

## Chemical detection of the predator *Notonecta irrorata* by ovipositing *Culex* mosquitoes

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**ABSTRACT:** We tested the oviposition response of *Culex* mosquitoes to the predator *Notonecta irrorata* in an outdoor artificial pool experiment employing equal numbers of control and predator pools. There was a strong oviposition avoidance by *Culex* of *Notonecta* pools; 83% of egg rafts were found in control pools during the period in which *Notonecta* were present. After removing *Notonecta*, mosquitoes continued to avoid ovipositing in the former *Notonecta* pools for two additional days suggesting a predator-released kairomone as the cue used by the mosquitoes to detect the presence of this predator. *Journal of Vector Ecology* 30 (2): 299-301. 2005.

**Keyword index:** Oviposition, predation risk, *Notonecta irrorata*, kairomone.

### INTRODUCTION

Natural selection should favor female mosquitoes, whose aquatic larvae are vulnerable to predation, that are able to detect aquatic predators and avoid ovipositing in sites with high risk of predation. A small but growing body of literature indicates that this is the case for some mosquito-predator systems. Experiments have shown evidence for oviposition avoidance of aquatic predators in the following mosquito-predator combinations: *Anopheles punctipennis*-bluegill sunfish (Petranka and Fakhoury 1991); *Culex pipiens* complex-mosquitofish (Angelon and Petranka 2002), *Culex pipiens quinquefasciatus*-*Notonecta hoffmani* (Chesson 1984); *Culex laticinctus*-*Notonecta maculata* (Kiflawi et al. 2003), *Culiseta longiareolata*-*Anax imperator* (Stav et al. 1999; Stav et al. 2000), *C. longiareolata*-*N. maculata* (Blaustein et al. 2004) and *C. longiareolata*-*Anisops sardea* (Eitam et al. 2002). In some cases (Angelon and Petranka 2002; Blaustein et al. 2004), but not all (Stav et al. 2000), one important cue used by the mosquitoes was predator-released kairomones.

In this study, we experimentally examined the possible oviposition habitat selection in response to risk of predation for another prey-predator combination: *Culex* species and the backswimmer *Notonecta irrorata* Uhler. We also experimentally determined whether any oviposition avoidance was due to a predator-released kairomone. *N. irrorata* is common in woodland pools near St. Louis, MO, where this study took place (L. Blaustein personal observation). It is a general predator that can potentially have large impacts on pond community structure (Wilbur 1997). Mosquito densities have been shown to be lower in experimental plots containing *N. irrorata* (Neri-Barbosa et al. 1997), but the contributions of this reduction due to direct consumptive predation and due to behavioral avoidance (i.e., oviposition habitat selection) have not been investigated.

### MATERIALS AND METHODS

On 7 September 2004 (day 0), eight circular red plastic tubs (45 cm diameter, 35 cm height) were set up in a residential area of St Louis, MO under a thick, mixed tree canopy of *Carya cordiformis*, *Ulmus americana*, and *Alnthus altissima*. Inter-pool distances ranged from 0.5 to 2 m. On the same day, 15 cm (volume = 24 L) of tap water and 60 g of dry leaf litter (consisting of a mixture of the three canopy species) were added to each pool.

We counted and removed mosquito (*Culex*) egg rafts for two days (days 1-2) prior to adding *Notonecta*. This "pre-*Notonecta* period" allowed us to determine if the pools randomly assigned to treatment and control were, by chance, different in attracting oviposition by the mosquitoes.

On day 2, we added to each *Notonecta* pool two adult *N. irrorata* collected from nearby natural ponds three days earlier. They were deprived of food during these three days prior to adding them to the experimental pools. This density was high enough to elicit strong oviposition avoidance responses by some mosquitoes to other *Notonecta* species in Israel (e.g., Eitam and Blaustein 2004). We did not observe any colonization of predators into the experimental pools. The absence of predator colonization was expected given considerable distance (>1 km) from any ponds (Wilcox 2001). Egg rafts collected the morning after *Notonecta* introduction (day 3) were excluded from analysis, an *a priori* decision because of the possibility that *Notonecta* kairomones could not sufficiently build up by the first night. The next four days (days 4-7) constituted the *Notonecta* period in which egg rafts were counted on the first, second, and fourth day of this period. As this short period demonstrated a highly significant effect (see Results), we terminated this part of the experiment. We then removed *Notonecta* from the pools and followed oviposition over the next six days (days 8-13). This "Post-*Notonecta* period" allowed us to assess whether any effects of *Notonecta* on oviposition remained even after the predators

Table 1. Analysis of variance (pre-*Notonecta* period) and repeated measures analyses of variance (*Notonecta* and post-*Notonecta* periods) comparing pools assigned to predator and control pools. Statistically significant *P*-values (<0.05) are in bold.

Period	Treatment			Time			Treatment x Time		
	d.f.	F	P	d.f.	F	P	d.f.	F	P
Pre- <i>Notonecta</i>	1,6	0.40	0.552	--	--	--	--	--	--
<i>Notonecta</i>	1,6	17.82	<b>0.006</b>	1,6	5.21	0.062	1,6	0.01	0.923
Post- <i>Notonecta</i>	1,6	0.60	0.468	2,12	2.26	0.146	2,12	6.52	<b>0.012</b>

were no longer present. Fewer egg rafts in former *Notonecta* pools would indicate a predator-released kairomone (Blaustein et al. 2004).

Because egg rafts were not always counted every day, we analyzed data as egg rafts per pool per two days. A one-way analysis of variance (ANOVA) was performed on egg raft data during the pre-*Notonecta* period. During the *Notonecta* and post-*Notonecta* periods, we analyzed data as repeated measures ANOVA. Data were natural-log transformed (ln[y+1]) due to violations of homogeneity of variance. Mauchly’s criterion test for sphericity indicated that no adjustments to degrees of freedom were necessary in within-subjects factors.

RESULTS

Of the six egg rafts collected in control pools, three were *Culex pipiens* and three were *Culex restuans*. Likewise, there were three of each species for the six egg rafts collected from predator pools. We thus refer to the egg raft counts generically as “*Culex*” egg rafts.

During the pre-*Notonecta* period, *Culex* egg raft

abundance was not significantly different in predator and control pools (Table 1, Figure 1). During the *Notonecta* period, egg raft abundance was considerably lower in the predator pools; 83% of the egg rafts were found in control pools during this period (Table 1, Figure 1). Upon removing the *Notonecta*, egg raft abundance was still considerably lower in the former predator pools during the first two days but not during four and six days after predator removal (Treatment x time interaction: *P*=0.012; Figure 1).

DISCUSSION

We demonstrated that *Culex* mosquitoes can detect and avoid the predatory backswimmer *Notonecta irrorata* in an artificial pool experiment. Because we identified larvae to species level from only a small fraction of egg rafts, and both *C. pipiens* and *C. restuans* were present, we can only be certain that at least one of the species responded to the predator when ovipositing. Growing evidence suggests that many mosquitoes can detect and avoid some, but not all, predators. Of the two species ovipositing in this experiment, at least some members of the *C. pipiens* complex have been shown to avoid

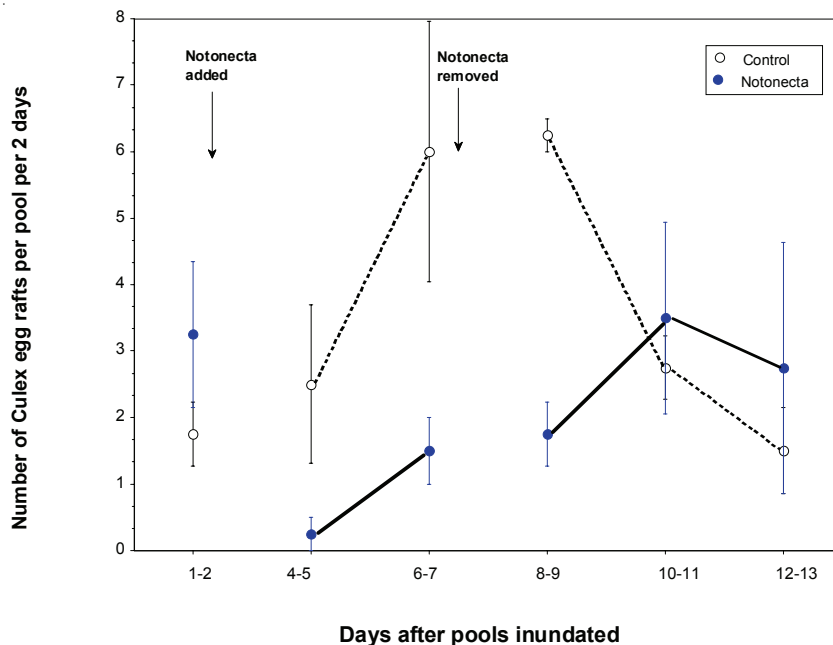


Figure 1. Mean *Culex* egg rafts per pool per two-day periods. Pools were created and filled with water on day 0. *Notonecta irrorata* were added on day 2 and removed on day 7. Error bars are ± one standard error.

mosquitofish (Angelon and Petranka 2002) and another notonectid species (Chesson 1984) when ovipositing. The effects of predators on oviposition habitat selection in *C. restuans* have not been investigated, although this mosquito has been shown to selectively oviposit in response to food level and conspecific density (Reiskind and Wilson 2002).

We also demonstrated that the cue is a chemical. The cue remained detectable for two and possibly three days after removal of the predator itself. Blaustein et al. (2004) demonstrated that a *Notonecta* released kairomone, under similar experimental conditions, remained detectable by *Culiseta longiareolata* for seven-eight days. This mosquito cannot chemically detect other predators such as odonates and urodeles (Stav et al. 2000, Blaustein 1999, Blaustein unpublished data). We suspect that the chemical(s) released by different backswimmer species and detected by different mosquitoes in California (Chesson 1984), Israel (Eitam et al. 2002, Blaustein et al. 2004), and now the midwestern U.S.A. is (are) the same or similar, although this remains to be determined.

These demonstrations of oviposition habitat selection by mosquitoes in response to predators have implications for how we experimentally assess the effects of aquatic predators on mosquito populations and the consequences of this behavior on mosquito populations (Spencer et al. 2002). Experiments assessing effects of predators on mosquito larvae that assume random oviposition when selective oviposition occurs likely overestimates the effect of the predator on mosquito populations. The kairomone, once chemically characterized, may be used as a mosquito control method. However, how effective a mosquito management method the application of such a kairomone to mosquito breeding habitats might be remains to be determined with empirical works and modeling.

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