderström, 1988). Given that both species share common prey resources and prefer

mosquito larvae, competition between and within these species is likely to be strong.

When we tested prey consumption, we found that prey mortality rates tended to decrease when a second predator was present. We observed cannibalism and intraguild predation in some of these treatments, and we expect that non-lethal negative predator–predator interactions occurred as well. However, despite these apparent trends, we found no significant differences in prey mortality due to multiple predators. Negative predator–predator interactions may not have a significant impact on selective predation of mosquito larvae.

When we quantified the occurrence of cannibalism within, and intraguild predation between, these two species, no strong differences emerged until day 18 of the experiment, when cannibalism in *A. punctatus* and intraguild predation occurred significantly more times than cannibalism in *A. disintegratus*. This was consistent with the observation that *A. punctatus* was slightly more aggressive than *A. disintegratus* in the pursuit of prey (L. Culler, pers. obs). Intraguild predation was symmetric, however, suggesting that one species was not dominant over the other. Competition for prey may be strongest between species and within *A. punctatus*.

In the constructed wetlands at the Jackson Lane Preserve, densities of *Agabus* reached up to 164 individuals m^{-2} on 10 March 2008 (M. DiPietro & L. Culler, unpubl. data). Smaller dytiscid larvae in the genus *Hydroporus* have been reported to reach densities of up to 641 individuals m^{-2} (Juliano & Lawton, 1990). Field studies manipulating densities of larval *Hydroporus* showed that competition or density-dependent cannibalism are common at high densities, because beetle survivorship dropped from 70% to 45% in the higher density treatments (Juliano & Lawton, 1990). Similar field experiments with *Agabus* could indicate the importance of competition, cannibalism and intraguild predation on predator populations. These interactions could reduce the overall impact on the prey community, and modified feeding behaviour by *Agabus* in the presence of other predators could indirectly affect prey consumption. Cannibalism and intraguild predation may also be important in regulating predator populations if the larvae hatch before preferred prey are present (Batzer & Wissinger, 1996). In the system studied here, preferred mosquito prey may not be present as

Agabus hatch, so cannibalism and intraguild predation may also initially support predator populations.

Implications for mosquito suppression

Conservation biological control, i.e. the manipulation of habitats to favour the natural enemies of pests, is a common goal in agricultural systems as a method to conserve biodiversity of natural enemies and reduce pest problems (Barbosa, 1998). Similarly, in wetland systems, abundant microcrustacean populations present when *Agabus* hatch may help to support and maintain predator populations before the preferred mosquito larvae hatch. *Agabus*, along with other native wetland predators that arrive later in the year, may work to suppress mosquito populations (Batzer & Wissinger, 1996).

Wetland management strategies that encourage colonisation by and maintain native wetland predator communities could help to reduce mosquito pest problems. For example, excluding fish that preferentially feed on larger invertebrates including many insect predators (Batzer *et al.*, 2000) could help to maintain native wetland predator communities. Also, structural complexity of wetlands may affect the ability of predators to suppress prey populations. Studies in terrestrial systems show that reducing complexity leads to an increase in intraguild predation and other antagonistic interactions, thereby diminishing predation pressure on prey populations (Finke & Denno, 2002). Vegetation and structural heterogeneity generally increase diversity and abundance of macroinvertebrates predators (Carlson *et al.*, 2004; Langellotto & Denno, 2004; Shrewsbury & Raupp, 2006; Mogi, 2007), and increased structural complexity may help in prey location and capture (Finke & Denno, 2002; Langellotto & Denno, 2004). Thus, constructing and restoring wetlands to have complex habitats consisting of a diverse assemblage of wetland plants, microtopographical features and coarse woody debris could lead to a greater ability of predators to suppress mosquito populations.

The polyphagous nature of macroinvertebrates poses a major obstacle for biological suppression of mosquitoes (Mogi, 2007). At the Jackson Lane Preserve, polyphagy by *Agabus* may actually contribute to suppression of mosquitoes because *Agabus* can persist on microcrustaceans until preferred mosquito prey hatch. Most importantly, both *Agabus* species

exhibited clear selective predation of mosquito larvae over other prey. Other biological control agents for mosquitoes, such as the mosquitofish, *Gambusia affinis*, do not exhibit selective predation (Linden & Cech, 1990), and may benefit mosquito larvae indirectly by reducing densities of their competitors (Blaustein & Karban, 1990). The species identity of mosquito prey can influence selective predation (Blaustein, 1998; Blaustein & Chase, 2007), thus any measure of the mosquito suppression potential of *Agabus* should include other mosquito species commonly found in constructed wetlands.

The results of this study contribute to the recent growing body of literature supporting the idea of using native insect predators for suppression of mosquito larvae (Chatterjee, Ghosh & Chandra, 2007; Chandra *et al.*, 2008; Mandal, Ghosh & Bhattacharjee, 2008). Predation may be one of the most important ecological forces controlling pest populations (Diabaté *et al.*, 2008), so studies of the ecological interactions between native wetland predators, such as *Agabus*, and mosquito larvae in constructed wetlands may lead to a better understanding of how to regulate and suppress mosquito populations. While macroinvertebrates are not recommended for inundative biological control (i.e. mass release of reared natural enemies) due to problems with production, storage and release, continued study of predator ecology may yield clues for the development of tools to suppress mosquito populations using macroinvertebrate predators (Mogi, 2007). Control strategies involving conservation of *Agabus* and other native mosquito-specific predators, would be preferred to introducing non-native generalist predators or the application of pesticides. For now, wetland management should focus on constructing wetlands with high ecological value in order to attract and conserve native wetland predators that can help to suppress mosquito populations.

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