

Geographic variation in *Culex* oviposition habitat selection responses to a predator, *Notonecta irrorata*

MATTHEW R. PINTAR,^{1,2} JASON R. BOHENEK^{1,3}

and WILLIAM J. RESETARITS Jr.¹ ¹Department of Biology and Centers for Water and Wetland Resources, and Biodiversity and Conservation Research, University of Mississippi, Oxford, Mississippi, U.S.A., ²Institute of Environment, Florida International University, Miami, Florida, U.S.A. and ³Olentangy River Wetland Research Park, School of Environment and Natural Resources, The Ohio State University, Columbus, Ohio, U.S.A.

Abstract. 1. Predators have effects on prey populations through both consumptive and non-consumptive effects. Predator's presence is expected to drive variation in prey oviposition habitat selection behaviour, but differences in biotic and abiotic characteristics of habitats, or trait variation, may produce geographic variation in species interactions.

2. We conducted a series of experiments in two geographic locations, Mississippi and Missouri, USA, to assess oviposition responses of *Culex* mosquitoes (prey) to the presence of *Notonecta irrorata* (predator). We first tested whether mosquitoes in each location respond to the presence of *N. irrorata*, with follow-up experiments to determine whether mosquitoes respond to variation in *N. irrorata* density, whether *N. irrorata* from each location generate different responses by the same *Culex* population, and whether diet and consumption of conspecifics affect oviposition.

3. We found that *Culex restuans* in Missouri had reduced oviposition when *N. irrorata* were present. In Mississippi, *C. restuans* did not respond to the presence of *N. irrorata* from either Mississippi or Missouri, to the variation in density of *N. irrorata*, or to *N. irrorata* that had been fed larval mosquitoes.

4. Our study documents the first instance of geographic variation in oviposition response of a prey species to a predator species.

Key words. Aquatic insects, geographic variation, habitat selection, oviposition, predator–prey, preference–performance.

Introduction

Predators are one of the most important factors governing the distribution and abundance of prey populations. In addition to direct, consumptive effects, predators also have non-consumptive effects on prey manifested in changes to behaviour, diet, morphology, and other characteristics (Peacor & Werner, 2001; Relyea, 2001; Preisser *et al.*, 2005; Winnie & Creel, 2007; Creel & Christianson, 2008; Peckarsky *et al.*, 2008). For many prey species, the lack of morphological or chemical defences necessitates predator avoidance behaviour for survival. Demographic habitat selection, where life cycle or life stage habitat choices are permanent or semi-permanent,

is a strategy to avoid predation used by colonising/ovipositing organisms, particularly for taxa with stages incapable of dispersal, such as larval aquatic insects (Abrams, 2007; Resetarits *et al.*, 2019). Habitat choice by prey should match their expected fitness in that patch (Rausher, 1983; Thompson, 1988; Craig *et al.*, 1989; Gripenberg *et al.*, 2010). Thus, effective habitat selection requires the detection, identification, and localisation of predators (Ferrari *et al.*, 2010), combined with assessment of the risk posed to an individual.

Ovipositing female mosquitoes are able to detect and respond to a range of aquatic predators, such as certain amphibians, fish, beetles, and water bugs, among other taxa (Petranka & Fakhoury, 1991; Tietze & Mulla, 1991; Stav *et al.*, 2000; Angelon & Petranka, 2002; Torres-Estrada *et al.*, 2009; Eveland *et al.*, 2016) (unpublished data). This includes responses by *Culex* and *Culiseta* mosquitoes to *Notonecta* (Hemiptera: Notonectidae) (Chesson, 1984; Eitam

Correspondence: Matthew R. Pintar, Florida International University, 3000 NE 151 St, North Miami, FL 33181. E-mail: matthew.pintar@gmail.com

& Blaustein, 2004; Sward-Arav *et al.*, 2016). *Notonecta* are aquatic, predatory water bugs that feed on vulnerable aquatic invertebrate prey (Streams, 1987; Pintar & Resetarits, 2021). They are especially efficient predators of mosquito larvae since both occupy the upper layer of the water column, and mosquito oviposition avoidance is a response to *Notonecta* predator-released kairomones (Ellis & Borden, 1970; Blaustein *et al.*, 2004; Silberbush *et al.*, 2010). Although the majority of mosquito–*Notonecta* habitat selection studies have focused on a single predator species (*N. maculata* Fabricius, 1794) in one geographic region (Israel) (Kiflawi *et al.*, 2003; Eitam & Blaustein, 2004; Silberbush *et al.*, 2010; Warburg *et al.*, 2011), few studies have explored mosquito–notonectid habitat selection interactions beyond this region or with other species (Chesson, 1984; Eitam *et al.*, 2002; Blaustein *et al.*, 2005). *Notonecta irrorata* Uhler, 1879 in the St. Louis region of Missouri, USA, is known to deter oviposition by native *Culex* species (Blaustein *et al.*, 2005).

Although the threat of predation is a dominant component of species interactions, outcomes of these interactions can be highly context-dependent, including outcomes dependent on geographic location (Chamberlain *et al.*, 2014). Across space, interactions can be a function of abiotic environmental conditions and/or biotic factors (Travis, 1996; Harley, 2003), all of which typically covary with latitude. Latitudinal variation in species interactions is documented among competing species (James *et al.*, 1997; Bertness & Ewanchuk, 2002), between plants and herbivores (Pennings & Silliman, 2005; Post, 2005), and between predators and prey (Jeanne, 1979; Fawcett, 1984; Stachowicz & Hay, 2000). Geographic variation in morphological and behavioural phenotypes has been documented within many insect species (Masaki, 1979; Mousseau & Roff, 1989; Huey *et al.*, 2000). Temperature and precipitation, among variation in other environmental characteristics, are typically responsible for generating spatial variation in phenotypes (Johnston & Bennett, 1996; Meiri *et al.*, 2005). With such clinal variation, there is selection for different phenotypes in different environments. This geographic variation in insect traits includes differences during oviposition that vary based on morphological capabilities of soil-ovipositing taxa (Herrmann *et al.*, 2010) as well as differences based on host plant specificity (Gotthard *et al.*, 2004). However, geographic differences in oviposition responses to predators have not been documented.

Here, we examined oviposition habitat selection by *Culex* mosquitoes in response to *N. irrorata* in two geographic locations, eastern Missouri and northern Mississippi, USA. We conducted a series of field mesocosm experiments to first assess whether mosquitoes in each location respond to *N. irrorata*, with follow-up experiments to determine whether (a) mosquitoes respond to variation in *N. irrorata* densities in Mississippi, (b) *N. irrorata* from each location generate different responses by the Mississippi population of mosquitoes, (c) diet and consumption of conspecific larvae can generate oviposition avoidance, and (d) *N. irrorata* are effective predators of *Culex* larvae in Mississippi. Given the effect of *N. irrorata* on mosquitoes in Missouri (Blaustein *et al.*, 2005) and the effects of notonectids in other regions (Kiflawi *et al.*, 2003), we expected ovipositing

mosquitoes to avoid patches where *N. irrorata* is present in all studies.

Methods

Study sites

We conducted a series of outdoor mesocosm experiments at two study sites using *N. irrorata* collected from both sites to assay responses by natural populations of mosquitoes. The first experiment was conducted at Tyson Research Center (hereafter Tyson) in St. Louis County, Missouri, and the remaining four experiments were conducted at the University of Mississippi Field Station (UMFS) in Lafayette County, Mississippi. Experiments 1 and 3 used *N. irrorata* collected from Tyson; Experiments 2, 3, and 5 used *N. irrorata* collected from UMFS; and Experiment 4 used *N. irrorata* from both Tyson and UMFS. Tyson is 465 km north-northwest of UMFS. Some methods vary between experiments due to material and space limitations, but methods within all individual experiments are controlled, enabling analysis within experiments and comparison of overall results among experiments. Additionally, we used Experiment 1 and other studies conducted in Mississippi to inform Experiments 2–4.

Experiment 1: habitat selection—Missouri

In Missouri, we constructed a rectangular array of 15 mesocosms (3 × 5) in an old field at Tyson on 30 June 2013. Mesocosms were blue plastic wading pools (70 litres; 0.85 m diameter) that were filled with filtered water from a nearby stream and then covered with window screen lids (1.3 × 1.13 mm openings) to prevent colonisation by other organisms. The mesocosm water was aged for 3 days to degrade or dissipate any chemical cues originating from the stream. At this time, each mesocosm received 250 g of leaf litter and 10 g of rabbit chow (Small World Rabbit Food, Manna Pro, St. Louis, MO, 40% protein) to stimulate productivity and attract mosquitoes to the array (Blaustein & Kotler, 1993; Relyea, 2002a; Binckley & Resetarits, 2008; Semlitsch & Boone, 2010).

The experiment was a randomised complete block design where each of three randomly assigned treatments were represented once per block (block = row = three mesocosms). The treatments consisted of controls, hydrocarbons, and *Notonecta*-conditioned water (NCW). Controls received no experimental alteration. Starting 3 July, both hydrocarbon and NCW additions were randomly added to respective treatment mesocosms each day and every other day, respectively. Hydrocarbon treatment consisted of a 5 ml mixture of synthetic notonectid kairomones dissolved in 95% ethanol. The synthetic kairomone mixture was composed of 30 µM of tricosane (Sigma-Aldrich #638-67-5) and 154 µM of heneicosane (Sigma-Aldrich #629-94-7). This dose is an approximate concentration that would be produced from five notonectids and represents the only known kairomones of notonectids (Silberbush *et al.*, 2010). NCW was created by housing five locally collected *N. irrorata* in each of five different 0.5 litre containers

of water for 24 h. Prior to water conditioning, all individuals were starved for 24 h. When not conditioning water, all captive *Notonecta* were fed frozen bloodworms (San Francisco Bay Brand, Inc., Newark, CA) for the duration of the experiment.

The window screen lids were sunk into the water to allow mosquitoes to oviposit in mesocosms on 3 July. Each mesocosm was checked daily for mosquito egg rafts (*Culex*), which were then collected individually in small cups and transferred to the laboratory for hatching and identification. Mosquito larvae were reared to fourth instars and identified to species using Darsie and Ward (2005). Two species comprised all individuals in our experiment, *Culex restuans* Theobald, 1901 and *Culex pipiens* × *Culex quinquefasciatus* (Silberbush and Resetarits unpublished data). The field experiment concluded after collections on 10 July.

Experiment 2: habitat selection—Mississippi

In Mississippi, we conducted two rounds of the same experiment to determine whether *N. irrorata* affects *Culex* oviposition. We used rectangular black plastic pools (~50 litres; 66 × 51 × 15 cm) as habitat patches and then filled them with unchlorinated well water and 100 g of dry hardwood leaf litter. Eight pools were arranged in a circle with a radius of 5 m (edge of each pool to centre of array), with pools 3.8 m from adjacent pools. We placed a single circular plastic pool (1 m diameter; ~110 litres) at the centre of the array filled with well water, 20 g of rabbit chow, and 500 g of hardwood leaf litter. This centre pool was covered with window screening to prevent oviposition, and its purpose was to provide additional cues from the decaying organic matter to attract mosquitoes to the array. This is in contrast to many other studies that place this organic matter within experimental mesocosms in which they directly measure oviposition or other biotic changes (Blaustein & Kotler, 1993; Relyea, 2002b; Binckley & Resetarits, 2008). However, high amounts of organic matter, and nutrient-rich manufactured materials like rabbit chow in particular, may influence oviposition and potentially interact with the perception of predator cues in patches (Pintar *et al.*, 2018). Therefore, our methods provide for the ability to attract mosquitoes to the array, while preventing interaction with cues in experimental pools that could lead to misinterpretation of results; hence, our results provide conservative estimates of effects. Treatment pools contained a cylindrical black plant pot 'cage' (32 cm diameter) with two screened sides and a screen lid to house *N. irrorata*. One of two treatments (controls; *N. irrorata* pools) was randomly assigned to the first pool and then alternated such that no pools of the same treatment were adjacent. *Notonecta irrorata* were collected from ponds at UMFS and were immediately added to pools (without being gut-cleared or fed).

The first round of the experiment was conducted in June 2015, and we placed two *N. irrorata* in each predator cage. We set up two arrays (blocks) simultaneously on 9 June and collected egg rafts on 10–17 June. The second round was conducted in October 2015, and we placed three *N. irrorata* within each predator cage. The first block was set up on 10 October and egg rafts were collected 11–14 October, while the second block

was set up on 13 October and egg rafts were collected 14–17 October. Other observations (Bohenek *et al.*, 2017; thousands of other egg rafts identified to species from unpublished data) showed that ~99% of *Culex* egg rafts oviposited at UMFS are *C. restuans*. Therefore, we collected a subset of egg rafts from these experiments, raised them to the fourth instar, and identified them to species (Darsie & Ward, 2005). All identified larvae (from 101 egg rafts) were *C. restuans*.

Experiment 3: habitat selection—density

We conducted another experiment in Mississippi to determine if *N. irrorata* density, rather than presence/absence, within a patch can affect *Culex* oviposition. Responses by colonising/ovipositing taxa in experimental mesocosms are largely a presence/absence response or a threshold response that occurs at a very low density of predators (Rieger *et al.*, 2004). *Notonecta irrorata* were collected on 23 May 2017 from one pond at UMFS (34°25'09.13"N, 89°23'37.76"W). On 24 May, we established mesocosms (blue plastic pools: 70 litres; 0.85 m diameter), linearly arranged and separated by 1 m edge-to-edge, each containing 250 g hardwood leaf litter. To reduce chances of additional notonectids colonising this experiment, the mesocosms were established at three sites (blocks) where previous observations indicated colonising notonectids were rare. Treatments consisted of three densities of *N. irrorata*: 0, 2, or 10 individuals per mesocosm (nine replicates per treatment, three replicates per block; $N = 27$). The *N. irrorata* densities represent low and high densities commonly encountered at UMFS, although *N. irrorata* densities can be higher. Treatments were randomly assigned to the first and second pools in each block, with treatments of the remaining pools systematically alternated so that each pool was adjacent to the two other treatments. The appropriate numbers of *N. irrorata* were randomly assigned, added to mesocosms on 24 May, and placed below the screens to prevent them from consuming any colonists. Mesocosms were covered with screening (1.3 × 1.13 mm openings) that was depressed below the water surface to separate *N. irrorata* from ovipositing mosquitoes and their eggs.

We removed and counted *Culex* egg rafts daily, but egg rafts were only found 26 May–6 June. We also checked mesocosms every day for colonising notonectids, which were removed to maintain treatment densities (only two colonised). The experiment was terminated on 28 June, when we searched through the leaf litter to determine *N. irrorata* survival, which was 100%. No *Culex* larvae were raised and identified, but based on prior experiments all were assumed to be *C. restuans*.

Experiments 4a and 4b: habitat selection—transplant experiments

Our final set of habitat selection experiments was transplant experiments where we directly compared the effects of *N. irrorata* from two populations (UMFS and Tyson) on the same population of *Culex* (UMFS). On 11 June 2018, we collected *N. irrorata* from UMFS, and on 12 June 2018 we collected

N. irrorata from Tyson and transported them to UMFS. We attempted to assay oviposition in mid-June, but there was no *Culex* activity. Thus, we maintained *Notonecta* from both populations in a lab at UMFS until mid-August.

The first transplant experiment (Experiment 4a) aimed to assess *Culex* oviposition in response to gut-cleared *N. irrorata*. The design of this experiment was similar to Experiment 2. We used 12 black plastic pools (~50 litres; 66 × 51 × 15 cm) arranged in a circle around a central bait pool (~110 litres). The treatment pools were 5 m from the centre of the array and were separated from each other by 2.6 m. There were three treatments: controls (no *N. irrorata*), Tyson (*N. irrorata* from Missouri), and UMFS (*N. irrorata* from Mississippi). The treatments of the first two pools were randomly assigned and then alternated around the array so that each pool was adjacent to the pools of the other two treatments. Each treatment pool contained well water, 100 g of hardwood leaf litter, and a cage. The bait pool contained well water, 20 g of rabbit chow, 500 g of hardwood leaf litter, and was covered with screening. To clear their guts, *N. irrorata* from both populations were not fed for 7 days prior to the start of the experiment. The first block was established on 12 August, contained two *N. irrorata* in predator pool cages, and had its egg rafts collected 13–16 August. Due to some mortality, the second block contained one *N. irrorata* per cage (to maintain equal numbers across all replicates in the block); the second block was established on 16 August, and egg rafts were collected 17–21 August.

The second transplant experiment (Experiment 4b) aimed to assess *Culex* oviposition in response to *N. irrorata* that were fed *Culex* larvae. Using a similar design to previous experiments, we set up nine black plastic pools (~50 litres; 66 × 51 × 15 cm) arranged in a circle around a central bait pool (~110 litres). Treatment pools were 5 m from the centre of the array and were separated from each other by 3.4 m. Treatment pools and bait pools were established in the same manner as the previous experiment, and the same three treatments (control, UMFS, Tyson) were randomly assigned as previously described. Pairs of *N. irrorata* were randomly assigned to each UMFS and Tyson pool, but only a single individual was in each cage at a time. Starting on 24 August, 24 h prior the establishment of the first block, we established holding containers (~2 litres) in the lab for each pair of *N. irrorata*. In the lab and for the duration of the experiment, *N. irrorata* from both UMFS and Tyson were fed 9 ml of *Culex* larvae (~100 individuals) per day that had been collected from Experiment 4a (UMFS) and raised in the lab. Using the paired *N. irrorata* system, we swapped each individual between the field and lab each day, such that every other day one individual was in the experimental pools while the other was in the lab with available food. The first block was established on 24 August and egg rafts were collected from 26 August to 29 August, while the second block was established on 29 August and egg rafts were collected from 30 August to 2 September. The same *N. irrorata* individuals were used in both blocks, but they were randomly reassigned to pairs and pools in the second block. A subsample of egg rafts from both transplant experiments was collected, raised to fourth instar, and identified to species; all egg rafts were *C. restuans* (Darsie & Ward, 2005).

Experiment 5: predation experiment

Finally, we conducted a predation experiment to verify that *N. irrorata* are effective predators of *Culex* larvae at UMFS. On 19 September 2017, we set up one 110-litre wading pool containing ~500 g hardwood leaf litter at UMFS, and covered it with window screening. On 20 September, we collected 38 *Culex* egg rafts from this pool. Egg rafts were raised in a greenhouse in individual 100 ml plastic containers filled with ~40 ml of water from the source pool and ~0.02 g of rabbit chow. On 26 September, larvae had reached the fourth instar, and we identified one larva from each egg mass to species. All identified larvae were *C. restuans*. The larvae from all 38 egg rafts were combined and mixed in a single container and then sorted into 12 groups of 100 individuals (all fourth instar).

We filled 12 clear plastic containers (34.6 × 21.0 × 12.4 cm) with 3.0 litres of unchlorinated well water to establish microcosms in a lab with a 12/12 h light/dark cycle (lights on 08.00 hours–20.00 hours) on 20 September. *Notonecta irrorata* were collected from the same pond as the density experiment at UMFS on 20 September and held in the lab without food until 26 September when we randomly assigned seven individuals to the microcosms (one *N. irrorata* per microcosm) and added them at 08.00 hours. This established our two treatments for the mosquito predation experiment: control (zero *N. irrorata*; $N = 5$) and predator (one *N. irrorata*; $N = 7$). The design was unbalanced because we expected no mortality from controls and some variation from predator replicates. We then randomly assigned the 12 groups of 100 *C. restuans* larvae to microcosms and added them at 09.00 hours on 26 September. We counted the number of larvae alive in each microcosm after 1 h (10.00 hours), 3 h (12.00 hours), and 23 h (08.00 hours on 27 September), after which the experiment was terminated.

Data analysis

All analyses of the habitat selection experiments (Experiments 1–4) were conducted in a similar manner. We summed the total number of egg rafts oviposited in each pool over the duration of each experiment, and then analysed the total number egg rafts with mixed-effects models fit with a Poisson distribution with treatment as a fixed effect and pool nested within block as a random effect. In the density experiment only, we set planned contrasts to first compare mesocosms with no *N. irrorata* to those that contained *N. irrorata* (both 2 and 10 per mesocosm) and second to compare mesocosms with two *N. irrorata* to those with 10 *N. irrorata*. For all other analyses, we compared the primary model (including treatment) to null model excluding treatment to obtain main effects of treatment. All analyses were conducted in R v 4.1.0 using the LME4 v 1.1-27 and MULTCOMP v 1.4-17 packages (Hothorn *et al.*, 2008; Bates *et al.*, 2015; R Core Team, 2021).

For Experiment 5, we analysed the number of larvae alive throughout the experiment with a repeated-measures ANOVA that included treatment, time, and the time × treatment interaction as fixed effects on the square root of transformed abundances of larvae.

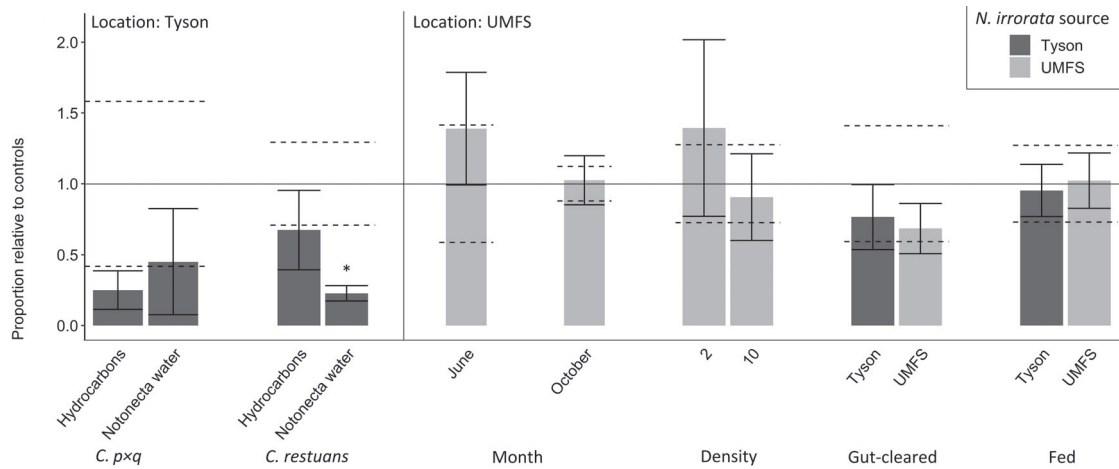


Fig. 1. Average proportion of egg rafts (\pm SE) oviposited in each treatment relative to controls for all habitat selection experiments. Experiments are ordered left to right in the same order they are presented in the text. Experiments on the left side were conducted at Tyson, while those on the right were conducted at UMFS. Results at Tyson are for both *Culex pipiens* \times *Culex quinquefasciatus* and *Culex restuans*; results at UMFS are all *C. restuans*. The colour of bars indicates the source location of *Notonecta irrorata* used in each experiment. The horizontal line at 1.0 represents the mean control value, while dashed lines indicate upper and lower standard error limits for controls in each experiment. The asterisk indicates a significant difference from the control. Tyson, Tyson Research Center; UMFS, University of Mississippi Field Station.

Results

Results of all habitat selection experiments are presented in Fig. 1 in the order (left to right) described here (and in the methods) and as a proportion of the controls within each experiment for direct comparison among experiments.

Experiment 1: habitat selection–Missouri

A total of 692 egg rafts were oviposited across the duration of the experiment: 384 *C. restuans* egg rafts and 306 *C. pipiens* \times *C. quinquefasciatus* egg rafts. There were no differences in *C. pipiens* \times *C. quinquefasciatus* oviposition across treatments ($\chi^2 = 2.27$, $P = 0.3218$), but there was a marginal difference in *C. restuans* oviposition across treatments ($\chi^2 = 5.55$, $P = 0.0625$). *Post hoc* Holm-adjusted Tukey's comparisons of treatments revealed that oviposition in pools containing *Notonecta*-conditioned water (NCW) was significantly lower than controls for *C. restuans*, while comparisons with the hydrocarbon water were not significant.

Experiment 2: habitat selection–Mississippi

In the June round of the experiment, a total of 172 egg rafts were oviposited, but there were no differences between treatments ($\chi^2 = 0.88$, $P = 0.3475$). In the October round of the experiment, a total of 994 egg rafts were oviposited, but again, there were no differences between treatments ($\chi^2 = 0.01$, $P = 0.9220$).

Experiment 3: habitat selection–density

A total of 716 *Culex* egg rafts were oviposited in the density selection experiment. The number of egg rafts oviposited in

each treatment did not vary between mesocosms with or without *N. irrorata* ($z = 0.310$, $P = 0.757$) or between those with 2 or 10 *N. irrorata* ($z = 0.049$, $P = 0.961$).

Experiments 4a and 4b: habitat selection–transplant experiments

During the gut-cleared experiment (Experiment 4a), a total of 570 egg rafts were oviposited, and there were no differences in oviposition rates among treatments ($\chi^2 = 0.29$, $P = 0.8666$). In the experiment when we fed *Culex* larvae to *N. irrorata* (Experiment 4b), a total of 681 egg rafts were oviposited, and there were no differences in oviposition rates among treatments ($\chi^2 = 0.18$, $P = 0.9144$).

Experiment 5: mosquito predation experiment

Notonecta irrorata began preying on mosquito larvae within the first hour of the mosquito predation experiment (Fig. 2). After 23 h, all *C. restuans* larvae were still alive in the controls, whereas only one larva survived in the seven predator replicates (out of 700 total larvae initially). Thus, in this experiment, there was a strong time \times treatment interaction ($F_{1,34} = 1979$, $P < 0.0001$), main effect of treatment ($F_{1,10} = 1184$, $P < 0.0001$), and main effect of time ($F_{1,34} = 2771$, $P < 0.0001$).

Discussion

We observed geographic variation in responses by ovipositing *Culex* to *N. irrorata* between populations in Missouri and Mississippi. First, it was shown previously that *Culex* in Missouri have lower oviposition rates in patches containing *N. irrorata*; however, Blaustein *et al.* (2005) only reported an overall

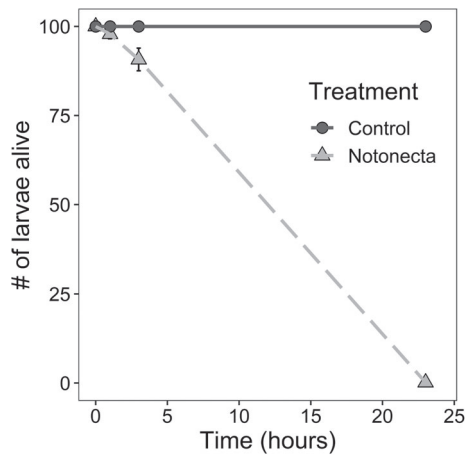


Fig. 2. Average number of *Culex restuans* larvae (\pm SE) in the control and *Notonecta irrorata* treatments alive at the start (0 h) of the predation experiment (Experiment 5) and after 1, 3, and 23 h.

avoidance among all *Culex* and likely did not consider individual species responses due to low total counts of egg rafts in their experiment. We verified that *Culex* in Missouri have lower oviposition rates in patches with *N. irrorata* kairomones, and we documented species-specific responses, with lower oviposition by *C. restuans* and no difference among treatments with *C. pipiens* \times *C. quinquefasciatus*. Second, across all of our experiments in Mississippi, we have documented that *C. restuans* in this population do not respond to *N. irrorata*. This includes experiments conducted in multiple seasons, across high and low *N. irrorata* densities, with *N. irrorata* that were fed and gut-cleared, and with *N. irrorata* from both Missouri and Mississippi (Fig. 1). To our knowledge, this represents the first documented instance of geographic variation of oviposition habitat selection responses in a single predator–prey system.

Geographic variation in habitat selection across two geographic locations in response to a highly effective predator (Fig. 2) is unexpected, given the fitness consequences. Prey avoidance behaviour should reflect the predation risk posed by a predator, especially among two common, native, widely occurring species like *C. restuans* and *N. irrorata* (Segev *et al.*, 2016). At UMFS, we conducted multiple experiments to verify this counterintuitive outcome and test alternative hypotheses. Our first alternative was to determine if there was a response to predator density, but high densities still produced no oviposition response by *C. restuans* at UMFS. Eitam and Blaustein (2004) observed a presence–absence response between *N. maculata* and ovipositing mosquitoes, and threshold responses have typically been observed by other ovipositing organisms in aquatic systems (Rieger *et al.*, 2004). Thus, given the outcome of our initial Mississippi experiments, this non-response to density was expected, given that densities eliciting avoidance elsewhere were typically low. Lastly, we simultaneously tested two additional hypotheses: (a) *Culex* oviposition responses to *N. irrorata* are a response to *N. irrorata* diet/consumption of *Culex* larvae and (b) *N. irrorata* from Missouri produce unique kairomones that those from Mississippi do not. In both cases, there were again no responses by *C. restuans* to any variation in *N. irrorata*.

Culex restuans at UMFS are capable of detecting, localising, and responding to predators present in aquatic habitats—we have documented that *C. restuans* avoid a wide range of fish species, ambystomatid salamanders, and adult predaceous diving beetles (Bohenek *et al.*, 2017; Pintar & Resetarits, 2020; unpublished data); however, there is considerable species-specific variation in whether *C. restuans* avoid a predator. If dietary or consumption-related cues play roles in this predator–prey interaction, we would expect cues of consumed conspecific larvae to be the most direct and informative cue of risk posed to larval mosquitoes within patches (Schoeppner & Relyea, 2005, 2009), but ovipositing *C. restuans* in Mississippi did not respond to predators that were fed larval mosquitoes.

Culex predator avoidance behaviour during habitat selection is also species-specific at Tyson, with reduced oviposition in response to some species, but not to others. Among tested fish species at Tyson, *C. restuans* reduced oviposition with *Gambusia affinis* (S.F. Baird & Girard, 1853), but not with *Lepomis cyanellus* Rafinesque, 1819 or *Aphredoderus sayanus* (Gilliams, 1824) (Eveland *et al.*, 2016; Silberbush & Resetarits, 2017)—these same patterns are observed at UMFS (unpublished data). The specific compounds that make up predator-released kairomones are unknown for the majority of taxa, including *N. irrorata*. The synthetic kairomone mixture used at Tyson (tricosane, heneicosane) represented kairomones produced by *N. maculata*, a Palearctic species (Silberbush *et al.*, 2010). Lack of response by both *C. restuans* and *C. pipiens* \times *C. quinquefasciatus* at Tyson to the synthetic kairomone mixture could indicate that *N. irrorata* do not produce these chemicals, produce them in different concentrations or ratios, or they do not act as kairomones in this predator–prey system. We did not test for responses by the Mississippi *Culex* population to the synthetic kairomones, but since they do not respond to the actual predator it is essentially moot.

Transplanting *N. irrorata* from Tyson to UMFS, maintaining *N. irrorata* from both populations in equivalent conditions prior to and during the experiments, and having equivalent non-responses to their presence by ovipositing *Culex* at UMFS suggest that there is nothing inherently physiologically different about the *N. irrorata* themselves. It is possible that *N. irrorata* in the two populations have different diets that elicit responses in Missouri but not Mississippi. However, in Missouri, we fed *N. irrorata* commercial bloodworms after 24 h without food, whereas Blaustein *et al.* (2005) deprived theirs of food for 3 days, yet we obtained equivalent results. In Mississippi, we used an array of feeding methods, including *C. restuans* larvae, gut-clearing for a week, transporting directly from natural ponds into mesocosms, and allowing *N. irrorata* to feed on organisms within mesocosms for up to 3 weeks, all with the same outcome. Furthermore, in the density experiment at UMFS, *N. irrorata* presence (but not density) affected colonisation of five aquatic beetle species in two families (Pintar & Resetarits, 2021). This suggests that differences in *C. restuans* oviposition in the two populations are not due to diet or other characteristics of *N. irrorata*, but rather to differences in *C. restuans*. One likely explanation is that there are differences in the sensory capabilities or recognition between *C. restuans* populations at UMFS and Tyson. Sensory differences could exist through (1)

the (in)ability to directly detect *N. irrorata* kairomones that are detected by other insect taxa (Pintar & Resetarits, 2021), (2) detection of the kairomones but differences in whether the kairomones are associated with predation risk, or (3) detection and recognition of the kairomones, but perceived importance of the risk posed by *N. irrorata* is low relative to other axes of patch quality, such as a shared predator (e.g. fish). Because we did not conduct a reciprocal transplant experiment in Missouri using Mississippi *N. irrorata*, we cannot exclude the possibility that each population produces unique chemical signatures. Replicating all studies at both sites would provide a more robust test of the nature of this geographic variation; however, all of our results suggest that *C. restuans* in Mississippi lack an evolved response to the presence of *N. irrorata*.

Although both *Culex* and *N. irrorata* strongly avoid many fish species, this does not necessarily mean they are both relegated to the same fishless patches. Based on patch size alone, *N. irrorata* prefer to colonise larger patches (Resetarits *et al.*, 2019) while *C. restuans* prefer to oviposit in smaller patches (Bohenek *et al.*, 2017). Thus, patch size can act as a niche dimension, potentially mediating the predatory effect of adult *N. irrorata* on larval *Culex*. Although *Notonecta* are probably a poor taxon to use for biocontrol of mosquito populations due to their vagility, our results here illustrating geographic variation in responses by ovipositing *Culex* to *N. irrorata* have potential implications for the use of other species in biocontrol. Some fish species, particularly *Gambusia* spp., are well known to have strong effects on both oviposition and larval survival within individual habitat patches (though not necessarily on regional abundances), while other fish species have a range of effects. But are these outcomes always true in predator–prey interactions in different systems? Our results indicate that, at least for some taxa, that answer is no. Therefore, there should be increased emphasis on ecological interactions in local populations to achieve the most effective methods for controlling some species.

Overall, we have documented the first instance of geographic variation in oviposition site selection by one prey species in response to a predator species. Although the mechanisms behind this geographic variation, as well as the spatial scope of the variation, need further study, there are clear consequences for these behavioural differences. At the population level, the lack of habitat preference at UMFS may be of relatively little importance at the population level due to the prevalence of *C. restuans* there. However, for an individual female, the lack of response to *N. irrorata* can be devastating, as they are potentially committing their entire lifetime's reproductive output to a habitat patch containing predators that can decimate their offspring. The oviposition response by *C. restuans* at Tyson enables females in this population to provide their offspring habitat patches devoid of these predators, at least initially. This illustrates how effective oviposition habitat selection is a critical component of predator–prey interactions, and females across an array of aquatic taxa make decisions to optimise fitness (McGuffin *et al.*, 2006; Vonesh *et al.*, 2009; Resetarits *et al.*, 2019). These habitat selection decisions are an important part of the factors driving patterns of species abundances, species distributions, and community structure across landscapes.

Acknowledgements

E. Biro, K. Medley, and K. Smith provided logistical support at Tyson Research Center. L. Eveland assisted with field work; A. Silberbush provided technical advice. Support was provided by the Henry L. and Grace Doherty Foundation, University of Mississippi, and University of Mississippi Field Station. Collection in Mississippi was approved by the Mississippi Department of Wildlife, Fisheries, and Parks (permits 0521162, 0525171, and 0502182). No collection permits were required in Missouri. This is publication #026 of the Center for Biodiversity & Conservation Research at the University of Mississippi.

The authors declare no conflicts of interest.

Data availability

The data that support the findings of this study are openly available in figshare at <https://doi.org/10.6084/m9.figshare.14706507>.

Author contributions

MRP, JRB, and WJR conceived and designed the experiments. MRP and JRB collected and analyzed the data. MRP wrote the manuscript with input from JRB and WJR.

References

- Abrams, P.A. (2007) Habitat choice in predator-prey systems: spatial instability due to interacting adaptive movements. *The American Naturalist*, **169**, 581–594.
- Angelon, K.A. & Petranka, J.W. (2002) Chemicals of predatory mosquitofish (*Gambusia affinis*) influence selection of oviposition site by *Culex* mosquitoes. *Journal of Chemical Ecology*, **28**, 797–806.
- Bates, D., Mächler, M., Bolker, B.M. & Walker, S.C. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**, 51.
- Bertness, M.D. & Ewanchuk, P.J. (2002) Latitudinal and climate-driven variation in the strength and nature of biological interactions in New England salt marshes. *Oecologia*, **132**, 392–401.
- Binckley, C.A. & Resetarits, W.J. (2008) Oviposition behavior partitions aquatic landscapes along predation and nutrient gradients. *Behavioral Ecology*, **19**, 552–557.
- Blaustein, L. & Kotler, B.P. (1993) Oviposition habitat selection by the mosquito, *Culiseta longiareolata*: effects of conspecifics, food and green toad tadpoles. *Ecological Entomology*, **18**, 104–108.
- Blaustein, L., Kiflawi, M., Eitam, A., Mangel, M. & Cohen, J.E. (2004) Oviposition habitat selection in response to risk of predation in temporary pools: mode of detection and consistency across experimental venue. *Oecologia*, **138**, 300–305.
- Blaustein, L., Blaustein, J. & Chase, J. (2005) Chemical detection of the predator *Notonecta irrorata* by ovipositing *Culex* mosquitoes. *Journal of Vector Ecology*, **30**, 299–301.
- Bohenek, J.R., Pintar, M.R., Breech, T.M. & Resetarits, W.J. (2017) Patch size influences perceived patch quality for colonising *Culex* mosquitoes. *Freshwater Biology*, **62**, 1614–1622.
- Chamberlain, S.A., Bronstein, J.L. & Rudgers, J.A. (2014) How context dependent are species interactions? *Ecology Letters*, **17**, 881–890.

- Chesson, J. (1984) Effect of notonectids (Hemiptera: Notonectidae) on mosquitoes (Diptera: Culicidae): predation or selective oviposition? *Environmental Entomology*, **13**, 531–538.
- Core Team, R. (2021) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.
- Craig, T.P., Itami, J.K. & Price, P.W. (1989) A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly. *Ecology*, **70**, 1691–1699.
- Creel, S. & Christianson, D. (2008) Relationships between direct predation and risk effects. *Trends in Ecology and Evolution*, **23**, 194–201.
- Darsie, R.F. & Ward, R.A. (2005) *Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico*. University Press of Florida, Gainesville, FL.
- Eitam, A. & Blaustein, L. (2004) Oviposition habitat selection by mosquitoes in response to predator (*Notonecta maculata*) density. *Physiological Entomology*, **29**, 188–191.
- Eitam, A., Blaustein, L. & Mangel, M. (2002) Effects of *Anisops sardea* (Hemiptera: Notonectidae) on oviposition habitat selection by mosquitoes and other dipterans and on community structure in artificial pools. *Hydrobiologia*, **485**, 183–189.
- Ellis, R.A. & Borden, J.H. (1970) Predation by *Notonecta undulata* (Heteroptera: Notonectidae) on larvae of the yellow-fever mosquito. *Annals of the Entomological Society of America*, **63**, 963–973.
- Eveland, L.L., Bohenek, J.R., Silberbush, A. & Resetarits, W.J. (2016) Detection of fish and newt kairomones by ovipositing mosquitoes. *Chemical Signatures in Vertebrates*, **13**, 247–259.
- Fawcett, M.H. (1984) Local and latitudinal variation in predation on an herbivorous marine snail. *Ecology*, **65**, 1214–1230.
- Ferrari, M.C.O., Wisenden, B.D. & Chivers, D.P. (2010) Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology*, **88**, 698–724.
- Gotthard, K., Margraf, N. & Rahier, M. (2004) Geographic variation in oviposition choice of a leaf beetle: the relationship between host plant ranking, specificity, and motivation. *Entomologia Experimentalis et Applicata*, **110**, 217–224.
- Gripenberg, S., Mayhew, P.J., Parnell, M. & Roslin, T. (2010) A meta-analysis of preference-performance relationships in phytophagous insects. *Ecology Letters*, **13**, 383–393.
- Harley, C.D.G. (2003) Species importance and context: Spatial and temporal variation in species interactions. *The Importance of Species: Perspectives on Expendability and Triage* (ed. by P. M. Kareiva and S. A. Levin), pp. 44–68. Princeton University Press, Princeton.
- Herrmann, D.L., Ko, A.E., Bhatt, S., Jannot, J.E. & Juliano, S.A. (2010) Geographic variation in size and oviposition depths of *Romalea microptera* (Orthoptera: Acrididae) is associated with different soil conditions. *Annals of the Entomological Society of America*, **103**, 227–235.
- Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.
- Huey, R.B., Gilchrist, G.W., Carlson, M.L., Berrigan, D. & Serra, L. (2000) Rapid evolution of a geographic cline in size in an introduced fly. *Science*, **287**, 308–309.
- James, A.C., Azevedo, R.B.R. & Partridge, L. (1997) Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. *Genetics*, **146**, 881–890.
- Jeanne, R.L. (1979) A latitudinal gradient in rates of ant predation. *Ecology*, **60**, 1211–1224.
- Johnston, I. A. & Bennett, A. F. (eds) (1996) *Animals and Temperature: Phenotypic and Evolutionary Adaptation*. Cambridge University Press, Cambridge, UK.
- Kiflawi, M., Blaustein, L. & Mangel, M. (2003) Oviposition habitat selection by the mosquito *Culiseta longiareolata* in response to risk of predation and conspecific larval density. *Ecological Entomology*, **28**, 168–173.
- Masaki, S. (1979) Climatic adaptation and species status in the lawn ground cricket III. Ovipositor length. *Oecologia*, **43**, 207–219.
- McGuffin, M.A., Baker, R.L. & Forbes, M.R. (2006) Detection and avoidance of fish predators by adult *Enallagma damselflies*. *Journal of Insect Behavior*, **19**, 77–91.
- Meiri, S., Dayan, T. & Simberloff, D. (2005) Biogeographical patterns in the Western Palearctic: the fasting-endurance hypothesis and the status of Murphy's rule. *Journal of Biogeography*, **32**, 369–375.
- Mousseau, T.A. & Roff, D.A. (1989) Adaptation to seasonality in a cricket: patterns of phenotypic and genotypic variation in body size and diapause expression along a cline in season length. *Evolution*, **43**, 1483–1496.
- Peacor, S.D. & Werner, E.E. (2001) The contribution of trait-mediated indirect effects to the net effects of a predator. *Proceedings of the National Academy of Sciences*, **98**, 3904–3908.
- Peckarsky, B.L., Abrams, P.A., Bolnick, D.I., Dill, L.M., Grabowski, J.H., Luttbegg, B. *et al.* (2008) Revisiting the classics: considering nonconsumptive effects in textbook examples of predator-prey interactions. *Ecology*, **89**, 2416–2425.
- Pennings, S.C. & Silliman, B.R. (2005) Linking biogeography and community ecology: latitudinal variation in plant-herbivore interaction strength. *Ecology*, **86**, 2310–2319.
- Petranka, J.W. & Fakhoury, K. (1991) Evidence of a chemically-mediated avoidance response of ovipositing insects to blue-gills and green frog tadpoles. *Copeia*, **1991**, 234–239.
- Pintar, M.R. & Resetarits, W.J. (2020) Aquatic beetles influence colonization of disparate taxa in small lentic systems. *Ecology and Evolution*, **10**, 12170–12182.
- Pintar, M.R. & Resetarits, W.J. (2021) Match and mismatch: integrating consumptive effects of predators, prey traits, and habitat selection in colonizing aquatic insects. *Ecology and Evolution*, **11**, 1902–1917.
- Pintar, M.R., Bohenek, J.R., Eveland, L.L. & Resetarits, W.J. (2018) Colonization across gradients of risk and reward: nutrients and predators generate species-specific responses among aquatic insects. *Functional Ecology*, **32**, 1589–1598.
- Post, E. (2005) Large-scale spatial gradients in herbivore population dynamics. *Ecology*, **86**, 2320–2328.
- Preisser, E.L., Bolnick, D.I. & Benard, M.F. (2005) Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, **86**, 501–509.
- Rausher, M.D. (1983) Ecology of host-selection behavior in phytophagous insects. *Variable Plants and Herbivores in Natural and Managed Systems* (ed. by R. F. Denno and M. S. McClure), pp. 223–257. Academic Press, New York.
- Relyea, R.A. (2001) The relationship between predation risk and antipredator responses in larval anurans. *Ecology*, **82**, 541–554.
- Relyea, R.A. (2002a) Costs of phenotypic plasticity. *The American Naturalist*, **159**, 272–282.
- Relyea, R.A. (2002b) Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. *Ecological Monographs*, **72**, 523–540.
- Resetarits, W.J., Pintar, M.R., Bohenek, J.R. & Breech, T.M. (2019) Patch size as a niche dimension: aquatic insects behaviorally partition enemy-free space across gradients of patch size. *The American Naturalist*, **194**, 776–793.
- Rieger, J.F., Binckley, C.A. & Resetarits, W.J. (2004) Larval performance and oviposition site preference along a predation gradient. *Ecology*, **85**, 2094–2099.
- Saward-Arav, D., Sadeh, A., Mangel, M., Templeton, A.R. & Blaustein, L. (2016) Oviposition responses of two mosquito species to pool size and predator presence: varying trade-offs between desiccation and predation risks. *Israel Journal of Ecology and Evolution*, **62**, 1–6.

- Schoeppner, N.M. & Relyea, R.A. (2005) Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. *Ecology Letters*, **8**, 505–512.
- Schoeppner, N.M. & Relyea, R.A. (2009) When should prey respond to consumed heterospecifics? Testing hypotheses of perceived risk. *Copeia*, **2009**, 190–194.
- Segev, O., Verster, R. & Weldon, C. (2016) Testing the link between perceived and actual risk of predation: mosquito oviposition site selection and egg predation by native and introduced fish. *Journal of Applied Ecology*, **54**, 854–861.
- Semlitsch, R.D. & Boone, M.D. (2010) Aquatic mesocosms. *Amphibian Ecology and Conservation: A Handbook of Techniques* (ed. by C. K. Dodd), pp. 87–104. Oxford University Press, Oxford.
- Silberbush, A. & Resetarits, W.J. (2017) Mosquito female response to the presence of larvivorous fish does not match threat to larvae. *Ecological Entomology*, **42**, 595–600.
- Silberbush, A., Markman, S., Lewinsohn, E., Bar, E., Cohen, J.E. & Blaustein, L. (2010) Predator-released hydrocarbons repel oviposition by a mosquito. *Ecology Letters*, **13**, 1129–1138.
- Stachowicz, J.J. & Hay, M.E. (2000) Geographic variation in camouflage specialization by a decorator crab. *The American Naturalist*, **156**, 59–71.
- Stav, G., Blaustein, L. & Margalith, Y. (2000) Influence of nymphal *Anax imperator* (Odonata: Aeshnidae) on oviposition by the mosquito *Culiseta longiareolata* (Diptera: Culicidae) and community structure in temporary pools. *Journal of Vector Ecology*, **25**, 190–202.
- Streams, F.A. (1987) Foraging behaviour in a notonectid assemblage. *American Midland Naturalist*, **117**, 353–361.
- Thompson, J.N. (1988) Evolutionary ecology of the relationship between oviposition preference and performance of off spring in phytophagous insects. *Entomologia Experimentalis et Applicata*, **47**, 3–14.
- Tietze, N.S. & Mulla, M.S. (1991) Biological control of *Culex* mosquitoes (Diptera: Culicidae) by the tadpole shrimp, *Triops longicaudatus* (Notostraca: Triopsidae). *Journal of Medical Entomology*, **28**, 24–31.
- Torres-Estrada, J.L., Rodríguez, M.H., Cruz-López, L. & Arredondo-Jimenez, J.I. (2009) Selective Oviposition by *Aedes aegypti* (Diptera: Culicidae) in response to *Mesocyclops longisetus* (Copepoda: Cyclopoidea) under laboratory and field conditions. *Journal of Medical Entomology*, **38**, 188–192.
- Travis, J. (1996) The significance of geographical variation in species interactions. *American Naturalist*, **148**, 1–8.
- Vonesh, J.R., Kraus, J.M., Rosenberg, J.S. & Chase, J.M. (2009) Predator effects on aquatic community assembly: disentangling the roles of habitat selection and post-colonization processes. *Oikos*, **118**, 1219–1229.
- Warburg, A., Faiman, R., Shtern, A., Silberbush, A., Markman, S., Cohen, J.E. et al. (2011) Oviposition habitat selection by *Anopheles gambiae* in response to chemical cues by *Notonecta maculata*. *Journal of Vector Ecology*, **36**, 421–425.
- Winnie, J. & Creel, S. (2007) Sex-specific behavioural responses of elk to spatial and temporal variation in the threat of wolf predation. *Animal Behaviour*, **73**, 215–225.

Accepted 28 May 2021

First published online 15 June 2021

Associate Editor: Dirk Mikolajewski