Illinois Water Resources Center Final Report

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Project Title: Influence of Water Quality and Stormwater Management on the Ecology of Mosquito-Borne Disease

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Problem and Research Objective:

Urban ecosystems are notoriously prone to outbreaks of mosquito-borne diseases due to the extensive adaptations by mosquito vectors to urban environments (Bradley and Altizer 2007). Mosquitoes undergo a ‘complex’ life cycle involving both aquatic juvenile stages (egg, larva and pupa) and an adult terrestrial stage. Mosquitoes that have adapted to reproduce in urban environments are those that are best able to utilize the many nutrient-rich aquatic habitats that urban development often provisions. Preventing juvenile mosquito development in these habitats is considered one of the most effective strategies for reducing adult mosquito abundance and public health risk due to mosquito-borne disease, and is thus a primary focus of urban mosquito control programs (Floore 2006). This is largely accomplished through the application of insecticides targeting the juvenile life stages (e.g. larvicides), and by modifying conditions or human behaviors that create or maintain productive environments for mosquito development (e.g. source reduction, public education, etc.). For these prevention programs to be effective requires identifying risk factors for vector mosquito production in urban aquatic habitats.

Infrastructure for managing urban stormwater runoff provides abundant aquatic habitats for *Culex pipiens* and *Cx. restuans*, the primary mosquito vectors of West Nile virus (WNV) in Illinois. These structures may be particularly prolific habitats for vector mosquitoes when receiving nutrient-enriched runoff or septic effluent. High-nutrient loading in aquatic habitats encourages oviposition by these species and can alleviate negative effects of larval competition on the rate of juvenile development and survivorship (Reiskind et al. 2004, Alto et al. 2012, Kraus and Vonesh 2012). Additionally, adult mosquitoes emerging from nutrient-rich environments are typically larger and possess greater nutrient reserves, which can enhance longevity, fecundity and dispersal range (Akoh et al. 1992, Drummond et al. 2006, Alto et al. 2012), and may ultimately alter their potential to acquire and transmit a pathogen.

In addition to altering conditions for developing mosquitoes, the retention of nutrient-rich runoff in stormwater management structures facilitates invasion by cattails (*Typha* spp.) and other emergent, aquatic macrophyte species tolerant of saturated, eutrophic conditions (McCormick et al. 2004, Kettenring et al. 2011). These invasive plants can competitively displace desired vegetation (e.g. turfgrass, native flood-tolerant or aquatic flora, etc.), resulting in dense, monospecific stands reaching several meters in height (Kominkova et al. 2000, Vaccoro et al. 2009), with an above-ground biomass often exceeding 1 kg / m^2 (Mason and Bryant 1975). To control this undesirable vegetation, managers of stormwater infrastructure may employ annual or semi-annual mowing, herbicide applications, or prescribed burning.

Emergent, aquatic plant growth and its management can have variable effects on the abundance and species composition of juvenile mosquitoes, depending on the plant species, type of aquatic habitat, and temporal concordance between vegetation management activities and hydroperiod (Walton 2012). In semi-permanent or permanent habitats (e.g., constructed wetlands, retention ponds), dense aquatic vegetation can provide juvenile mosquitoes refuge from predation (De Szalay and Resh 2000), and is therefore often characterized as a significant risk factor for the production of vector mosquitoes. Reducing the density of emergent vegetation has been shown to be an effective environmental management tool for diminishing juvenile mosquito abundance in these settings (De Szalay et al. 1995, Thullen et al. 2002, Lawler et al. 2007). However, vegetation management practices can also promote eutrophic conditions that support high juvenile mosquito densities when the resulting plant litter is later inundated and decomposes, particularly in ephemeral or seasonally flooded aquatic environments (Jiannino and Walton 2004, Walton and Jiannino 2005). Thick accumulations of plant litter in these habitats can also alter drainage, creating fragmented, nutrient-rich habitats that obstruct the movement or functional response of predators that regulate juvenile mosquito populations (Berkelhamer and Bradley 1989).

The overall objectives of this study were to examine the potential consequences to vector mosquito production resulting from colonization of two common stormwater management tools (ditches and dry detention basins) by one of the most widespread and invasive, aquatic macrophytes groups in urban landscapes (cattails), and from actions taken to control these invasive macrophytes (mowing), and to evaluate whether these effects of invasive vegetation or vegetation management on the larval ecology of vector mosquitoes are mediated by water quality. The specific goals of this study were:

Goal 1. Conduct a field survey of stormwater ditches and dry detention basins to evaluate relationships among plant composition (grasses vs. cattails), plant management (mowing), water quality and the abundance of juvenile *Culex* spp. mosquitoes.

Goal 2. Determine whether the type of plant detritus in aquatic habitats (cattails vs. turfgrass), or exogenous orthophosphate enrichment, influence the oviposition response of *Culex* spp. mosquitoes, and whether differences in the responses of *Cx. pipiens* and *Cx. restuans* to these factors are consistent with differences in the distribution of their juveniles in storm water BMPs observed in Goal 1.

Goal 3. Compare the effects of plant detritus type (cattails vs. turfgrass) and concentration in aquatic habitats on the duration of juvenile development, adult body size and adult longevity of *Cx. pipiens* and *Cx. restuans*. Determine whether these parameters in *Cx. pipiens* are influenced by exogenous orthophosphate enrichment of cattail infusions.

Methodology:

Goal 1.

A total of 36 stormwater management structures (16 dry detention basins and 20 drainage ditches) were selected in the communities of Urbana, Champaign and Savoy, IL, based on dominant plant species composition and the presence of standing water in the early spring (≈1 month prior to sampling juvenile mosquitoes). Grasses (primarily turfgrass) were the dominant vegetation at 11 sites, and the remaining 25 sites were colonized by cattails. Vegetation within 17 of the study sites was mowed at least once during the study period as part of existing municipal or private maintenance programs.

Juvenile mosquito abundance and water quality at each study site were assessed at ≈2 week intervals, from 3 June to 16 August, 2013 (total 5-6 inspection dates per site). When standing water was present, a sample of mosquito larval and pupae was collected (20 dips per sample), and specimens were counted and identified to species (larvae) or genus (pupae). Hand-held meters were used to collect field measurements of dissolved oxygen, pH, conductivity, salinity and total dissolved solids. A water sample was collected from each mosquito sampling location, transported to the lab on ice and analyzed immediately for total reactive phosphorus and nitrates by colorimetry (Hach 2009). The relative influences of various site characteristics (e.g. vegetation type, mowing history, type of structure) on larval abundance were evaluated as fixed effects in a General Linear Mixed Model (GLMM). Water quality measures and lagged intervals of cumulative rainfall and mean daily air temperature in the two weeks preceding sampling, were explored as covariates. A spearman correlation analysis was also used to compare relationships between juvenile mosquito abundance and each water quality parameter.

Goal 2.

Two field assays was performed to compare how the type and quantity of nutrient enrichment in aquatic habitats affects the oviposition response of *Cx. pipiens* and *Cx. restuans*. Each assay was performed at 5 replicate sites in Urbana, IL. At each site, ovitraps representing each of seven treatments were randomly assigned to sheltered locations at least 10 m apart along the margins of woody vegetation. Ovitraps were prepared by submerging a quantity of plant substrate in 8 liters of tap water in a 19 L pail. Egg rafts were collected daily from the water surface of ovitraps and the position of each ovitrap was rotated among locations within each study site. At the end of each week, ovitrap positions were re-randomized. First instar larvae hatching from field-collected egg rafts were identified to species. For both *Cx. pipiens* and *Cx. restuans*, the daily numbers of egg rafts in each ovitrap, as a proportion of the daily total collected at each site, were fit to a GLMM with a binomial distribution and logit link function.

Trap location nested within study site was included as a random effect. Post-hoc, pairwise comparisons between the average proportion of egg rafts collected in each treatment were performed using a sequential Bonferoni adjustment.

In the 1st experiment, we compared oviposition by *Culex* spp. mosquitoes in infusions prepared from cattail or turfgrass clippings (4.5 g / L) and receiving one of three levels of orthophosphate enrichment: a) no enrichment, b) weekly addition of 0.27 mg PO_4-P / L / week, and c) a single dose of 0.81 mg PO_4-P / L added at the start of the assay. A seventh treatment consisted of weekly enrichment with 0.27 mg PO_4-P / L / week in the absence of a plant substrate. The weekly enrichment rate used in this assay (0.27 mg PO_4-P / L) is comparable to the median concentration of reactive phosphorus measured in storm water BMPs contemporaneously with a high abundance of *Cx.* spp. larvae in Goal 1 (>3 per dip; Fig. 1). Collections were made 04 September to 01 October, 2013.

In the 2nd experiment, we varied the amount of plant substrate used in each trap, comparing 0.75, 1.5 and 4.5 g / L of turfgrass clippings, and 0.75, 1.5, 4.5 and 9 g / L of cattail clippings. Collections of *Culex* spp. egg rafts were made 25 June to 21 July, 2014.

Goal 3.

Three laboratory experiments were performed to examine how nutrient enrichment of the aquatic environment from plant detritus and exogenous orthophosphate inputs affect the duration of juvenile development, and the size and longevity of adult mosquitoes. Experiments were performed by adding newly-hatched larvae to 340 ml of a plant infusion (with or without the addition of orthophosphate) that was allowed to decompose for 3 days prior to the experiment. After completing larval development, individual pupae were transferred from the plant infusion to screen-covered, 50 ml plastic centrifuge tubes provisioned with water-soaked cotton balls and observed every 12 hours to record the time of adult eclosion and time of death. After death, the length of one wing was measured as a proxy for adult body size (van den Huevel 1963).

The first laboratory experiment examined the effect of high orthophosphate enrichment (0.81 mg PO_4-P / L) on the juvenile development rate, adult size and adult longevity of *Cx. pipiens* reared in an infusion of decomposing cattails (4.5 g / L). Experiments were performed at 2 larval densities (10 and 20 per replicate). Each treatment (+/- orthophosphate enrichment x 2 larval densities) was replicated four times in this experiment.

The remaining laboratory experiments evaluated the influence of plant substrate type (cattails vs. turfgrass) and concentration on the juvenile development rate adult size and adult longevity of *Cx. pipiens* (Exp. 2) and *Cx. restuans* (Exp. 3). In both experiments, infusions were prepared using three concentrations of cattails (1.5, 4.5, and 9.0 g / L), and two concentrations of turfgrass (0.75 and 1.5 g / L). Additional concentrations of turfgrass (0.375 g / L) and cattails (0.75 g / L) were added in experiment 3 (*Cx. restuans*). In both experiments, 10 newly hatched larvae were added to each replicate, and each treatment was replicated 5 times.

Principle Findings and Significance.

Goal 1.

1) A substantial increase in the abundance of juvenile mosquitoes (*Cx.* sp. pupae and larvae of *Cx. pipiens* and *Cx. restuans*) occurred immediately following mowing of vegetation in stormwater structures (Fig. 2). Abundance of *Cx.* spp. juveniles was low in habitats with unmanaged vegetation or vegetation mowed >2 weeks previously. The class of structure (dry detention basin or ditch) was not a significant influence on the abundance of mosquito juveniles.
2) Dominant plant type mediated the response of *Cx. pipiens* to mowing. The abundance of *Cx. pipiens* larvae and *Cx.* sp. pupae were much greater in recently mowed turfgrass habitats than in recently mowed habitats colonized by cattails (Fig. 2a, 2c). The abundance of *Cx. restuans* larvae was not significantly influenced by plant type (Fig. 2b).
3) Total reactive phosphorus concentration varied greatly among samples (Fig. 1), and was positively correlated with the abundance of *Cx. restuans* larvae (P=0.004) and *Culex* sp. pupae (P=0.038). The abundance of *Cx. pipiens* larvae was positively correlated with reactive phosphorus concentration in dry detention basins (P=0.036), but not in ditches (P>0.05). Relationships between juvenile mosquito abundance and nitrate concentration were not significant.

Goal 2.

1) In the first field assay where a single concentration of plant substrate was evaluated (4.5 g / L), the type of substrate had asymmetrical effects on the oviposition behavior of *Cx. pipiens* and *Cx. restuans* in the absence of orthophosphate enrichment (open bars, Fig. 3). Consistent with our observations in Goal 1, *Cx. pipiens* deposited a greater proportion of egg rafts in turfgrass infusion and *Cx. restuans* preferred to oviposit in cattail infusions. Similar results were observed when oviposition preferences were compared over a range of turfgrass and cattail concentrations (Fig. 4). The greatest proportion of *Cx. pipiens* egg rafts were collected in the highest concentration of turfgrass substrate (4.5 g / L), whereas *Cx. restuans* deposited a significantly greater proportion of egg rafts in the highest concentration of cattails (9.0 g / L).
2) Weekly enrichment of plant infusions with PO_4-P (0.27 mg / L / wk) did not affect the oviposition behavior of either species (light grey bars, Fig. 3). However, both species responded positively to cattail infusions enriched with a single, large orthophosphate dose (0.81 mg PO_4-P / L; dark grey bars, Fig. 3).

Goal 3.

1) Enrichment of cattail infusions with 0.81 mg PO_4-P / L did not significantly affect the juvenile development rate of *Cx. pipiens* males or females (Table 1).
2) Orthophosphate enrichment of cattail infusions had a significant negative effect on the longevity of adult *Cx. pipiens* males (P=0.006), but only a marginally negative effect on their wing length (P=0.059). The mean wing length of adult *Cx. pipiens* females was not

significantly affected by orthophosphate enrichment, and enrichment only had a marginally positive effect on adult female longevity (P=0.051).

3) At a comparable concentration of plant substrate (1.5 g / L), the rate of juvenile development (Fig. 5), and the size and longevity of adult mosquitoes (Fig. 6) were greater when *Cx. pipiens* and *Cx. restuans* were reared in turfgrass infusions, compared with cattail infusions. The lowest concentration of cattail substrate (0.75 g / L) did not provide sufficient resources for juvenile development of *Cx. restuans*; average survivorship of larvae to pupation was <10 %.

These finding demonstrate that mowing of vegetation in stormwater management structures during the growing season substantially increases the immediate risk of these habitats supporting vector mosquito production. Conversely, ditches and dry detention basins with unmanaged vegetation were associated with a relatively low abundance of *Culex* spp. juveniles. These results indicate that caution should be used when conducting maintenance activities that result in the deposition of plant debris in locations likely to collect standing water. Vegetation management practices applied during the growing season should be coordinated with public health agencies so that larval surveillance, and if necessary insecticide treatments, can be implemented to prevent production of vector mosquitoes. When possible, vegetation management should be performed in the early spring or late fall, when enrichment of aquatic habitats is unlikely to coincide with the peak seasonal activity of vector mosquitoes and WNV transmission risk.

Results from our study indicate that colonization by cattails does not appear to increase public health risks associated with mowing of vegetation in dry detention basins and ditches. In recently mowed structures, the presence of cattails was associated with a lower relative abundance of larvae of *Cx. pipiens*, the primary epidemic vector of WNV. Addition of cattail detritus resulted in aquatic habitats that were less attractive to gravid *Cx. pipiens* than adding turfgrass detritus, although this difference was less apparent when cattail infusions were enriched with a high concentration of orthophosphate, or when a high concentration of cattail detritus was present (9 g / L). Aquatic habitats containing cattail detritus generally provided a poorer quality environment for the juvenile development, and produced adult *Cx. pipiens* and *Cx. restuans* mosquitoes with a lower resistance to starvation (i.e. reduced longevity when deprived of food) – decreasing the likelihood of mosquitoes produced by these habitats surviving long enough to acquire and transmit a pathogen.

Similar to previous studies (Mercer et al. 2005, Gingrich 2006, Young et al. 2014), we observed a positive association between reactive phosphorus and juvenile mosquito abundance. Average mosquito abundance and reactive phosphorus concentration were both highest in recently mowed habitats. Although we did not attempt to identify the source of phosphorus loading, it is likely that these relatively high reactive phosphorus concentrations were a result of both direct inputs from runoff and endogenous release from decomposing plant detritus deposited by mowing, and represents an overall increase in nutrient availability in mowed structures. However, we regard the study design in Goal 2 and Goal 3 (experiment 1) to represent a realistic scenario for possible interactions between cattail detritus and exogenous inputs of biologically-active phosphorus from stormwater runoff. The P concentrations used in the lab and field experiments (0.27 and 0.81 mg PO_4-P / L) are within the moderate to high range of soluble reactive P reported from urban stormwater runoff (Waschbusch et al. 1999), and comparable or greater P loading may occur in stormwater BMPs intercepting highly-enriched runoff from sources such as fertilized lawns or compost amendments used for erosion control (Waschbusch et al. 1999, Faucette et al. 2005).

Notable Achievements.

This study provides the first evidence of invasive aquatic plants and their management mediating the abundance and species composition of mosquitoes of public health significance in two common urban stormwater management tools; ditches and dry detention basins. Our study identifies a potential mechanism for the observed effects of plant species composition effects on juvenile vector communities in these habitats (i.e. oviposition preference) and demonstrates that that plant species composition can also influence important traits of adult mosquitoes produced in stormwater management structures. Our results also suggest an interaction between plant type and high exogenous phosphorus enrichment in stormwater infrastructure that could alter the species composition or abundance of juvenile vectors.

Students Supported With Funding. These funds helped to support an indenependent study by an undergraduate student (Ellie Moen) who assisted with the collection of field data described under Goal 1. Funds were also used to provide materials required for field and laboratory assays (Goals 2 and 3) that were performed as independent research projects by one undergraduate student, Ellie Moen, and one first-year veterinary medicine student, Matt Holland.

Publications and Presentations. One manuscript describing the results in this report is currently in review in the journal Ecological Applications, and a second if being prepared and will be submitted to another peer-reviewed journal for publication. Research results also have been resented at the Annual Meeting of the Illinois Mosquito and Vector Control Association.

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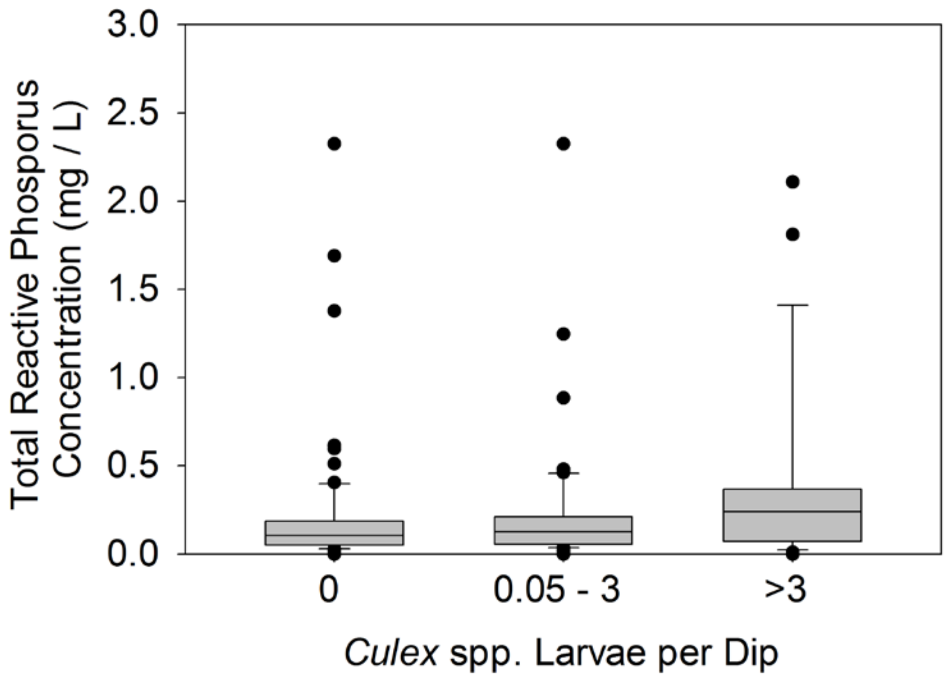

Figure 1. Box plot comparison of total reactive phosphorus concentration by larval mosquito abundance class.

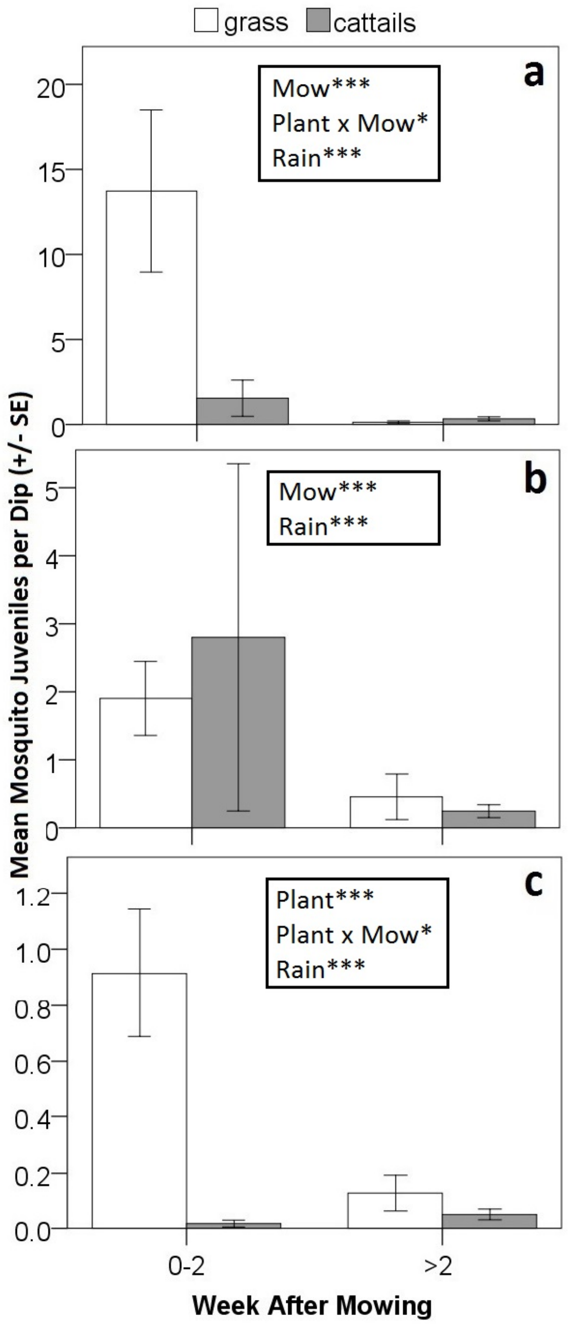

Figure 2. Effect of plant type and recent mowing on the average numbers of *Cx. pipiens* larvae (a), *Cx. restuans* larvae (b), and *Culex* sp. pupae (c) collected from stormwater ditches and detention basins, 03 June to 16 August, 2013 (observations from dry sites excluded from calculation of average values). Significant effects and interactions in GLMMs are shown for each group [**Plant** = dominant plant type (grass or cattails), **Mow** = vegetation mowed ~≤ 2 weeks prior to sampling, **Rain** = cumulative rainfall 0 to 8 days prior to sampling; * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001].

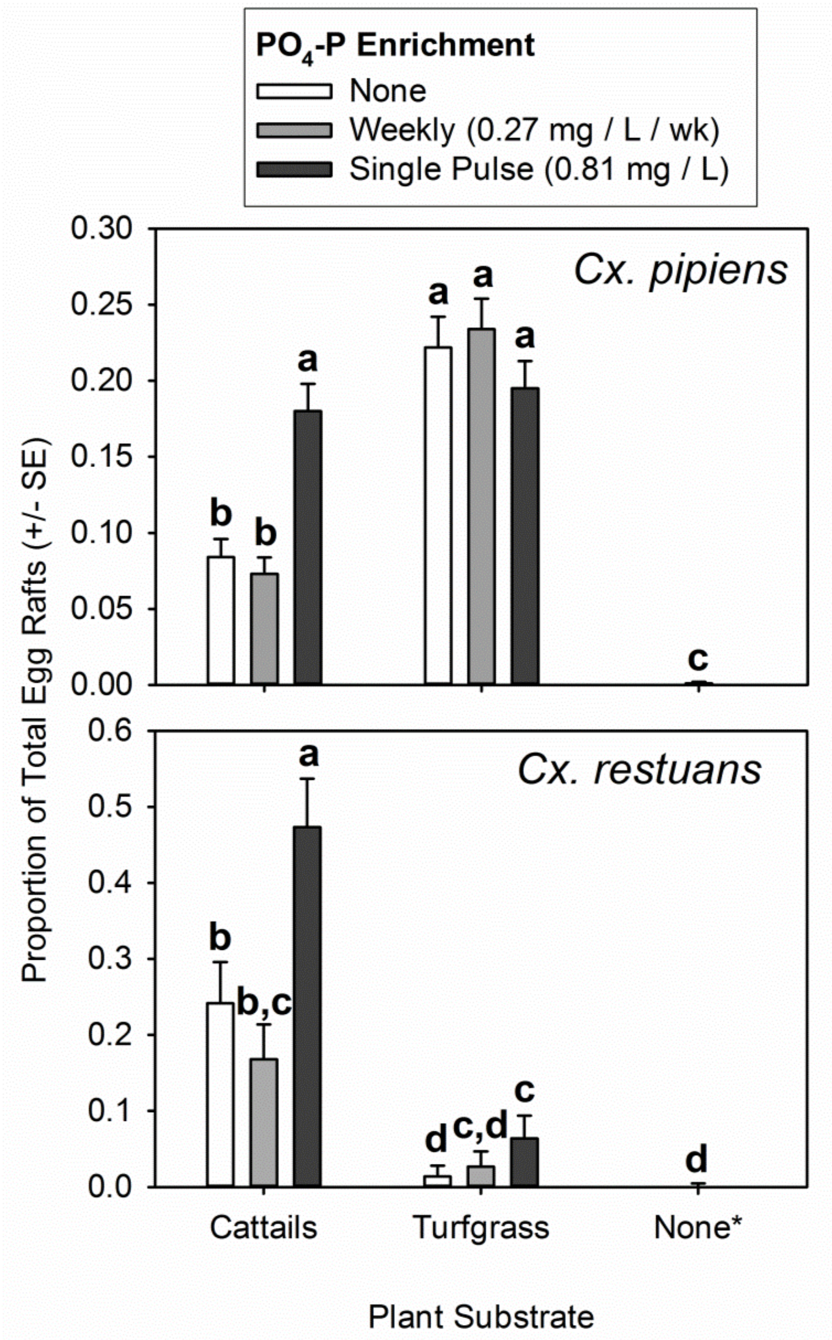

Figure 3. Influence of orthophosphate enrichment on the proportion of eggs rafts of Cx. *pipiens* and *Cx. restuans* collected ovitraps baited with either cattails or turfgrass clippings (4.5 g / l). For each species, columns with the same letter are not significantly different (P>0.05; post-hoc test in GLMM with Bonferroni correction).

* Traps without plant substrate were enriched with 0.27 mg PO_4-P / L / week.

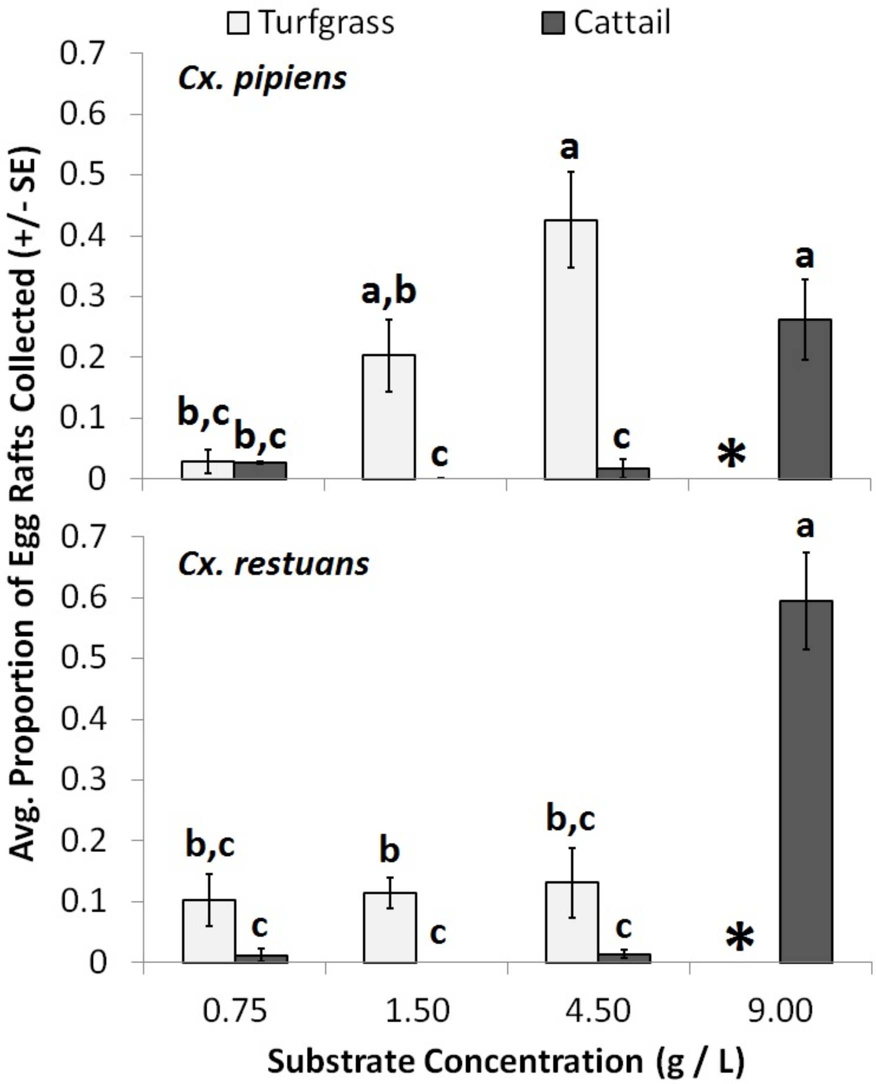

Figure 4. Proportion of *Cx. pipiens* and *Cx. restuans* egg rafts collected from infusions containing varying concentrations of turfgrass or cattail substrate. Turfgrass was not evaluated at the 9.0 g / L concentration (indicated by the asterisk in figure). For each species, columns with the same letter are not significantly different (P>0.05; post-hoc test in GLMM with Bonferroni correction).

Table 1. General linear models evaluating the effects of larval rearing density and orthophosphate enrichment (0.81 mg PO_4-P / L) of cattail infusions (4.5 g / l) on the duration of juvenile development, adult longevity and wing length of male and female *Cx. pipiens*.

Response Variable	Parameter[1]	Males			Females		
		Coeff. (± 95% CI)	t	p-value	Coeff. (± 95% CI)	t	p-value
1 / duration of juvenile development	Intercept	0.098 (0.002)	52.8	<0.001	0.084 (0.003)	32.5	<0.001
	Orthophosphate	0.000 (0.002)	<0.1	0.946	0.003 (0.003)	0.9	0.377
	Larval Density	0.004 (0.002)	1.8	0.089	**0.011 (0.003)**	**3.7**	**0.003**
1 / adult longevity	Intercept	0.581 (0.017)	34.6	<0.001	0.397 (0.026)	15.2	<0.001
	Orthophosphate	**-0.064 (0.019)**	**-3.3**	**0.006**	0.065 (0.030)	2.2	0.051
	Larval Density	**-0.186 (0.019)**	**-9.6**	**<0.001**	-0.007 (0.030)	-0.2	0.817
wing length	Intercept	0.361 (0.004)	100.4	<0.001	0.283 (0.004)	75.2	<0.001
	Orthophosphate	-0.009 (0.004)	-2.1	0.059	0.003 (0.004)	0.7	0.511
	Larval Density	**-0.039 (0.004)**	**-9.4**	**<0.001**	**-0.014 (0.004)**	**-3.2**	**0.007**

[1] The interaction term (orthophosphate x larval density) was excluded from the final models as it was not a significant effect for any of the response variables for males or females.

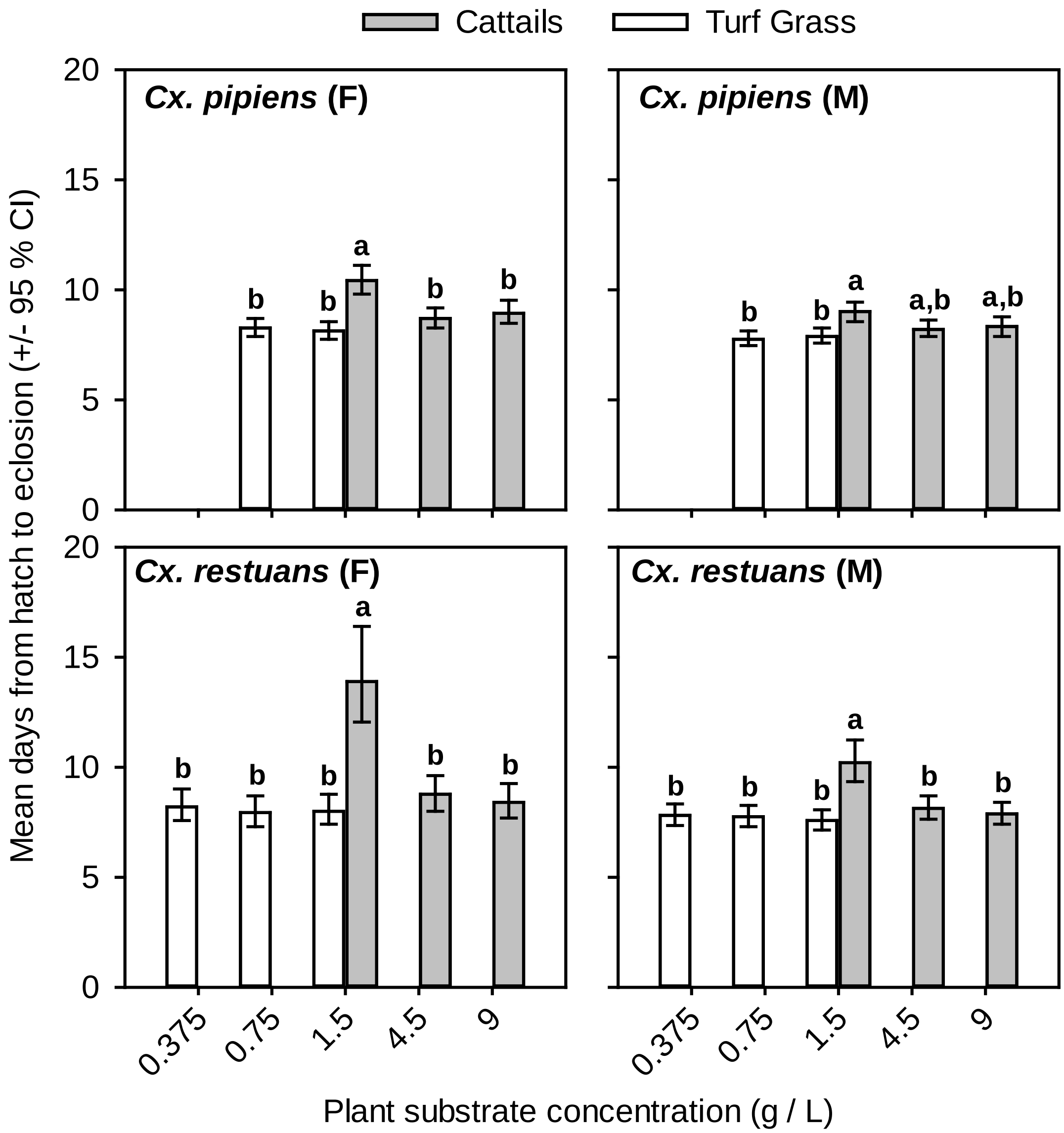

Figure 5. Juvenile development time of male (M) and female (F) *Cx. pipiens* and *Cx. restuans* reared in infusions containing varying concentrations of turfgrass or cattail substrates. Columns within each group that share the same letter were not significantly different (P>0.05; post-hoc test with sequential Bonferroni correction in GLM using 1 / Days to Eclosion as the response variable).

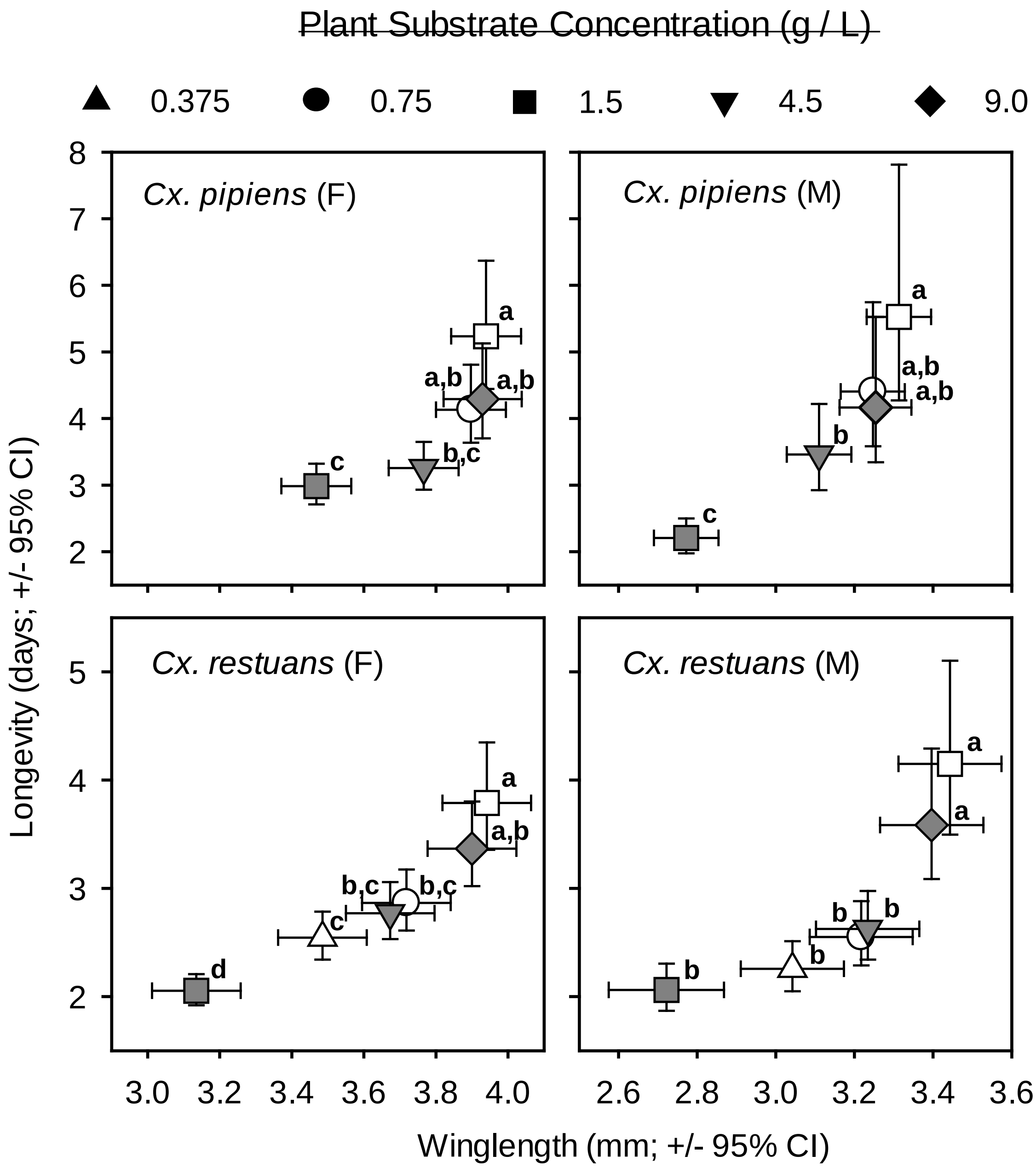

Figure 6. Average wing length and longevity of adult male (M) and female (F) *Cx. pipiens* and *Cx. restuans* reared in infusions containing varying concentrations of turfgrass (open symbols) and cattails (closed symbols). Average longevity within treatments for each group that share the same letter were not significantly different (P>0.05; post-hoc test with sequential Bonferroni correction in GLM using 1 / days surviving as the response variable). A significant linear relationship was observed between adult winglength and transformed adult longevity (1 / days surviving) for male (R^2 = 0.546) and female (R^2 = 0.512) *Cx. pipiens*, and male (R^2 = 0.436) and female (R^2 = 0.509) *Cx. restuans*.