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Seasonal Weather, Nutrients, and Conspecific Presence Impacts on the Southern House Mosquito Oviposition Dynamics in Combined Sewage Overflows

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ABSTRACT Combined sewage overflows have created favorable conditions for the establishment of the southern house mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae), larvae in natural creeks that would otherwise be unsuitable for the development of this mosquito species. Here, we show the results from a seminatural experiment carried over the three seasons of mosquito activity (spring, summer, and fall) in Tanyard Creek, Atlanta, GA. In this study we manipulated the amount of nutrients by further enriching combined sewage overflow water, and tracked weather variables, organic nutrient concentration, exposure time to conspecifics, and the number of egg rafts collected in experimental containers. We found season and nutrient enrichment to be the most important variables explaining the differences in egg rafts counts. Further analyses suggest that temperature may also play a role in seasonal oviposition patterns. The results from this study suggest that nutrient enrichment and adequate temperatures are important factors shaping *Cx. quinquefasciatus* oviposition seasonality in combined sewage overflows.

KEY WORDS *Culex quinquefasciatus*, Atlanta, habitat selection, linear mixed effects model, combined sewage overflow

The house mosquito, *Culex pipiens* sensu lato (Diptera: Culicidae), is a complex of species traditionally associated with degraded and polluted water bodies (Britton 1914, Wada and Ofuji 1962, Kumada et al. 1972), drainage systems (Scorza 1972, Rajagopalan et al. 1975, Munstermann and Craig 1977), and septic tanks (de Meillon et al. 1967a, Mackay et al. 2009, Burke et al. 2010). However, colonization of natural creeks has been reported for members of this species complex in urban areas, including the red house mosquito *Cx. pipiens pallens* Couquillett in Nagoya-shi (Kumada et al. 1972) and Saga-shi, Japan (Mogi and Okazawa 1990, Mogi et al. 1995, Mogi and Sota 1996) and the southern house mosquito *Culex quinquefasciatus* in Atlanta, GA (Calhoun et al. 2007). This is a situation of significant human and veterinary health concern given that many members of this complex are vectors of several arboviruses, such as West Nile virus (Turell et al. 2001, Richards et al. 2010), St. Louis Encephalitis virus (Monath and Tsai 1987, Richards et al. 2009); and also parasites, like filarial worms (Sasa 1965) and avian malaria *Plasmodium* spp. (Hegner 1929). Therefore, understanding the ecological changes allowing increased mosquito densities is critical to mitigate or eliminate the threat of mosquito-borne disease outbreaks.

The city of Atlanta, GA, uses a combined sewage and storm water system to treat and dispose wastewater. A combined sewer is a single pipe sewer system in which storm water runoff and domestic sewage are mixed and treated together. After heavy rainfall events, large volumes of untreated wastewater, rich in organic matter, exceed the maximum capacity of the combined sewer system (CSS) and discharge into urban streams in combined sewage overflows (CSO) (Calhoun et al. 2007, Chaves et al. 2009). In Atlanta, CSO streams are located in residential areas with high human population densities, creating both a pest problem and a potential risk of vector-borne disease by providing high-nutrient breeding grounds for *Cx. quinquefasciatus* that significantly increases their abundance (Calhoun et al. 2007). One of the reasons for the increased abundance of mosquitoes in these habitats could be the increased concentration of organic nutrients in the water that can enhance mosquito productivity (Mogi and Okazawa 1990) and oviposition habitat selection (Chaves et al. 2009). For example, ammonium [NH_4^+] is the most common form of environmental nitrogen in cultivated land and

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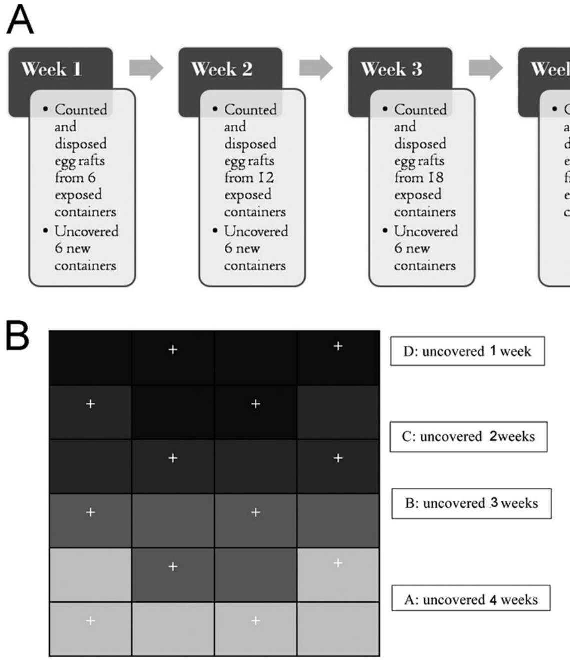

Fig. 1. Experimental design. (A) Timeline of the procedure carried out during each season. (B) Spatial arrangement of the containers and conspecific exposure time, CE. + symbols indicate which containers had the nutrient enrichment.

often can be found downstream from water treatment plants where sewage is discharged (Leisnham and Slaney 2005). Ikemoto and Sakaki (1979) found a positive correlation between ammonium concentration in stagnant water and mosquito abundance. Furthermore, Sunish et al. (1998) showed ammonium to be an ovipositional attractant. Mechanistically, NH_4^+ and PO_4^{-3} (phosphate) may promote microbial activity related to larval foraging (Carpenter 1982, Walker et al. 1991, Sunish and Reuben 2001).

Our previous studies have shown that both *Cx. quinquefasciatus* oviposition rates (Chaves et al. 2009) and the fitness of larvae (Chaves et al. 2011b) were enhanced in habitats containing nutrient-rich water from CSOs. Like other insects with aquatic larvae, *Cx. quinquefasciatus* influence offspring fitness with their choice for oviposition sites, because juveniles have limited mobility to colonize new habitats (Mangel 1987, Spencer et al. 2002, Reiskind and Wilson 2004). Thus, the discriminatory process of habitat selection may be related to a variety of factors including, but not limited to, nutrient availability, conspecific presence, and weather variability. Beyond conspecific presence, Kiflawi et al. (2003a) suggested that gravid mosquitoes avoid negative interactions with other species when choosing among reservoirs for oviposition. When given the choice between high or low densities of competitors and predators, the majority of females oviposited in pools with a lower density of both (Blaustein and Kotler 1993, Kiflawi et al. 2003b). In these studies, overused habitats were predicted to deter further conspecific oviposition. The hypothesis that mosquitoes selectively oviposit to avoid potential competitors for the emerging larvae received support from other research showing that changes in oviposition behavior are related to chemical cues and pheromones emitted by members of the same species (Bentley and Day 1989, Millar et al. 1994, Takken 1999).

Abiotic environmental factors also appear to be important for oviposition. The abundance and proliferation of *Cx. pipiens* s.l. populations is seasonal (Makiya 1973, Spielman 2001). Seasonal abundance and concomitant changes in mosquito body size, fecundity, and oviposition behavior are associated with weather variability (Nayar and Sauerman 1970, Bock and Milby 1981, Barker et al. 2010). Temperature and rainfall are correlated with *Culex* spp. oviposition at the interannual (Strickman 1983, 1988), monthly (Day et al. 1990), daily (Chaves and Kitron 2011), and hourly (de Meillon et al. 1967b, Macdonald et al. 1981, Beehler et al. 1993) time scales.

Here, we examined the impact of nutrient dynamics, conspecific presence, climatic factors, and their corresponding interactions on the oviposition of *Cx. quinquefasciatus* in urban aquatic habitats. Our research goals were: 1) to evaluate chemical measures of water quality in isolated pools versus free-flowing stream and describe the association between water chemical measurements and oviposition; 2) to investigate whether oviposition is hindered, enhanced, or unaffected by the exposure to conspecifics across seasons; 3) to evaluate the effects of temperature and precipitation on oviposition; and lastly 4) to determine how these biotic and abiotic factors interact as predictors of *Cx. quinquefasciatus* oviposition.

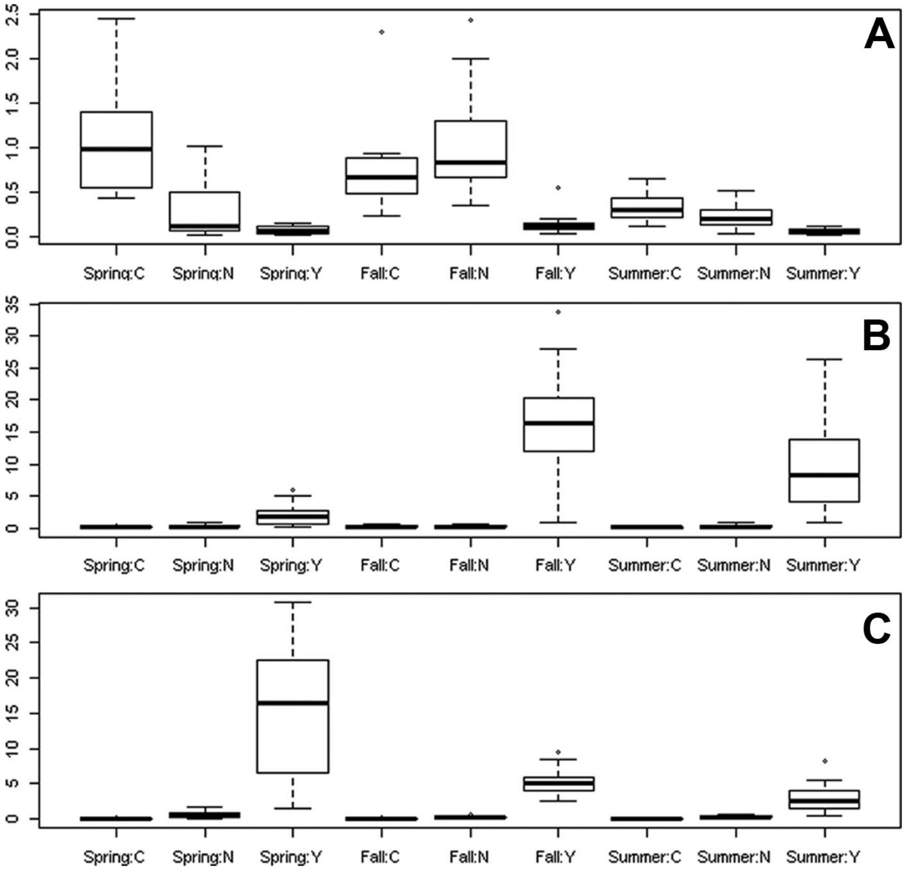

Fig. 2. Boxplots of nutrient concentration (milligrams per liter) in protein-enriched (yes [Y]) and nonenriched (no [N]) containers and stream (C) over the spring, fall, and summer sampling seasons. (A) Nitrate [NO_3^-], (B) ammonium [NH_4^+], and (C) phosphate [PO_4^{3-}]. In the boxplots bold line inside the box represents the median of the distribution. Bottom quadrant of box represents first quartile, and top quadrant represents third quartile; 50% of the data lies inside the box. The other 50% are represented by dashed lines and circles.

Materials and Methods

Study Site. Our study site was an embankment parallel to Tanyard Creek, a CSO-polluted stream flowing through various northwest Atlanta, GA, neighborhoods (Calhoun et al. 2007). At the time of this study a sewage overflow from the closest treatment facility was triggered by any continuous precipitation above 3 mm.

Experimental Design. We conducted a seminatural experiment to capture variability from the natural environment while also manipulating some conditions. Our experimental setup consisted of 24 dark blue 18.93 liters (5 gallons) Rubbermaid (Columbus, OH) containers positioned in a four-row by six-column arrangement (Fig. 1). Each container was filled with 3 liters of water collected from Tanyard Creek within 48 h after an overflow event. Although overflow duration and amount of nutrient release varies, CSO water is always rich in organic matter (Bernhardt et al. 2008). Twelve of the 24 containers were added an additional 12 g of crushed Purina One (St. Louis, MO) dog biscuits (21% protein content). This served as an ovipositional attractant (Chaves et al. 2009). The purpose of this addition was to determine if there were differences in the number of gravid mosquito females choosing between enriched and nonenriched habitats to oviposit egg rafts. This experimental setup was placed during three different seasons of mosquito activity in the study site: 6–30 October (fall) and repeated on 7 April through 1 May 2009 (spring), and 1–24 June 2009 (summer). At the beginning of each season, three enriched and three nonenriched containers were exposed for oviposition, and the remaining 18 containers were covered with a clear plastic tarp to prevent insects, debris, detritus, leaf litter, and rainfall from invading the experimental habitats (Fig. 1A). The spatial arrangement of the containers is shown in Fig. 1B. Here we placed enriched containers next to nonenriched containers provided one of our previous studies show that oviposition *Cx quinquefasciatus* is spatially finely grained (Chaves et al. 2009) and to avoid the clustering of containers with nutrients. At the beginning of the experiment each container had 3 liters of CSO water. Containers had openings to allow

Table 1. Analysis of variance (ANOVA) for the effects of season, age, and enrichment

Factor	df	Sum squares	Mean square	F	P (>F)
[NO_3^-]					
Season	2	751.7	375.85	30.08	2.094e-11***
Week	1	48.44	48.44	3.88	0.05117
Treatment	3	1,925.11	962.56	77.03	2.2e-16***
Season:week	6	4.63	2.31	0.19	0.83118
Season:treatment	2	954.45	238.61	19.10	2.682e-12***
Week:treatment	3	10.45	5.22	0.42	0.6592
Season:week:treatment	6	1.85	0.46	0.04	0.99735
Residuals	126	1,574.45	12.5		
[NH_4^+]					
Season	2	832.71	416.36	43.16	5.272e-15***
Week	1	460.13	460.13	47.70	2.181e-10***
Treatment	3	2,621.31	1,310.66	135.87	2.2e-16***
Season:week	6	124.68	62.34	6.46	0.002129**
Season:treatment	2	1,114.8	278.7	28.89	2.2e-16***
Week:treatment	3	4,45.64	222.82	23.10	2.840e-09***
Season:week:treatment	6	1,36.47	34.12	3.53	0.009021**
Residuals	126	1,215.39	9.65		5.272e-15***
[PO_4^{-3}]					
Season	2	4.80	2.40	23.16	2.716e-09***
Week	1	0.27	0.27	2.59	0.1096
Treatment	3	9.09	4.55	43.83	3.555e-15***
Season:week	6	0.45	0.23	2.19	0.1162
Season:treatment	2	5.29	1.32	12.77	9.315e-09***
Week:treatment	3	0.17	0.08	0.77	0.4635
Season:week:treatment	6	0.63	0.16	1.551	0.2029
Residuals	126	13.07	0.10		

Season (three levels: spring, summer, and fall); age (week, with four levels corresponding to weeks 1 through 4); and enrichment (whether nutrients were added, with three levels: yes, no, and Tanyard Creek control samples) on the concn of: ammonium [NH_4^+], nitrate [NO_3^-], and phosphate [PO_4^{-3}].

water runoff in case liquid volume increased to over 4 liters because of rainfall in exposed containers. During each of the three following weeks, six new containers were exposed, three with nutrient enrichment and three without the enrichment (Fig. 1A). The purpose of gradually uncovering the containers was to quantify the effects of conspecific presence, which we expected to weigh more heavily in containers with longer exposure time than in those uncovered later on the experiment. Thus, conspecific exposure time, a key variable inherent for the aim of understanding the impact of conspecifics, is defined as the maximum length of time in weeks for which a group of containers was exposed. During the 4 wk of observations each season, egg rafts were removed every 3 d from all open containers. Observations were made every 3 d based on our previous results, that showed a periodicity of 3 d in the oviposition rhythm of *Cx. quinquefasciatus* in Atlanta (Chaves and Kitron 2011). To account for the possibility of broken egg rafts, we counted partial rafts that seemed to fit into a larger oval raft as one unique egg raft. During the entire study, we did not observe other insects in the oviposition traps, and all larvae developed in the lab from a fraction (at least one raft per container) of the field caught egg rafts were identified as *Cx. quinquefasciatus.* We also checked whether egg rafts were seamed (i.e., whether egg rafts had a longitudinal seam) or not and in all cases we did not find deviations from the 1:1 ratio of seamed and unseamed egg rafts reported for *Culex pipiens* s.l. by Weber and Weber (1985).

To measure nutrient levels, a 15 ml surface water sample was collected from each uncovered container and three 15 ml surface water samples were collected from various points along the stream, placed on ice, and filtered to remove solid materials during each sampling visit. Continuous flow colorimetric assays measuring ammonium, nitrate, and phosphate concentrations were completed for all water samples by a separate lab at the University of Georgia (see Supp Material 1 for a detailed description of the tests [online only]).

Rainfall and air temperature data for all visits were collected from the Weather Underground (http://www.wunderground.com/) Weather Station KGAATLAN32, within a three KM radius of our study site, and used to assess the influence of weather variability oviposition activity. For rainfall, we accumulated rainfall records corresponding to the 3 d between visits (i.e., inter visit time, IVT), and estimated the average temperature of the IVT (average temperature in the analyses) and recorded the maximum and minimum temperature during the IVT (respectively, maximum and minimum temperature in the analyses).

Statistical Analysis. To quantify the effects of the season (season: spring, summer, or fall), medium age (1, 2, 3, or 4 wk), and nutrient addition (treatment: yes [Y] or no [N]) on the concentration of chemical nutrients, an analysis of variance (ANOVA) (Faraway 2004) was performed for the concentration of nitrate [NO_3^-], ammonium [NH_4^+], and phosphate [PO_3^{-3}] as a function of season, medium age, and nutrient enrichment.

To account for the lack of independence, or pseudoreplication, associated with taking multiple measurements and samples from the same containers, lin-

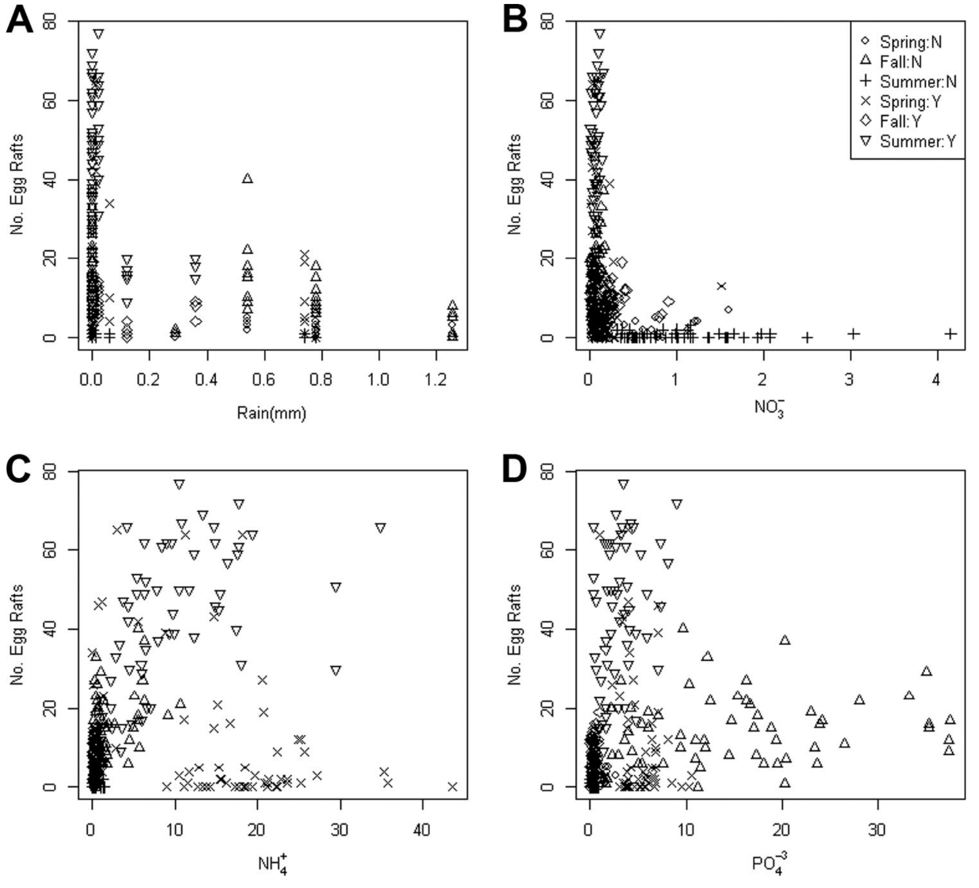

Fig. 3. *Culex quinquefasciatus* egg raft number as function of (A) rainfall, (B) nitrate [NO_3^-], (C) ammonium [NH_4^+], and (D) phosphate [PO_4^{3-}]. Concentrations are in milligrams per liter. Symbols indicate the season and whether rafts were collected in a nuntrient enriched (Y) or nonenriched pool (N).

ear mixed effects (LME) models were used to analyze the egg rafts count data (Chaves 2010). We also want to clarify that we did not use this analytical tool for the water samples provided data and residuals from exploratory mixed effects model behaved as if they were statistically independent, something that makes unnecessary the use of mixed effects models (Venables and Ripley 2002, Chaves 2010). These models analyzed the number of egg rafts oviposited as function of season, nutrient enrichment, temperature, precipitation, and exposure time as fixed factors. Random factors included the nested variability because of the season, the week, and the visit (i.e., the weekly observations, mathematically expressed as S|W|V) and in a simplified form not nested by season (i.e., W|V). Linear mixed effects models (LMEMs) were fitted with restricted maximum likelihood, REML (Faraway 2006).

The Akaike Information Criterion (AIC) was used to select the minimum number of variables able to satisfactorily explain the egg rafts observations. The model with the minimum AIC is the best model, that is, the one that maximizes the variability of the data with the minimum number of parameters (Faraway 2004). Parameter inference for the best model was based on a Markov Chain Monte Carlo (MCMC) where uninformative priors were assigned to model parameters; then, for each parameter, the 95% high probability distribution (HPD), the Bayesian equivalent to maximum likelihood confidence limits, was generated by sampling the posterior distribution of the samples generated via MCMC (Faraway 2006).

We also used regression trees to capture any possible nonlinearities in the impacts of the environmental variables on the number of collected egg rafts. Briefly, a regression tree is a set of rules developed from the independent variables, that is, the environmental variables that we measured, that can best recreate the observed pattern in the response variable (Olden et al. 2008). Moreover, regression trees lack assumptions that restrict their use under circumstances of lack of independence in observations (Faraway 2006). Regression trees have also been previously used in mosquito studies (Hu et al. 2006, Chaves et al. 2011a). All of the analyses were performed using the R Statistical Language (R Development Core Team, Vienna, Austria).

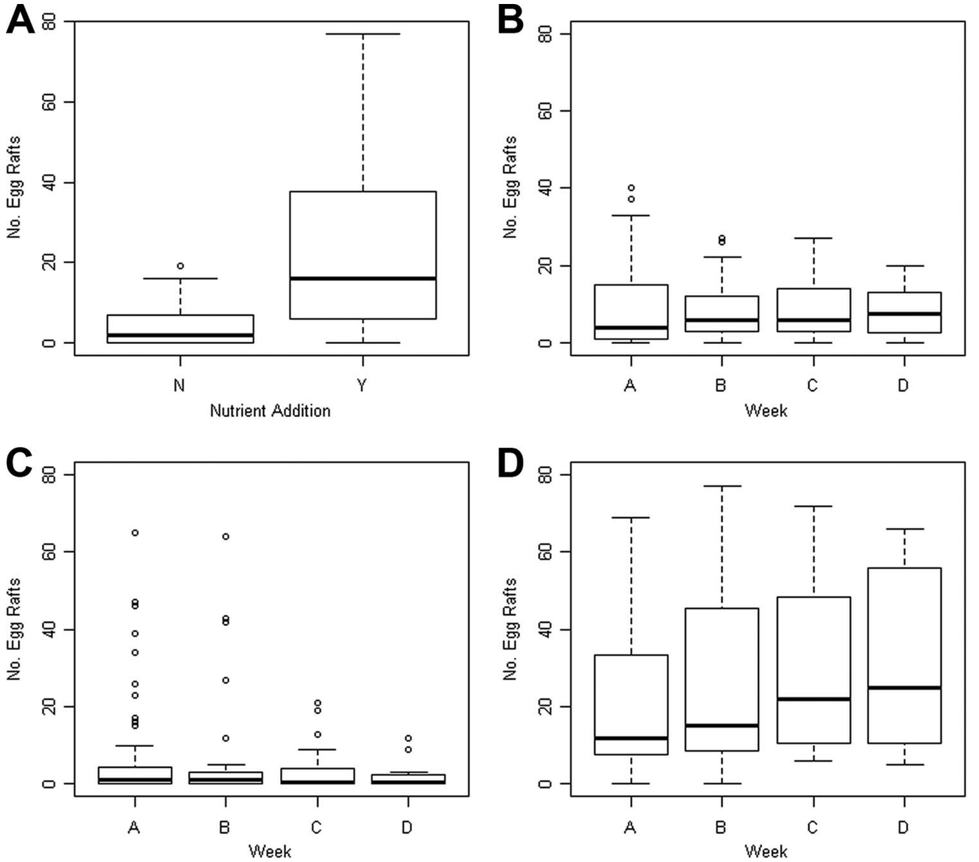

Fig. 4. Boxplots of *Culex quinquefasciatus* egg raft number as function of (A) nutrient enrichment (yes [Y], no [N]) and conspecific exposure time during, (B) spring season, (C), fall season, and (D) summer season. For the explanation of the boxplot lines see Fig. 2 and for the interpretation of the X axis in B, C, and D see Fig. 1.

Results

Nutrient measurements from the creek and the enriched and nonenriched containers are shown in Fig. 2. Nitrates were significantly higher in the creek than in the containers over the seasons (Fig. 2A; Table 1), while ammonium was higher in the enriched containers, especially during the summer and fall, with the differences across season and enrichment being statistically significant (Fig. 2B; Table 1). Phosphate followed a similar pattern to that observed for ammonium, with the highest concentrations in the enriched pools, with statistically significant ($P < 0.05$; Table 1) differences across the seasons (Fig. 2C; Table 1).

Oviposition was highly variable during low rainfall; however, a decreasing trend in the number of egg rafts was noted as the 3 d cumulative rainfall increased (Fig. 3A). Similar patterns were observed regarding the variability in the number of oviposited egg rafts as function of concentration of the different nutrients, nitrate (Fig. 3B), ammonium (Fig. 3C), and phosphate (Fig. 3D). However, no clear trends in the number of oviposited egg rafts counted during each visit as function of nutrient concentration were evident in any of the cases. Nevertheless, high nitrate concentration (>1 mg/L; Fig. 3B) and phosphate concentration (>10 mg/L; Fig. 3D) were associated with a decrease in the average number of oviposited egg rafts. The average number of egg rafts was higher in the nutrient enriched habitats (Fig. 4A), and number of egg rafts changes during the seasons, with the lowest variability observed during the spring (Fig. 4B), the most extreme variability, that is, with many outliers, observed during the fall (Fig. 4C), followed by the widest variability in the summer (Fig. 4D). On average, the largest numbers of egg rafts were oviposited during the summer (Fig. 4D), followed by the spring (Fig. 4B), and then fall (Fig. 4C). In both spring (Fig. 4B) and fall (Fig. 4C), the longer the conspecific exposure time, the larger the number of oviposited egg rafts. By contrast, the opposite pattern was observed in the summer, where the larger number of egg rafts was observed in the containers with the least conspecific exposure (Fig. 4D). These changes in the impact of conspecific exposure time may be associated with temperature.

The process of model selection (Table 2) showed that nutrient enrichment was a better predictor of oviposition than the specific concentration of the dif-

Table 2. Linear mixed effects model selection

Covariates	Nested random factors	AIC
Null: no covariates	Visit week	3,094
Null: no covariates	Visit week season	2,970
Enrichment + season*CE + rain	**Visit week season**	**2,721**
Enrichment + season + rain + CE	Visit week season	2,737
Enrichment + temp(avg) + rain + CE	Visit week season	2,746
Enrichment + temp(max) + rain + CE	Visit week season	2,747
Enrichment + temp(min) + rain + CE	Visit week season	2,749
Enrichment + temp(avg)*CE + rain	Visit week season	2,755
Enrichment + temp(max)*CE + rain	Visit week season	2,756
Enrichment + temp(min)*CE + rain	Visit week season	2,758
$[NO_3^-] + [NH_4^+] + [PO_4^{-3}]$ + season*CE + rain	Visit week season	2,868
$[NO_3^-] + [NH_4^+] + [PO_4^{-3}]$ + season + rain + CE	Visit week season	2,864
$[NO_3^-] + [NH_4^+] + [PO_4^{-3}]$ + temp(avg) + rain + CE	Visit week season	2,870
$[NO_3^-] + [NH_4^+] + [PO_4^{-3}]$ + temp(max) + rain + CE	Visit week season	2,866
$[NO_3^-] + [NH_4^+] + [PO_4^{-3}]$ + temp(min) + rain + CE	Visit week season	2,873
$[NO_3^-] + [NH_4^+] + [PO_4^{-3}]$ + temp(avg)*CE + rain	Visit week season	2867
$[NO_3^-] + [NH_4^+] + [PO_4^{-3}]$ + temp(max)*CE + rain	Visit week season	2,835
$[NO_3^-] + [NH_4^+] + [PO_4^{-3}]$ + temp(min)*CE + rain	Visit week season	2,858

All models were fitted with REML, and the response variable was the no. of *Culex quinquefasciatus* egg rafts. Covariates (Fixed Factors) included whether a pool was enriched or not (enrichment), season (spring, summer, or fall), rainfall (rain), temp (avg = average, max = maximum, and min = minimum), conspecific exposure time (CE), and nutrient concn ($[NO_3^-]$, $[NH_4^+]$, and $[PO_4^{-3}]$). Random Factors included the error that was assumed to be normally distributed and the nested random factors that accounted for the medium age (either by nesting the sampling visits within a week or by additionally nesting the visits within a week within a given season). Model with the lowest AIC is in **bold.**

ferent nutrients. Similarly, the interaction between season and conspecific exposure time was a better predictor than any of the different dimensions of temperature (maximum, minimum, or average) of the number of recorded egg rafts. Rain was a significant factor. Model selection (Table 2) also showed the more detailed nested structure of variability to be the best for the model. Parameter estimates for the best model can be seen in Table 3. Rainfall had a significantly negative impact on the number of counted egg rafts, and the number of egg rafts significantly increased when containers were enriched and during the summer (Table 3). The variability because of the age of the medium and the visits (S|W|V and S|W variance) are close to two thirds of the size of the unexplained variability (i.e., error variance) of the model, and account for close to 40% of the overall variability in the model.

Figure 5 shows how the average number of egg rafts follows temperature changes during the three seasons, while in the summer the number decreased after relatively large rainfall events. Nevertheless, the mixed effects model was unable to capture this relationship, or any of the observed relationships with organic nutrient concentration (Fig. 3), because of their nonlinear association with the number of oviposited egg

Table 3. Parameter estimates for the best model explaining the no. of *Culex quinquefasciatus* egg rafts

Parameter	Estimate	SE	Lower 95% HPD	Upper 95% HPD
Intercept (spring, enrichment:N, CE-A)	1.43	4.38	−6.06	9.07
Enrichment:Y	18.74[a]	1.1	16.51	20.96
Fall	−1.83	5.92	−11.87	8.31
Summer	11.17[a]	5.98	0.82	21.01
CE-B	−0.78	2.45	−5.56	4.19
CE-C	−1.61	2.87	−7	4.54
CE-D	−0.62	3.78	−7.75	7.61
Rain	−6.75[a]	3.48	−13.41	−0.68
Season fall:CE-B	2.48	3.46	−4.74	8.96
Season summer:CE-B	0.97	3.46	−5.91	7.75
Season fall:CE-C	4.65	4.06	−3.99	12.31
Season summer:CE-C	0.34	4.06	−7.25	8.96
Season fall:CE-D	2.78	5.34	−8.84	13.06
Season summer:CE-D	1.9	5.34	−8.59	12.95
S|W|V variance	10.45			
S|W variance	55.04			
Residual variance	109.78			

The covariates were: enrichment:N (nonenriched) and enrichment:Y (enriched). CE is the conspecific exposure time where A, B, C, and D are groups for the decreasing exposure time (see Fig. 1 for further details). S|W|V represents the variance observed during the visits in a week and S|W represents the variance observed during the sampling weeks of a given season.

[a] Statistically significant P (<0.05).

rafts. By contrast, the regression tree (Fig. 6) was able to explicitly capture the impact of nutrient concentration on oviposition, as well as other nonlinear relationships. In regression trees the more important variables the ones at the most basal branches (Olden et al. 2008), meaning that $[NH_4^+]$, nutrient enrichment and Tmax were the most important variables explaining the patterns of oviposition measured by egg rafts counts (Fig. 6). In Fig. 6, when $[NH_4^+]$ was above 2 mg/L mosquitoes oviposited more egg rafts, specially at high temperatures (Tmax ≥32.5°C or Tmin >12°C when Tmax <32.5°C) with >48 egg rafts per container or visit, and when $[NH_4^+]$ was below 2 mg/L, enrichment was the most important factor explaining differences in the number of recorded egg rafts (Fig. 6). Nonenriched containers had between ≈2–9 egg rafts (with differences driven by temperature), while enriched containers had between ≈8–19 egg rafts (with the larger number associated with low rainfall, that is, below 0.18 mm/3 d). Interestingly, the impacts of temperature varied according to the season. If we follow the right branches of the tree we see than when $[NH_4^+]$ > 2 mg/L, Tmax <32.5°C, and Tmin <12°C the number of egg rafts in the fall is slightly below three, while in the fall can be close to 19 (Fig. 6).

Discussion

Pioneering studies in mosquito ecology established that poor water quality is associated with outbreaks of the house mosquito in many places around the world (Britton 1914, Wada and Ofuji 1962, Kumada et al. 1972, Scorza 1972, Rajagopalan et al. 1976), and more recently with the colonization of urban creeks (Mogi

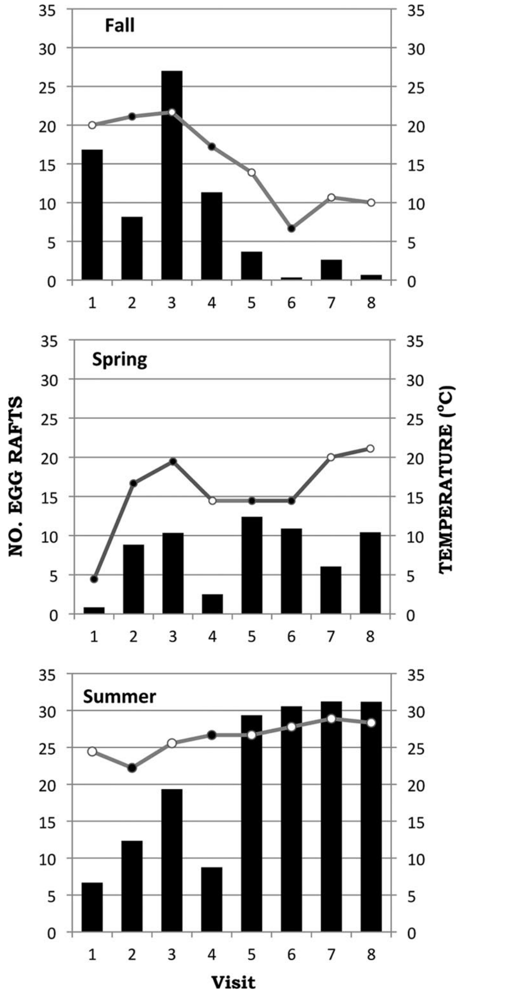

Fig. 5. *Culex quinquefasciatus* average number of egg rafts per visit (black bars) and temperature (gray line) across seasons (each season consisted of eight visits, two per week). Black circles represent rainfall of >0.25 mm.

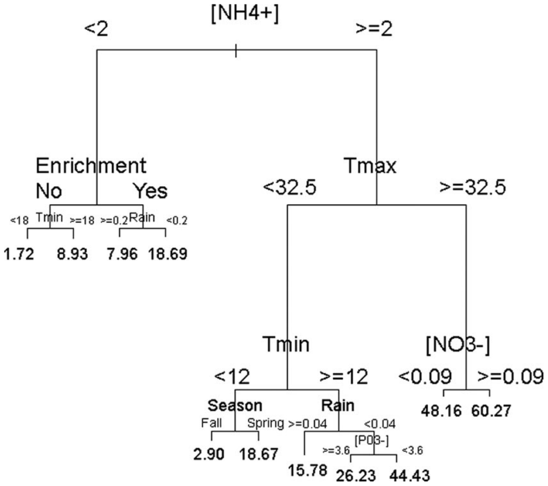

Fig. 6. *Culex quinquefasciatus* regression tree for the number of egg rafts as function of the environmental variables. For the model $R^2 = 0.85$. Values in the terminal nodes are estimated number of egg rafts. In the middle of each branch is the covariate name and in the vertices are the threshold values for each subsequent branch in the tree. Covariates included: enrichment (yes [Y] or no [No]), ammonium [NH4+], nitrate [N03−], and phosphate [PO4−] concentration in milligrams per liter; season (spring, fall, and summer); rainfall (rain); temperature, in °C, which included: average (Tavg), maximum (Tmax), and minimum (Tmin). For this tree all predictions were cross-validated, that is, each observation was taken out once when building the tree, and the minimum split size, that is, minimum number of observations per node, was 18 (5% of the total observations).

et al. 1995, Mogi and Sota 1996, Calhoun et al. 2007). In both of these cases oviposition plays a major role as the defining event that ensures the selection of suitable larval habitat for mosquito fitness maximization (Chaves et al. 2009). Our results suggest that stagnant waters along creek edges, like our experimental containers, may become excellent larval habitats for the house mosquito because of a chemical composition that is rich in both phosphate and ammonium, both of which have higher concentration in these habitats than in the free flowing water on the main course of the creek, where nitrates have higher concentration than the experimental containers. As previous studies have shown for both culicine (Beehler and Mulla 1993, Beehler et al. 1994, Beehler and Mulla 1995) and anopheline mosquitoes (Sunish et al. 1998, Sunish and Reuben 2001) these two organic molecules are very important for bacteria that may serve as food sources for larval mosquitoes (Carpenter 1982, Beehler et al. 1994) or oviposition attractants (Ikeshoji 1966, Rockett 1987, Beehler et al. 1994). Additionally, nutrient rich media, which is also known to enhance the attractiveness of larval habitats (Beehler and Mulla 1993, 1995), is associated with an increased fitness of larval mosquitoes, by increasing the pupation and emergence rates, and the size of the emerging adults (Chaves et al. 2011b).

However, the chemical analysis of our water samples also showed that our nutrient enrichment treatment increased $[NH_4^{+}]$ and $[PO_4^{-3}]$ more than twofold when compared with the contents of the containers that were not enriched. These results have two implications: either our manipulation increased organic contents to unrealistic levels, or can be valid if the isolated pools created after a combined overflow have an additional enrichment. Thus, the relevance of our results depends on further observations of isolated larval habitats that can be created of the borders of the creeks after a CSO. As previous studies have shown, the proper canalization of urban creeks (Mogi et al. 1995, Mogi and Sota 1996) avoided stagnation and reduced the potential for colonization of urban creeks by the house mosquito. These studies have also shown that river segments prone to stagnation have characteristics similar to those of our experimental containers.

The results of this and our previous work (Chaves et al. 2009, Chaves and Kitron 2011) do not indicate a strong role for apical droplet pheromones in the oviposition of the house mosquito (Bruno and Laurence 1979, Millar et al. 1994). Instead, overall nutrient contents seem to be a major determinant of mosquito larval habitat selection. However, the impact of the egg raft pheromones may be density-dependent and act in a nonlinear fashion. For example, our results showed that only during the summer season egg raft oviposition rates declined with time since container exposure. However, the lack of larvae removal confounds the separation of a chemical effect and the presence of conspecifcs. Nevertheless, the lack of differences in attractiveness between containers where larvae were removed and present (Chaves et al. 2009) suggests there may be a chemical signal mediated factor on oviposition habitat selection of the house mosquito. However, in a previous study (Chaves and Kitron 2011), we found that regime shifts in nutrient concentration cannot be compensated by the cues that might be generated by newly oviposited rafts. We observed that after strong rainfall events able to change the chemical concentration of nutrients in experimental containers, the number of oviposited egg rafts was never as high as before the regime shift in nutrient concentration at the experimental oviposition containers (Chaves and Kitron 2011).

Our results showed that seasonal patterns of oviposition by the southern house mosquito vary strongly across the seasons, at least for one study year, with an increased activity during the summer. Similar observations have been made for the red house mosquito *Cx. pipiens pallens* in Shanghai, China (Suenaga et al. 1982). Although the differences between seasons were marked, our linear mixed effects models were unable to find a significant relationship with any of the temperature measurements we considered in our analysis, Fig. 5 shows abrupt changes in the number of oviposited egg rafts for colder temperatures in both the spring and fall seasons, which indeed are very nonlinear according to the tree regression of Fig. 6. However, studies on *Culex nigripalpus* Theobald in southern Florida mosquitoes have shown that rainfall maybe a major determinant of the seasonal patterns of oviposition (Day et al. 1990). We consider these differences to be possibly related to the strength of seasonality effects on different climatic factors. While in southern Florida there is a stronger seasonality in rainfall when compared with Atlanta, the temperature seasonality in Atlanta is stronger than in southern Florida, which is one of the scenarios where Schmalhausen's law, an evolutionary principle stating that organisms are very sensitive to changes in an environmental factor when they are stressed by any environmental factor, comes into effect (Chaves and Koenraadt 2010). However, further studies comparing many sites are necessary to fully understand the impacts of different degrees of variability across environmental factors and to understand why seasonal drivers can be so variable across landscapes and whether they can change interannually. Finally, as a summary of our studies on the oviposition of the southern house mosquito in CSOs of Atlanta, GA, we can robustly affirm that such habitats are primarily chosen because of their enrichment (Chaves et al. 2009), that they are associated with fitness advantages for the emerging mosquitoes (Chaves et al. 2011a), that at intermediate time scales oviposition dynamics are strongly associated with rainfall-induced regimes shifts in water quality (Chaves and Kitron 2011), and this study indicates that temperature was an important factor shaping the differences observed in oviposition across the seasons within the year of mosquito activity we studied.

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