

Complex Effects of Superior Competitors and Resources on *Culex restuans* (Diptera: Culicidae) Oviposition

Marta G. Grech^{1,3} and Steven A. Juliano²

¹Centro de Investigación Esquel de Montaña y Estepa Patagónica (CIEMEP), CONICET and UNPSJB, Facultad de Ciencias Naturales, Sede Esquel, Roca 780 (9200), Esquel, Chubut, Argentina, ²School of Biological Sciences, Illinois State University, Normal, IL 61790-4120, and ³Corresponding author, e-mail: mgrech@comahue-conicet.gob.ar

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Abstract

A growing body of literature on mosquito oviposition behavior supports the hypothesis that females place eggs in habitats that provide best available opportunity for growth, development, and maturation of their offspring. We conducted a field experiment to evaluate *Culex* oviposition behavior in response to the interspecific competitor *Aedes triseriatus* (Say) (Diptera: Culicidae) larvae, and resources in the form of quantity of plant detritus, and dissolved nitrogen (TN) and phosphorus (TP) derived from that detritus. We tested a set of specific predictions: 1) As a poorer competitor, *Culex* will avoid ovipositing in containers with superior interspecific competitors; 2) *Culex* choose oviposition habitats that contain greater amount of resources for the microbial food of their offspring; 3) Sufficiently high resource abundance can override avoidance of oviposition in containers with interspecific competitors. *Culex restuans* Theobald (Diptera: Culicidae) was the only species ovipositing, and the oviposition responses changed over time. The effect of resources was more important in driving oviposition decisions at the beginning and end of the experiment. The amount of resources, as manifest by TN and TP concentrations, had differential effects on oviposition. At the beginning females laid more eggs in containers with low detritus, which had the highest TN. After that, females preferred those containers with high detritus, which had low TN and high TP. The effect of competitors was important only during the middle of the experiment. Paradoxically, even as a poorer competitor *Cx. restuans* preferentially oviposited in containers with late-instar *Ae. triseriatus*, suggesting that the presence of successful heterospecifics indicates a good quality larval habitat.

Key words: oviposition behavior, interspecific competitors, resources

Oviposition behavior is an important aspect of the life history of mosquitoes. Oviposition site location and selection decisions by female mosquitoes are critical for individual reproductive success and maintenance of mosquito populations, and may have important implications for vector abundance, distribution and dynamics (Blaustein 1999, Spencer et al. 2002, Kershenbaum et al. 2012). Mosquito species exhibit different and complex oviposition strategies. Females of some genera deposit individual eggs (e.g., genera *Anopheles* and *Toxorhynchites*) or eggs rafts (e.g., genus *Culex*) on the water surface. Others genera like *Aedes*, *Ochlerotatus*, and *Psorophora* lay eggs on a substrate above the water line, or attached to the vegetation below water surface (e.g., genus *Mansonia*) (Clements 1999). The entire range of available habitats where mosquitoes may oviposit, and ultimately where larvae must succeed in completing development, includes natural and artificial containers (e.g., phytotelmata, rock pools, and human-made containers) and bodies of fresh water (e.g., ponds and wetlands) (Washburn 1995,

Juliano 2009). All these habitats differ widely in their suitability in terms of food resources, presence of predators and competitors, and physical and chemical characteristics (Washburn 1995, Forattini 2002).

Gravid female choice of appropriate oviposition sites usually involves visual, tactile and olfactory cues associated with biotic and abiotic factors (Bentley and Day 1989). Mosquito females may be sensitive to a variety of cues about aquatic habitat quality like color, substrate texture, and temperature (Clements 1963), and also chemicals of different origins (e.g., from larvae, pupae, eggs, bacteria, wood, and plant infusions) that act as attractants and repellents (Bentley and Day 1989).

Our knowledge of mosquito oviposition is largely derived from work on species of container-inhabiting *Aedes*, whose egg-laying behavior differ from *Culex* species (Clements 1999). Observational and experimental studies show that gravid females in the genera *Culex*, *Culiseta*, and *Anopheles*, which deposit their eggs on the

water surface, avoid sites with predators (e.g., backswimmers, tadpole shrimp, toad tadpoles, dragonfly nymphs, and fishes) (Chesson 1984, Petranka and Fakhoury 1991, Blaustein 1999, Stav et al. 1999, Vonesh and Blaustein 2010). The presence of conspecific immature stages also affects oviposition decisions. The presence of eggs or larvae could indicate the suitability of a potential oviposition site (e.g., habitat permanence and stability) but could also indicate future competition (Edgerly et al. 1998, Wasserberg et al. 2014). Many studies have demonstrated the effect of conspecifics on *Culex* oviposition, but results have been inconsistent, with some positive effects of conspecific larvae, eggs, or water that previously held larvae and eggs for *Culex quinquefasciatus* (Say; Diptera: Culicidae) (Dadd and Kleinjan 1974, Laurence and Pickett 1985, Mokani and Shine 2003, Barbosa et al. 2007, Wachira et al. 2010) and *Culex tarsalis* Coquillett (Diptera: Culicidae) (Osgood 1971). Other studies showed negative effects of conspecifics for *Cx. tarsalis* and *Cx. quinquefasciatus* (Reisen and Meyer 1990), and *Culex restuans* Theobald (Diptera: Culicidae) (Reiskind and Wilson 2004), or mixed results for *Culex annulirostris* Skuse (Diptera: Culicidae) and *Culex molestus* Forskal (Diptera: Culicidae) (Dhileepan 1997), and *Culex quinquefasciatus* Say (= *Culex pipiens fatigans* Wiedemann [Diptera: Culicidae]) (Bruno and Laurence 1979).

Culex (Reiskind and Wilson 2004, Reiskind et al. 2004, Chaves et al. 2009, Nguyen et al. 2012, Allgood and Yee 2017) and *Culiseta* females (Blaustein and Kotler 1993) prefer nutrient enriched habitats for oviposition, and *Culex* larvae can survive in eutrophic habitats containing high amounts of organic material (Vinogradova 2000). Experimental studies indicated that the addition of dog food to the water to make the habitat nutrient rich increases some fitness components of *Culex* (e.g., survival of larvae, the proportion of larvae that pupate, the size of pupae and emerging adults, and the sex ratio) (Reiskind et al. 2004, Chavez et al. 2011). However, it seems that the type and amount of specific nutrients available in aquatic habitats (e.g., NO₃, NH₄ and PO₄ concentrations), are also important, having differential effects on immature development (Noori et al. 2015).

Although several studies examined the effect of competitors, predators, and food amount in isolation (Wasserberg et al. 2013), relatively few studies have tested the interdependence of food or resource levels and the perception of predation risk by mosquito larvae (Fincke et al. 1997, Beketov and Liess 2007) and ovipositing females (Wasserberg et al. 2013). The combined effects of food resources and interspecific competitors for oviposition decisions remains virtually unstudied. In this study, we test in the field whether *Cx. restuans* select oviposition habitat in response to abundances of the interspecific competitor *Aedes triseriatus* (Say; Diptera: Culicidae) and plant detritus as a nutrient source for the microorganisms that are the principal food of larvae (Merritt et al. 1992). *Cx. restuans* is a native species that is an enzootic vector of West Nile virus among birds in North America, and may also act as a bridge vector between birds and mammals (Nasci et al. 2001, Apperson et al. 2002). *Culex* and *Aedes* species commonly use water holding natural and human-made container habitats, and they can coexist in urban environments (Leisnham et al. 2014). Many *Culex* use a wider range of aquatic habitats, including natural and artificial bodies of fresh water (Forattini 2002), compared to container-dwelling *Aedes*. Laboratory competition experiments have shown that *Culex* are typically inferior competitors for resources compared to invasive *Aedes albopictus* (Skuse) (Diptera: Culicidae), and probably *Aedes* in general (Carrieri et al. 2003, Costanzo et al. 2005, Murrell and Juliano 2012, Yee and Skiff 2014). Excluding *Aedes* oviposition in the field increases abundance and developmental success of *Culex* larvae in containers, suggesting that *Culex* are competitively suppressed by

Aedes. This pattern is consistent with the hypothesis of colonization-competition tradeoff between *Culex* and *Aedes* (Murrell and Juliano 2013). Although *Culex* are known to prefer nutrient enriched containers for oviposition, and to avoid ovipositing in habitats with conspecifics (Reiskind and Wilson 2004), and predators (Blaustein 1999), *Culex* oviposition responses to interspecific competitors is unstudied. Thus it is unknown whether *Aedes* larvae influence *Culex* habitat choice at oviposition, and if *Culex* avoid habitats with superior interspecific competitors like *Aedes* (Murrell and Juliano 2013).

In this study, we test the general hypothesis that female *Culex* place eggs in containers that provide best available opportunity for growth, development, and maturation of their offspring. We conducted a field experiment to evaluate *Culex* oviposition behavior in response to interspecific competitors (using *Ae. triseriatus* larvae) and resources (in the form of plant detritus). Specifically, we generated a set of specific predictions about the effect of competitors, resources, and their interaction on *Culex* oviposition:

- 1) Because *Culex* larvae are poor competitors, *Culex* females will avoid ovipositing in containers with superior interspecific competitors, and this avoidance will increase as the number of interspecific competitors increases from none to low to high. Thus, *Culex* egg rafts deposited will decrease as number of *Aedes* larvae increases.
- 2) *Culex* females choose oviposition habitats that contain greater amount of resources that fuel microbial growth that is the food of their offspring. Thus, more *Culex* egg rafts will be deposited in containers with greater detritus resources.
- 3) Sufficiently high resource abundance can override the avoidance of oviposition in containers with interspecific competitors. The effects of abundant resources are thus postulated to be more important than competitors in driving females' oviposition decisions. This predicts a significant interaction of competitors and resources on oviposition.

Materials and Methods

Study Area

The study site was located at ParkLands Foundation's Merwin Nature Preserve, Lexington, Illinois (40° 39' 10" N, 88° 52' 21" W), in a heavily wooded, largely undisturbed area. This preserve is an upland deciduous forest dominated by white oak (*Quercus alba*) and shagbark hickory (*Carya ovata*), and the surrounding area is agricultural. Deer, squirrels, and multiple species of birds are residents of these woods.

Field Experiment

To evaluate *Culex* oviposition behavior in response to resources and competitors we conducted a field experiment. Eighteen 7.6 liter white buckets were divided into six treatments of three replicates each. Each treatment was defined by combinations of two amounts of plant detritus as a source of nutrients that fuel microbial growth, providing food for developing mosquito larvae (timothy hay [*Phleum pratense*, Oxbow Animal Health]: low = 1.4 g/liter; high = 7 g/liter), and one of three abundances of newly hatched (~24 h old) *Ae. triseriatus* larvae (no larvae; low density = 200 larvae; and high density = 400 larvae). A 4 cm diameter overflow hole was drilled into the side of the container 5 cm from the top and covered with 0.5 mm nylon mesh to prevent filling beyond the desired volume of 5 liters due to natural inputs of rain, and also to limit water level rise, and thus to limit flooding-induced hatching of *Aedes* eggs that may be

laid on container walls above the water line. Buckets were placed in a 6 × 3 array, at least 5 m apart, under closed canopy.

On 6 July 2015, each bucket was filled with 5 liters rain water collected from rain barrels placed adjacent to trees at Merwin Preserve, and detritus was added to each bucket. Hay was placed in nylon mesh (0.25 mm) bags tied with nylon cords, and weighted with black gravel housed in small plastic case, to keep bags submerged. Buckets were covered with a mesh to exclude ovipositing mosquitoes and left in the field 2 d to allow bacterial growth. On 8 July (day 0 of the experiment), we uncovered each bucket and added 24-h old *Ae. triseriatus* larvae in the appropriate numbers for each treatment. *Ae. triseriatus* used in this experiment were from a colony originally collected from Tyson Research Center, Eureka, Missouri, and maintained in the laboratory for about 1 yr. They likely represented several overlapping generations. The day before the experiment, eggs from this colony were synchronously hatched in 0.2 g/liter nutrient broth solution. The newly hatched larvae were rinsed to remove nutrient solution, and then separated into aliquots of 200 and 400 larvae and transferred to the field.

From 9 July 2015 to 19 July 2015 each bucket was checked daily for *Culex* egg rafts. Rafts were counted, removed, and transferred to the laboratory in 24-well tissue culture plates with one raft in each well. From each raft, 15 hatched larvae were reared (14:10 [L:D] h, 25°C, 80% relative humidity) to fourth instar for identification, with each larva housed in a 4-dram glass vial containing 0.3 g/liter liver powder and reverse osmosis (RO) water. When pupae began to appear in those buckets that initially had *Ae. triseriatus* larvae, the experiment ended. During the entire experiment, buckets received natural inputs of rain, and volumes were roughly stable at the bottom of the overflow holes.

At the beginning (day 0, 8 July), middle (day 6, 14 July) and end (day 11, 19 July) of the experiment, samples of water were removed (25 ml) from each bucket for measurements of total phosphorus (TP) and total nitrogen (TN). Samples were taken back to the laboratory and stored frozen (−20°C) until analyzed. We used traditional persulfate digestion followed by the Lachat Method for ammonium molybdate based assay method, as indicated by the QuickChem method—10-115-01-1-f to assay TP. We used alkaline persulfate digestion, followed by a boric acid addition, and then assay of TN as nitrate using the hydrazine reduction method. At each of these times (*i*), we also counted the number of *Aedes* individuals as pupae (P_i), young larvae (1st and 2nd instar combined = $L12_i$), and older larvae (3rd and 4th instar combined = $L34_i$). Larvae that died were left in containers, contributing to the detritus available.

Statistical Analysis

We report analyses of both effects of initial manipulations of plant detritus and *Ae. triseriatus* larvae, and effects of measures of dissolved N and P and number and stage of surviving *Ae. triseriatus*. Both of these analyses are informative, but because dissolved nutrients and surviving *Ae. triseriatus* larvae and their size and stage changed over the course of the experiment, we emphasize analyses of effects of measured dissolved nutrients and numbers and stages of surviving larvae at three different periods, rather than initial detritus amounts and initial number of larvae.

We analyzed effects of resources and competitors on *Culex* oviposition behavior using generalized linear mixed-effects models (GLMM) with a Poisson family distribution and log link function, employing an information theoretic approach for model comparison (Burnham and Anderson 2002). First, we divided the original

data set (total of 11 d) into three periods: Period 1: days 0 to 4 (8–12 July); Period 2: days 5 to 8 (13–16 July); and Period 3: days 9–11 (17–19 July) that corresponded to the times at which we took TP and TN measurements, and counted *Ae. triseriatus*. We tested models for each period that included the number of surviving immature *Aedes* of each age/stage class ($L12_i$, $L34_i$, P_i), and TP and TN concentrations as fixed effects, and the random effect of bucket. The summations of egg rafts across days within each time period ($i = 1, 2, 3$) were the response variables in three separate linear model analyses.

Models were evaluated with information theoretic procedures allowing multiple model comparisons to be made, and the most parsimonious of these models to be identified (Burnham and Anderson 2002). We tested three models that included abundances of immature *Aedes* in stages with non-zero abundance at the time of the count: model I) number of larvae $L12_i$, TP_i , and TN_i concentrations for Period 1; model II) number of larvae $L12_i$ and $L34_i$, TP_i , and TN_i concentrations for Period 2; model III) number of larvae $L34_i$ and pupae P_i , TP_i , and TN_i concentrations for Period 3. Models with all possible combinations of predictor variables were considered, and this resulted in 7 and 15 *a priori* candidate models for time 1, and time 2 and 3, respectively. Akaike's information criterion (AICc) with a correction for small sample sizes was calculated for each model. We initially tested models with all interactions of the fixed effects, and these models always yielded a poorer AICc than reduced models with only main effects. Model comparisons were made with $\Delta AICc$, which is the difference between the AICc for model *i* and the best model (i.e., with the lowest AICc value). Models with $\Delta AICc \leq 2$ have some substantial support from the data (Burnham and Anderson 2002). The AICc weight of a model (w_i) is a measure of the weight of evidence that model *i* is the best model in the set of all models considered. To evaluate the relative importance of predictor variables, AICc model weights were summed across all models that contained the parameter being considered. Predictor variables with good support have high parameter likelihood values (near 1) (Burnham and Anderson 2002). Finally, model-averaging was used to calculate parameter estimates and standard errors, based on AICc weights (Anderson 2008). To supplement parameter likelihood evidence of important effects, we also calculated 95% confidence intervals limits (CL) of parameter estimates. Statistical analyses were carried out using R software, Version 3.2.4, lme4 (Bates et al. 2015), and MuMIn (Bartoń 2016) packages (R Core Team 2016).

The effects of treatments (six combinations of resources [low = 1.4 g/liter; high = 7 g/liter of plant detritus] and competitors [without *Ae. triseriatus* larvae; low density: 200 larvae; and high density: 400 larvae]) on *Culex* oviposition were analyzed using Generalized linear models (GLM) with a Poisson family distribution and log link function. Models were run for each Period (1, 2, 3), and the summation of eggs rafts for each period were the response variables in three separate linear model analyses (R software, Version 3.2.4).

GLM with a Gamma family distribution and log link function was used to test the effects of resources (low and high plant detritus), competitors (without larvae; 200 and 400 larvae), and their interactions on TP and TN measurements. Models were run for each Period, and post-hoc tests were performed to evaluate significant differences among group means (R software, Version 3.2.4, agricolae package; de Mendiburu 2015). Also, the effect of treatments (four combinations of resources [low and high plant detritus] and competitors [200 and 400 larvae]) on proportion of surviving *Ae. triseriatus* at the

end of the experiment (Period 3) was analyzed, using GLM with a Binomial family distribution and logit link function (R software, Version 3.2.4).

Results

Nutrients Levels and Survival of *Ae. triseriatus*

In general, TN concentration was greater in low plant detritus treatments during the whole experiment (Fig. 1), whereas TP followed the opposite pattern, being greater in high plant detritus containers (Fig. 2). During Period 2, after *Ae. triseriatus* larvae addition, TN concentration increased in low-detritus containers (mean_{lowPD} = 5.25 mg/liter), regardless larval density, with the effect of resources significant (Fig. 1; Table 1). Then during Period 3, a general decrease in TN content was observed, with the highest values in containers with low resources and presence of *Ae. triseriatus* larvae, compared with those without competitors, and those of high resources (Fig. 1; Table 1). High-detritus containers had similar low TN concentrations across treatments for both Periods, 2 and 3

(Fig. 1; Table 1). Although, greatest TP concentrations occurred during Period 2 for high-detritus containers with *Ae. triseriatus* larvae, compared with those containers without larvae (Fig. 2), the effect of competitors was not significant (Fig. 2; Table 1). Only the main effect of resources was significant for TP concentrations during the whole experiment. Low-detritus containers always had mean TP concentrations <3000 µg/liter, across treatments (Fig. 2; Table 1).

Overall, of 3,600 *Ae. triseriatus* larvae initially added, 1,573 survived through 11 days of the experiment. During Period 2, the majority of larvae were in the 3rd and 4th instar, and a few of them still remain in the 1st and 2nd instar (Table 2). In Period 3 at the end of the experiment, about 2/3 of surviving individuals were larvae in L34 age class, and 1/3 were pupae (Table 2). Only the main effect of resources was significant for proportion of surviving *Ae. triseriatus* at the end of the experiment (Table 3). Mean survival was greatest in lowPD_L400 treatment across time, followed by lowPD_L200 treatment (Fig. 3). High-detritus containers yielded lower survival values, averaging only ~25% of *Ae. triseriatus* larvae surviving in those treatments (Fig. 3).

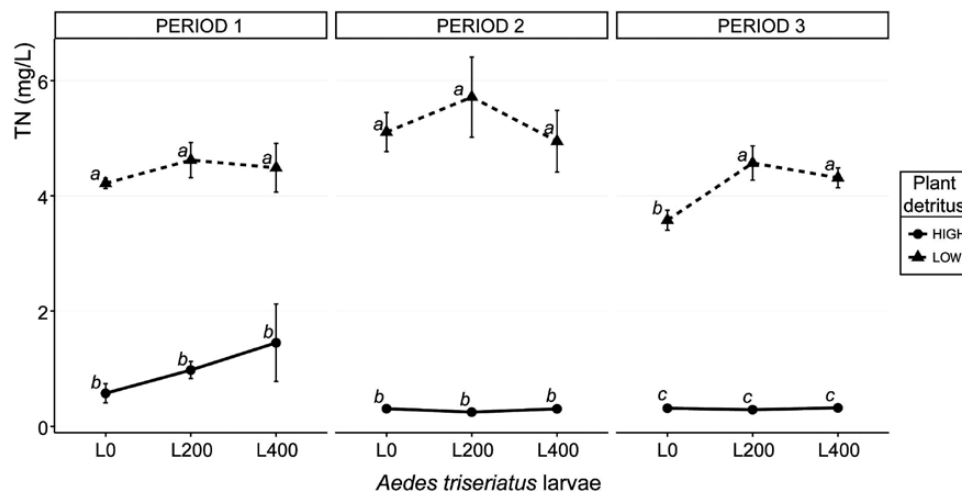


Fig. 1. Mean (\pm SE) TN measurements (mg/liter) at three periods for combinations of detritus resources (low: 1.4 g/liter; high: 7 g/liter plant detritus) and competitors (L0: without *Aedes triseriatus* larvae; L200: 200 larvae; L400: 400 larvae). Period 1: measurements taken pre- *Ae. triseriatus* larvae addition; Periods 2 and 3: measurements taken post-larvae addition. Different letters indicate significant differences among means.

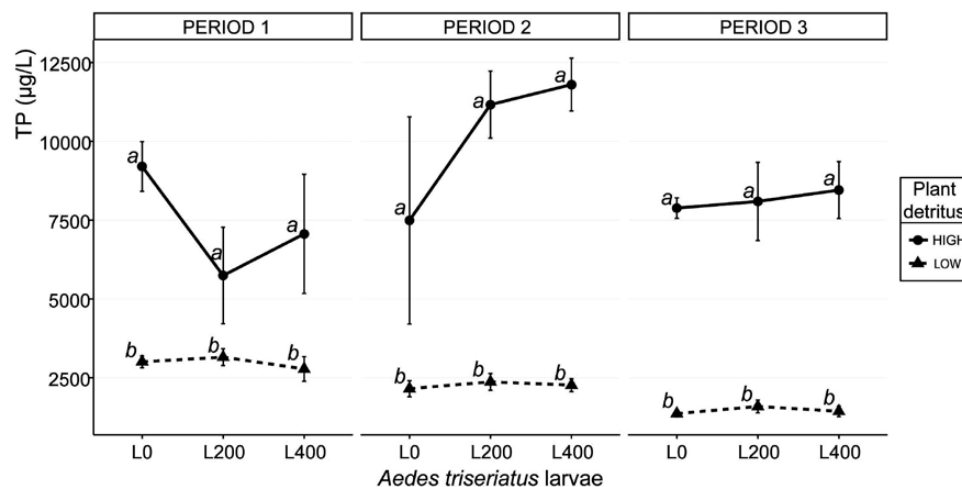


Fig. 2. Mean (\pm SE) TP measurements (µg/liter) at three periods for combinations of detritus resources (low: 1.4 g/liter; high: 7 g/liter plant detritus) and competitors (L0: without *Aedes triseriatus* larvae; L200: 200 larvae; L400: 400 larvae). Period 1: measurements taken pre- *Ae. triseriatus* larvae addition; Periods 2 and 3: measurements taken post-larvae addition. Different letters indicate significant differences among means.

Table 1. Generalized linear models results for the effect of treatments (six combinations of resources [low and high plant detritus] and competitors [0, 200 and 400 larvae]) on TN and TP measurements at three periods

Period	Factor	TN			TP		
		df	F value	P value	df	F value	P value
1	Resources (R)	1	139.9	<0.0001	1	23.1	0.0002
	Error	16			16		
2	Resources (R)	1	250.6	<0.0001	1	43.7	<0.0001
	Competitors (C)	2	0.5	0.6375	2	1.4	0.2927
	R*C	2	0.6	0.5413	2	1.2	0.3424
	Error	12			12		
3	Resources (R)	1	885.9	<0.0001	1	158.3	<0.0001
	Competitors (C)	2	5.1	0.0244	2	0.1	0.8814
	R*C	2	5.5	0.0203	2	0.1	0.9051
	Error	12			12		

Water samples for Period 1 taken before addition of *Aedes triseriatus*; samples for Periods 2 and 3 taken after addition of *Ae. triseriatus*. Significant effects are shown in bold.

Table 2. Total number of *Aedes triseriatus* individuals of each stage across 3 Periods, during July 2015

Stage	Period		
	1	2	3
L12	3,600	14	—
L34	—	1,801	1,023
P	—	—	560
Total individuals	3,600	1,815	1,573

L12: young larvae; L34: old larvae; P: pupa.

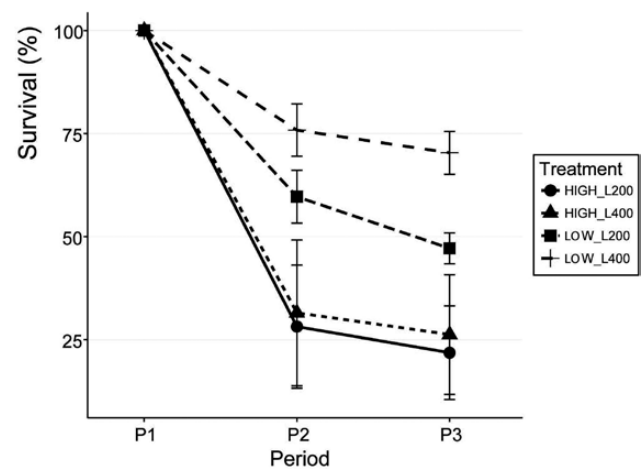
Table 3. Generalized linear model result for the effect of treatments (four combinations of resources [low and high plant detritus] and competitors [200 and 400 larvae]) on proportion of surviving *Aedes triseriatus* at Period 3 (17–19 July)

Factor	Survival		
	df	F value	P value
Resources (R)	1	12.7	0.0074
Competitors (C)	1	1.9	0.1951
R*C	1	0.9	0.3646
Error	8		

Significant effects are shown in bold.

Cx. restuans Egg Rafts

A total of 643 egg rafts were collected during the experiment, and *Cx. restuans* was the only species ovipositing. The mean number of collected egg rafts (mean over all buckets during the period) increased markedly from Period 1 (overall mean \pm SE: 4.9 ± 5.9 rafts) to Period 2 (21.2 ± 16.4 rafts), and then decreased during Period 3 (9.6 ± 9 rafts; Fig. 4). During Period 1, only resource significantly affected the mean number of *Cx. restuans* egg rafts (Table 4) which was greater in treatments with low detritus, with a maximum lowPD_L200 (Fig. 4). During Period 2 none of the manipulated variables significantly affected number of egg rafts (Table 4). There was a tendency for greater *Cx. restuans* oviposition in containers with high detritus, with the exception of treatment lowPD_L400, which was similar to high-detritus treatments (Fig. 4). Greatest number of rafts were laid in highPD_L200 and highPD_L400 treatments (Fig. 4). During

**Fig. 3.** Survival (%) of *Aedes triseriatus* larvae (mean \pm SE) at three periods as function of treatments (four combinations of resources [low: 1.4 g/liter; high: 7 g/liter plant detritus] and competitors [L200: 200 larvae; L400: 400 larvae]).

Period 3, the patterns were similar to those in Period 2 (Fig. 4), and the effect of resource was significant (Table 4). Mean number of egg rafts was greater in high-detritus treatments (Fig. 4). Over the entire experiment, females laid fewest egg rafts (overall mean = 5.8) in containers with low detritus with no larvae of *Ae. triseriatus* (Fig. 4).

Models for *Cx. restuans* Oviposition

The best model of *Cx. restuans* oviposition differed among the three Periods. For Period 1 the best model included only TN₁ concentration (Table 5). In contrast, for Period 2, the best model included both the number of larvae L34₂ of *Ae. triseriatus* and TN₂ concentration (Table 5). For Period 3 there were two models with substantial support (Δ AICc ≤ 2). The best model included only TP₃, and the next best included only TN₃ (Table 5). Model-averaged parameter estimates and confidence intervals indicated little support for an effect of number of larvae of *Ae. triseriatus* at the beginning and at the end of the experiment (Table 6). Only for Period 2 did the number of larvae L34₂ have a strong and positive effect on the summation of *Cx. restuans* egg rafts during that period (Table 6). In contrast, model parameters for nutrient concentrations showed a different pattern, yielding an important positive for TN₁ during Period 1, and

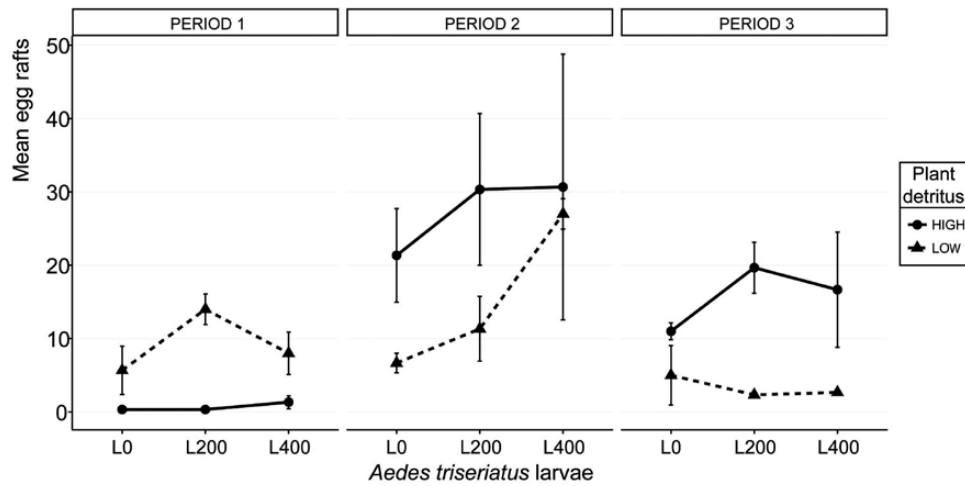


Fig. 4. Mean (\pm SE) number of *Culex restuans* egg rafts at three periods for combinations of detritus resources (low: 1.4 g/liter; high: 7 g/liter plant detritus) and competitors (L0: without *Aedes triseriatus* larvae; L200: 200 larvae; L400: 400 larvae).

Table 4. Generalized linear models results for the effect of treatments (six combinations of resources [low and high plant detritus] and competitors [0, 200 and 400 larvae]) on *Culex restuans* oviposition activity for each period considered

Period	Factor	<i>Cx. restuans</i> egg rafts		
		df	F value	P value
1	Resources (R)	1	26.9	0.0002
	Competitors (C)	2	2.2	0.1581
	R*C	2	2.5	0.1273
	Error	12		
2	Resources (R)	1	2.8	0.1214
	Competitors (C)	2	1.3	0.3036
	R*C	2	0.4	0.6959
	Error	12		
3	Resources (R)	1	15.2	0.0021
	Competitors (C)	2	0.3	0.7495
	R*C	2	1.1	0.3615
	Error	12		

Significant effects are shown in bold.

important negative effect for TN_{12} in Period 2, and an important positive effect of TP_{13} in Period 3 (Table 6).

Discussion

The results of our field study showed that the *Cx. restuans* oviposition responses change with changing conditions in these containers. Further, the effect of resources was more important in driving females' decisions than the effect of interspecific competitors at the beginning and at the end of the experiment, and the effect of competitors was important only for Period 2, when most *Ae. triseriatus* were late instar larvae and thus large and active. Finally, the effect of resources was complex; the amount of plant detritus added affected both total N and total P, but not in the same way, and oviposition responses to dissolved nutrients changed over time. Dissolved N was attractive early in the experiment, but dissolved P was attractive late in the experiment. These results do not support our prediction about the effect of interspecific competitors, and suggest that even as a poorer competitor *Cx. restuans* does not avoid ovipositing in containers with *Ae. triseriatus*. In fact, a strong positive effect

of older *Ae. triseriatus* larvae on the number of egg rafts laid by *Cx. restuans* was observed in the middle of the experiment. The resource prediction is supported by some but not all the data. We predicted that *Cx. restuans* would prefer oviposition habitats containing greater amounts of resources. The amount of resources, as quantified by TN and TP concentrations, had differential effects on oviposition behavior. At the beginning of the experiment females laid more eggs in containers with low detritus, which were those with highest TN content. Then, from Period 2 until the end of the experiment, *Cx. restuans* females preferred those containers with high detritus, which had low TN and high TP concentrations. Thus, *Cx. restuans* does favor oviposition in containers with greater dissolved nutrients, but because the two main dissolved nutrients that we measured covary, and because the response appears to favor different nutrients in different circumstances, they do not always oviposit preferentially in containers with the greatest amount of plant detritus.

The oviposition behavior of *Cx. restuans* in response to conspecific competitors and resources was studied previously in a set of experiments under field conditions (Reiskind and Wilson 2004). These authors observed a pattern that differed from ours in response to cues of future competition, where female *Cx. restuans* preferred habitats without conspecific larvae over habitats with conspecific competitors. However, in the present study *Cx. restuans* display a different strategy, and do not avoid habitats with interspecific competitors. A positive response of *Culex* oviposition was only observed when *Ae. triseriatus* larvae were mostly in the L34 age class; young competitor larvae had no effect on *Culex* oviposition habitat choice. Moreover, those containers with low detritus in which larvae of competitors were absent were the least preferred. This result is consistent for other *Culex* species, such as *Cx. annulirostris*, which showed greater oviposition preference for sites with 4th instar conspecifics rather than sites containing younger conspecifics (Dhileepan 1997). These authors also suggest that chemicals of larval origin or visual stimuli could be involved in *Cx. annulirostris* oviposition site selection. Their results and ours suggest that abundant late stage mosquito larvae may be a cue to good sites for larval development. The preference for oviposition in sites with abundant late stage larvae, which may be competitors for offspring, may seem a paradox; however later stage larvae are closer to pupation, when they will no longer compete for resources with younger larvae that hatch from deposited eggs.

Table 5. Generalized linear models explaining variation in *Culex restuans* oviposition activity (all groups, by period)

Period	Models	Explanatory variables	K	AICc	ΔAICc	W _i
1	1	TN _{t1}	2	80.52	0	0.67
	2	TN _{t1} , TP _{t1}	3	83.31	2.78	0.17
	3	L12 _{t1} , TN _{t1}	3	83.77	3.24	0.13
	4	TP _{t1}	2	95.24	14.72	0.00
	5	L12 _{t1} , TP _{t1}	3	98.41	17.88	0.00
	6	L12 _{t1}	2	104.37	23.84	0.00
2	Full	L12 _{t1} , TN _{t1} , TP _{t1}	4	87.09	6.57	0.03
	1	L34 _{t2} , TN _{t2}	3	141.89	0	0.51
	2	L34 _{t2} , TP _{t2}	3	144.86	2.97	0.12
	3	L12 _{t2} , L34 _{t2} , TN _{t2}	4	145.63	3.74	0.08
	4	L34 _{t2} , TN _{t2} , TP _{t2}	4	145.79	3.9	0.07
	5	L34 _{t2}	2	146.52	4.62	0.05
	6	L12 _{t2} , L34 _{t2}	3	147.62	5.73	0.03
	7	L12 _{t2} , TN _{t2}	3	148.12	6.23	0.02
	8	TN _{t2}	2	148.45	6.56	0.02
	9	L12 _{t2} , L34 _{t2} , TP _{t2}	4	148.51	6.62	0.02
	10	TP _{t2}	2	148.56	6.67	0.02
	11	L12 _{t2} , TP _{t2}	3	149.07	7.18	0.01
	12	L12 _{t2}	2	150.33	8.44	0.01
	13	L12 _{t2} , TN _{t2} , TP _{t2}	4	151.57	9.68	0.00
14	TN _{t2} , TP _{t2}	3	151.60	9.71	0.00	
Full	L12 _{t2} , L34 _{t2} , TN _{t2} , TP _{t2}	5	150.27	8.38	0.008	
3	1	TP _{t3}	2	106.15	0	0.414
	2	TN _{t3}	2	107.87	1.72	0.175
	3	TN _{t3} , TP _{t3}	3	109.01	2.86	0.10
	4	L34 _{t3} , TP _{t3}	3	109.45	3.3	0.08
	5	TP _{t3} , P _{t3}	3	109.51	3.36	0.08
	6	L34 _{t3} , TN _{t3}	3	110.23	4.08	0.05
	7	TN _{t3} , P _{t3}	3	110.74	4.59	0.04
	8	L34 _{t3} , TN _{t3} , TP _{t3}	4	112.58	6.43	0.02
	9	L34 _{t3} , TP _{t3} , P _{t3}	4	112.64	6.49	0.02
	10	TN _{t3} , TP _{t3} , P _{t3}	4	112.84	6.69	0.01
	11	L34 _{t3} , TN _{t3} , P _{t3}	4	113.72	7.57	0.01
	12	L34 _{t3} , P _{t3}	3	121.11	14.96	0.00
	13	P _{t3}	2	124.71	18.56	0.00
	14	L34 _{t3}	2	127.04	20.89	0.00
Full	L34 _{t3} , P _{t3} , TN _{t3} , TP _{t3}	5	116.81	10.66	0.002	

Models with all possible combinations of predictor variables are provided in decreasing order of importance. k: total number of parameters in the model. AICc: Akaike's information criterion (corrected for small sample size). ΔAICc: difference in AICc between the best model and the model indicated. W_i: model weight. Explanatory variables: number of *Aedes triseriatus* young larvae (L12), old larvae (L34) and pupae (P), and TP and TN measurements.

A growing body of literature on mosquitoes indicates that because conspecifics are competitors, their presence in a habitat indicates a low quality oviposition site, and could result in negative density-dependent effects on progeny fitness (e.g., affects survival, growth, and development rates) (Kitron et al. 1989, Reisen and Meyer 1990, Kiflawi et al. 2003, Reiskind and Wilson 2004, Munga et al. 2006). On the other hand, the presence of conspecific, particularly later instar conspecifics, could indicate suitable habitats for oviposition because of low predation risk, high food availability for offspring, site permanence and stability, and appropriate physical and chemical conditions (Wilmot et al. 1987, Dhileepan 1997, Edgerly et al. 1998, Mokany and Shine 2003, Wong et al. 2011, Yoshioka et al. 2012, Wasserberg et al. 2014). Thus, ovipositing females should face a trade-off between the cost of future intraspecific competition and the risk of choosing low-quality habitats for their progeny. Some recent studies evaluating this trade-off between these two opposites forces suggest a density-dependent shift in the effect of conspecific larvae or eggs on oviposition response, with positive effects at low to intermediate densities and negative effects at intermediate- to high-conspecific densities (Wachira et al. 2010,

Wasserberg et al. 2014). The effect of heterospecifics on oviposition still remains poorly understood. A similar density dependent pattern was observed for *Anopheles gambiae* Giles (Diptera: Culicidae) and *Cx. quinquefasciatus* species, where female *An. gambiae* laid more eggs in sites containing low numbers of *Cx. quinquefasciatus* egg rafts, but avoid ovipositing in sites with high numbers of *Cx. quinquefasciatus* (Wachira et al. 2010). We observed a positive effect of older interspecific competitors on *Cx. restuans* oviposition during Period 2, however, this effect was similar for low- and high-competitor densities and differed from the treatment in which larvae of interspecific competitors were absent (Fig. 4).

Previous studies found that nutrient enriched habitats are preferred by gravid *Culex* females, and such habitats also increased some fitness components of larvae, pupae and adults (Reiskind and Wilson 2004, Reiskind et al. 2004, Chaves et al. 2009, 2011, Nguyen et al. 2012, Allgood and Yee 2017). However, it is important to evaluate these responses for both the amount of resources and the type of nutrient added to the rearing water (Noori et al. 2015). In our study, a pronounced shift in *Cx. restuans* egg laying behavior in response to plant detritus as a source of nutrients was observed,

Table 6. Parameter estimates (β) (\pm SE), parameter likelihoods, and explanatory variables with confidence intervals (CL) are shown (CL excluding zero are reported in bold font)

Period	Explanatory variables	$\beta \pm$ SE	Parameter likelihood	CL: lower	CL: upper
1	TN _{t1}	0.81 \pm 0.22	1	0.34	1.29
	TP _{t1}	0.0001 \pm 0.0003	0.19	-0.0005	0.0007
	L12 _{t1}	0.0004 \pm 0.001	0.16	-0.002	0.003
2	L34 _{t2}	0.004 \pm 0.001	0.88	0.001	0.007
	TN _{t2}	-0.16 \pm 0.062	0.72	-0.2	-0.03
	TP _{t2}	0.00004 \pm 0.0004	0.26	-0.00005	0.0002
3	L12 _{t2}	-0.02 \pm 0.2	0.18	-0.5	0.4
	TP _{t3}	0.0002 \pm 0.00006	0.72	0.00008	0.0004
	TN _{t3}	-0.3 \pm 0.2	0.41	-0.7	0.04
	L34 _{t3}	0.002 \pm 0.003	0.18	-0.005	0.009
	P _{t3}	-0.0008 \pm 0.007	0.16	-0.02	0.01

Explanatory variables: number of *Aedes triseriatus* young larvae (L12), old larvae (L34) and pupae (P), and TP and TN measurements.

from a preference for low nutrient containers at the beginning to preference for high nutrient containers from Period 2 until the end of the experiment. Moreover, those high-detritus containers yielded lower survival values for *Ae. triseriatus* (~25%) (Fig. 3), suggesting that this condition is sub-optimal for the interspecific competitors because of unfavorable physical and chemical conditions associated with high levels of resources. This result could indicate greater detritus creates suitable habitats for *Cx. restuans* oviposition, not only by providing food for developing larvae but also because these habitats may be sub-optimal for competitors.

Gravid *Culex* females are attracted to hay and grass infusions (Isoe et al. 1995, Reiskind and Wilson 2004, Allan et al. 2005) and the degree of attractiveness of infusions changes over time, in association with changes in bacterial metabolic activity (Maw 1970, Isoe et al. 1995, Ponnusamy et al. 2010). In container and tree-hole mosquito habitats, leaf material inputs are first incorporated into microbial biomass or modified by microbial activity (Kaufman et al. 2002). Microbial production in these habitats can be resource limited primarily by availability of labile organic carbon, and secondary by inorganic nutrients such as phosphorous or nitrogen (bottom-up nutrient effect) (Kaufman et al. 2002). Also the dynamics of nutrients (e.g., nitrite, nitrate, ammonium, phosphate) are complex and dependent on inputs of leaf and animal detritus, nutrient cycling process, and excretion by mosquito larvae, among others (Walker et al. 1991). In our study, values of TN and TP varied among treatments and times, and according to the best-fitting models these nutrients have differential attractive effects on *Cx. restuans* oviposition. During the whole experiment, TN and TP concentrations were negatively related across detritus treatments with greater TN in low-detritus containers and TP in high-detritus containers. More plant detritus as source of nutrients was translated into higher TP concentration in these aquatic systems, and those containers containing high amounts of TP were preferred for oviposition by females *Cx. restuans* during Period 3. Although TP values fluctuated over time, only the initial detritus resource amount used in this experiment had a significant effect on TP. TP was not influenced by the abundance of *Ae. triseriatus* larvae added to the containers (Table 1).

Our field study assessed the combined effects of plant detritus as source of resources, and *Ae. triseriatus* larvae as interspecific competitors on *Cx. restuans* oviposition behavior. The results showed that, in artificial container habitats, *Cx. restuans* oviposition pattern change over time with changes in chemical composition of the water, abundance and stage of larvae, and amount of detritus. Future studies should test for microbial activity effects on *Cx.*

restuans oviposition behavior in conjunction with competitor density and stage, and detritus amount. Weather may contribute to the temporal pattern of *Cx. restuans* oviposition activity. During periods 1, 2, and 3 precipitation at the nearest National Oceanic and Atmospheric Administration weather station (Lexington, IL) was 89, 0, and 15 mm, respectively, which is inversely related to the temporal pattern of oviposition (Fig. 4). Such temporal variation is unlikely to account for the effects of the treatments within each period. These additional variables could help us to understand how multiple effects can influence mosquito oviposition patterns. Despite the documented avoidance of oviposition by *Culex* in sites with biotic threats from predators, we find no evidence that *Cx. restuans* oviposition is inhibited by the presence of a superior interspecific competitor. Indeed, the significant attraction of *Cx. restuans* to containers with late stage *Ae. triseriatus* larvae is more consistent with the alternative—that presence of successful heterospecifics is indicative of a good-quality larval habitat. This suggests the hypothesis that the presence of nearly mature larvae of a competitor may be a cue to a habitat that will shortly reach a successional stage suitable for *Cx. restuans* larvae, after those *Aedes* larvae have pupated. Understanding the importance of oviposition decisions in multi-species communities will be potentially useful for developing models of mosquito population dynamics, and improving current mosquito control strategies.

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