

Small-stature emergent macrophytes and crepuscular sprinkler disturbance reduce mosquito abundance in wetland mesocosms

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ABSTRACT: The impact of emergent macrophyte species and crepuscular sprinkler disturbance on mosquito abundance over a 2-year period was measured in wetland mesocosms. Mosquito oviposition and abundance of immature mosquitoes and aquatic invertebrates were monitored in monotypic plots of small-stature (height of mature stands <1.5 m) alkali bulrush (*Schoenoplectus maritimus*) and large-stature (height of mature stands > 2 m) California bulrush (*Schoenoplectus californicus*) without or with daily sprinkler showers to deter mosquito egg laying. Relative to wetlands without operational sprinklers, oviposition by culicine mosquitoes was reduced by > 99% and immature mosquito abundance was reduced by > 90% by crepuscular sprinkler applications. Mosquito abundance or distribution in wetlands did not differ between the two bulrush species subjected to the sprinkler treatment. Alkali bulrush wetlands without daily sprinkler treatments contained more egg rafts but significantly fewer mosquito larvae than did California bulrush wetlands. Predaceous damselfly naiads were 3-5 times more abundant in alkali bulrush than in California bulrush. Stem density, rate of spread, and autumnal mortality of alkali bulrush were higher than for California bulrush. Replacement of large emergent macrophytes by smaller species may enhance the efficacy of integrated mosquito management programs to reduce mosquito-transmitted disease cycles associated with multipurpose constructed wetlands used worldwide for water reclamation and habitat restoration. *Journal of Vector Ecology* 38 (2): 379-389. 2013.

Keyword Index: *Schoenoplectus*, bulrush, constructed wetlands, oviposition deterrence, top-down effects, Zygotera.

INTRODUCTION

Wetlands support diverse communities of flora and fauna, buffer against natural disasters, and play key roles in biogeochemical nutrient cycling and pollutant removal (Mitsch and Gosselink 2000, Kadlec and Wallace 2008). In recent years, increasing numbers of wetlands have been constructed as less costly alternatives to traditional wastewater treatment systems and to fulfill multipurpose goals such as recreation, environmental education, and mitigation of habitat loss from human activities (Kadlec and Wallace 2008). The simultaneous fulfillment of such diverse goals can be especially challenging for wetlands treating wastewaters. Balancing ecological services requires an understanding of the ecological interactions that are influenced by the design and manipulation of complex wetland ecosystems. For example, some of the design features and management practices for wetlands used to treat ammonia-rich municipal and agricultural wastewaters can concomitantly generate large populations of disease-vectoring and/or pestiferous mosquitoes (Knight et al. 2003, Walton 2012). Mitigation of vector production can require time-consuming and expensive mosquito abatement programs.

Integrated mosquito management (IMM) involves a combination of strategies that can include the modification of the physical and ecological structure of the aquatic developmental sites of mosquitoes, education and outreach, use of biorational agents (e.g., mosquito-specific bacterial larvicides, hormonal insect growth regulators) and/or biological control agents (e.g., larvivorous fish) against immature mosquitoes and, if necessary, the application of

chemical insecticides. Wetland management and design strategies that reduce refuge and resources available for immature mosquito development are important components of IMM for constructed treatment wetlands (Knight et al. 2003, Sanford et al. 2005, Thullen et al. 2005, Walton 2012). Mosquito control strategies that focus on the aquatic stages of the mosquito life cycle can target those developmental sites that have the potential to produce large numbers of adult mosquitoes and will reduce the need to use chemical insecticides against adult mosquitoes that disperse from constructed wetland developmental sites into the surrounding landscape.

Alkali bulrush, *Schoenoplectus* (= *Scirpus*) *maritimus* (L.) Lye, is a widely distributed wetland species (USDA-NRCS 2011) that may be more amenable than large macrophytes to IMM strategies for some constructed wetlands. Structural complexity above and below the water surface, spatial and temporal growth patterns, and suitability as a food source for herbivores are among the plant characteristics that can influence mosquito abundance (Collins and Resh 1989, Walton et al. 1990, Knight et al. 2003, Jiannino and Walton 2004). California bulrush, *Schoenoplectus californicus* (C.A. Meyer) Palla, is a large-stature (height > 2 m) emergent macrophyte planted commonly in managed wetlands in the western United States. Other large-stature (height > 2 m) emergent macrophytes used frequently in treatment wetlands include bulrushes (*S. acutus* (G.H.E. Muhlenberg ex J. Bigelow), *S. americanus* (Persoon) Volkart ex Schinz and R. Keller), cattail (*Typha* spp.) and common reed (*Phragmites australis* (Cav.) Trin. ex Steud.). Dense stands of large-stature emergent macrophytes often reduce the efficacy of

mosquito management efforts (Walton et al. 1990, Walton and Workman 1998, Thullen et al. 2002, Knight et al. 2003). Comparatively low height (<1.5 m), shallow roots, pliable stems, an annual cycle that naturally reduces above-ground plant biomass, and rapidly sinking dead plant biomass of alkali bulrush are expected to reduce the cost of vegetation management and mosquito abatement as well as enhance the efficacy of ecologically-based mosquito control strategies.

In this study, we compared mosquito oviposition and immature abundance in replicate plots containing either *S. maritimus* or *S. californicus*. Bulrush species was cross-classified with the absence or presence of a sprinkler treatment to assess the deterrent of mosquito oviposition by disturbance from sprinklers. The invertebrate communities within the four treatments also were examined. The null hypotheses that mosquito egg laying, immature mosquito abundance and invertebrate community structure did not differ among the four treatments were tested.

MATERIALS AND METHODS

Study Site

Replicate monotypic stands of *S. maritimus* and *S. californicus* were established at the University of California-Riverside Aquatic and Vector Control Facility (Riverside, CA, USA). Seeds collected from three bulrush populations in Riverside County were separated from their siliceous outer husks and refrigerated at 4° C in peat moss for 3 months. After cold storage, seeds of *S. maritimus* (53 g, ~22,000 seeds) and *S. californicus* (53 g, ~80,000 seeds) were planted in separate containers and cultivated as monotypic stands under field conditions.

Twenty-four plastic wading pools (diameter = 1.25 m, height = 0.3 m) were distributed among six earthen ponds (3.7 x 7.2 m). Water was supplied continuously to the ponds from a reservoir supplied with groundwater. Water depth was maintained in each pond by a float valve. Four 2.54 cm diameter holes were drilled at a height of 0.2 m along the perimeter of each pool to facilitate water exchange between the inside of the wading pool and the pond. Water depth in each pond was maintained below the upper lip of the wading pools in year 1 (depth ~ 0.3 m). Bulrush roots occluded the holes in the sides of the wading pools by the end of the first field season; therefore, water levels (depth ~0.32 m) were maintained above the lip of the wading pools during year 2 of the study.

Young plants of each species were transplanted to the wading pools that had been filled with 0.15 m of steam-autoclaved soil. Nine clumps of a single bulrush species (five stems per clump) were planted into each wading pool in November, 2007. Pools on the north side of the ponds contained *S. californicus* to minimize shading of *S. maritimus* in adjacent pools (Figure 1). The two pools nearer the inflow spigot were placed 3 m from the other pair of pools. Each pool of a pair was separated from the other pool and the pond sides by ~0.25 m. Open water was maintained in areas outside of wading pools by removal of newly emerged vegetation.

Sprinklers were placed at the center of each pond (Figure

1). A 360°-flow sprinkler head (Toro Company, Bloomington, MN, USA) was attached at the apex of vertical 3 m tall PVC pipe (diameter = 1.27 cm [½ inch]). Non-operational sprinklers were positioned in the ponds without the sprinkler treatment. Sprinkler flow in each of the three ponds with operational sprinklers was adjusted to reach the perimeter of the pond. In-line timers (9100 series model, DIG Corp, Vista, CA, USA) regulated water flow from the reservoir. Sprinklers were operated for 3 h in the evening (beginning at sunset) and 1 h in the morning (beginning 1 h before sunrise) when *Culex* spp. oviposition was most likely to peak (Logen and Harwood 1965, Schreiber et al. 1989, Beehler et al. 1993). Timer programs were adjusted twice monthly to account for changes in daylength. Six plastic washtubs (volume: 11.5 or 15 liters; Rubbermaid, Fairlawn, OH, USA) were tethered to the pools and floated in the corners of each pond and in the center between paired pools to assess whether water from the sprinklers was blocked by plant growth across time. On the day before egg rafts were enumerated in the plots, any water in the tubs was removed. The volume of water in each tub was measured the following morning. Sprinkler treatments were run March – October, 2008 in year 1 and May – October, 2009 in year 2. The sprinkler treatment was reassigned among the ponds at the start of year 2.

Mosquitoes and invertebrates

Mosquitoes were sampled during the periods that sprinklers were activated. Mosquitoes also were sampled on 10 March 2008 and 11 May 2009 before sprinkler applications began for year 1 and year 2, respectively. After 3 weeks of sprinkler operation, surveys of mosquito abundance in bulrush habitat commenced on 1 April 2008. *Culex* spp. egg rafts floating on the water surface were counted twice monthly within floating PVC quadrats (0.09 m²) taken at three equidistant locations along a transect in each pool. Transects radiated from the sprinkler at the pond center to the corner of the pond perimeter to examine the relationship between egg raft distribution and the distance from the sprinkler. Bulrush stems were carefully manipulated by hand to ensure egg rafts were counted with minimal disturbance of plant growth.

Immature mosquitoes were sampled 5 days after an egg raft survey, but less frequently than was mosquito egg-laying. Standard 350 ml dippers (Service 1993) were used to sample immature mosquitoes and associated invertebrates within the emergent vegetation. Dipper samples taken within emergent vegetation can provide trends for populations of mosquitoes and vegetation-dwelling macroinvertebrates that are similar to the trends of abundance for these taxa observed in collections taken using nets along transects through the vegetation (Mulla 1990). A dipper sample was taken at each location used in the egg raft survey. Each sample was concentrated by filtration through a 148 µm mesh screen and preserved with 70% ethanol.

Immature mosquitoes were categorized as small larvae (1st and 2nd instars), large larvae (3rd and 4th instars), or pupae. Small larvae and pupae were identified to genus and large larvae to species using Meyer and Durso (1998). Other

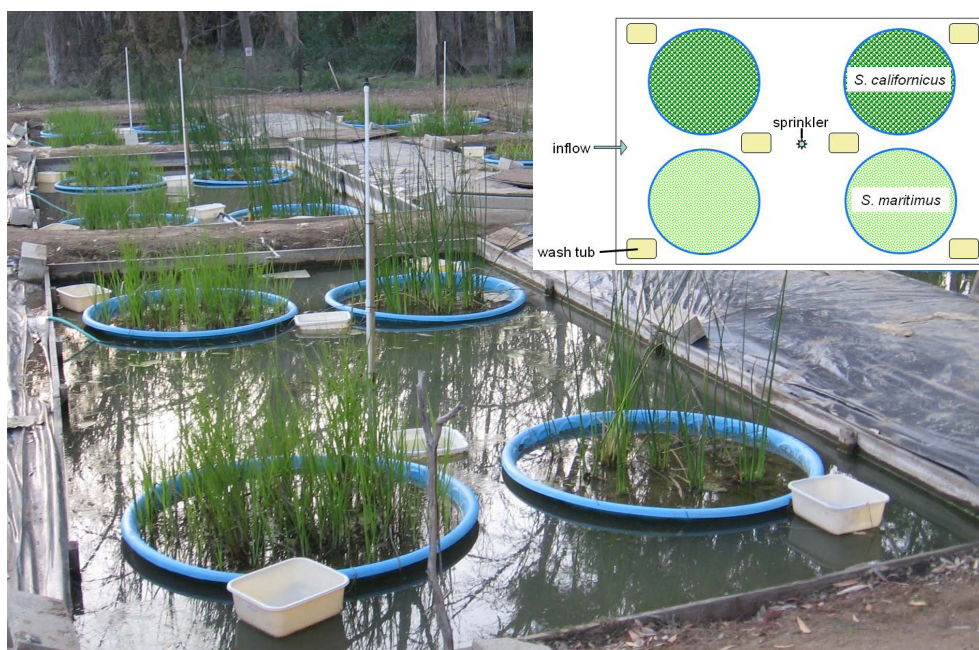


Figure 1. Study ponds and a schematic of the bulrush plots in a pond (insert) at the U.C.-Riverside Aquatic and Vector Control Facility. North is indicated by N.

invertebrates collected in dipper samples were enumerated and identified using Merritt and Cummins (1996) and Pennak (1989).

Bulrushes

For each bulrush species, the densities of live standing, dead standing, and dead floating stems were assessed monthly in year 1 and four times during year 2 within 0.09 m² PVC quadrats. Plant heights were measured for up to fifteen stems within the three quadrats in each pool. The height of each plant was measured from the base at the soil substrate to the apex of the aerial shoot. Digital photographs were taken from the top of a 2.4-m folding ladder and the percent of surface area of each pool covered by plant biomass was estimated using Image-Pro Plus (v. 3.0, Media Cybernetics, Bethesda, MD, USA). Percent cover was calculated for newly transplanted stands in 2007. Image analysis of percent cover was not carried out in 2009 because bulrush growth had covered the surfaces of all pools for both species and dense algal mats formed within the few open areas within the bulrush limited the precision of digital processing.

Statistical analyses

The effects of bulrush species and sprinkler treatment on mosquito egg laying and abundance were tested using repeated-measures Multivariate Analysis of Variance (MANOVA) across dates or ANOVA on single dates and between years (SYSTAT, ver. 9: SPSS Inc., 1998). Mosquito counts were $\log_{10}(x+1)$ transformed and mosquito life stages were analyzed separately (egg rafts, small larvae, and large larvae) by season (spring vs summer in 2008) and year. Pupae were too rare for statistical analyses. Bulrush characteristics (densities of live, dead standing, and dead floating stems; stem height; percent cover) were analyzed separately for each year. Data with non-normal distributions were analyzed using Kruskal-Wallis ANOVA (KW-ANOVA) by date or year.

Mosquito data excluded from statistical analysis included: (1) one replicate from April 2008 when water flow through a timer was clogged and sprinkler flow did not reach the vegetation and (2) the first date of 2009 before sprinklers were turned on.

Canonical Correspondence Analysis (CCA) (CANOCO, ver. 4.5, ter Braak and Smilauer 2002) was used to generate an ordination biplot that summarized the spatial and temporal associations among invertebrate taxa collected in dipper samples. The nominal environmental variables included sample year, presence/absence of sprinkler treatment, bulrush species, location of pool relative to the pond inflow, and position of the sample within pools. Rare invertebrate groups (<5 individuals collected) were removed from the CCA model. Variance of invertebrate data was normalized using the square-root transformation.

RESULTS

Mosquitoes

Culex spp. were the most common (97% of the total number) immature mosquitoes collected and were predominantly (91%) 1st and 2nd instars. Large *Culex* larvae (3rd and 4th instars) were common, albeit at lower abundances (8% of total), and were identified ($n = 847$) as *Culex tarsalis* Coquillett (85%) and *Culex stigmatosoma* Dyar (15%). *Culex* pupae (1%) were relatively rare.

Anopheles larvae were rare (<1% of total) in year 1; however, larvae were common and steadily increased in abundance during year 2, representing 28% of the total mosquito numbers by the end of 2009. *Anopheles hermsi* Barr and Gupta vanij was the sole *Anopheles* species identified.

Prior to activating the sprinklers, larval mosquito abundance was similar across the six ponds on 10 March 2008 (~10 1st and 2nd instars per dipper sample) and again at the start of 2009 (ANOVAS, $P > 0.05$). In wetlands without

Table 1. Results of Kruskal-Wallis ANOVAS to evaluate the significance of sprinkler treatments on the abundance of egg rafts, small larvae (1st and 2nd instars), and large larvae (3rd and 4th instars) of mosquitoes in 1.2 m² wetland mesocosms.

Life stage	Year	Season	df	U-Value	P-value
Egg rafts	2008	Spring	1	810	<0.001
		Summer	1	864	<0.001
Small larvae	2009	Summer	1	793	<0.01
	2008	Spring	1,56*	342.97*	<0.001
		Summer	1	738	0.02
Large larvae	2009	Summer	1	973	<0.001
	2008	Spring	1	576	<0.01
		Summer	1	720	0.04
	2009	Summer	1	758	0.02

* F test, F-value

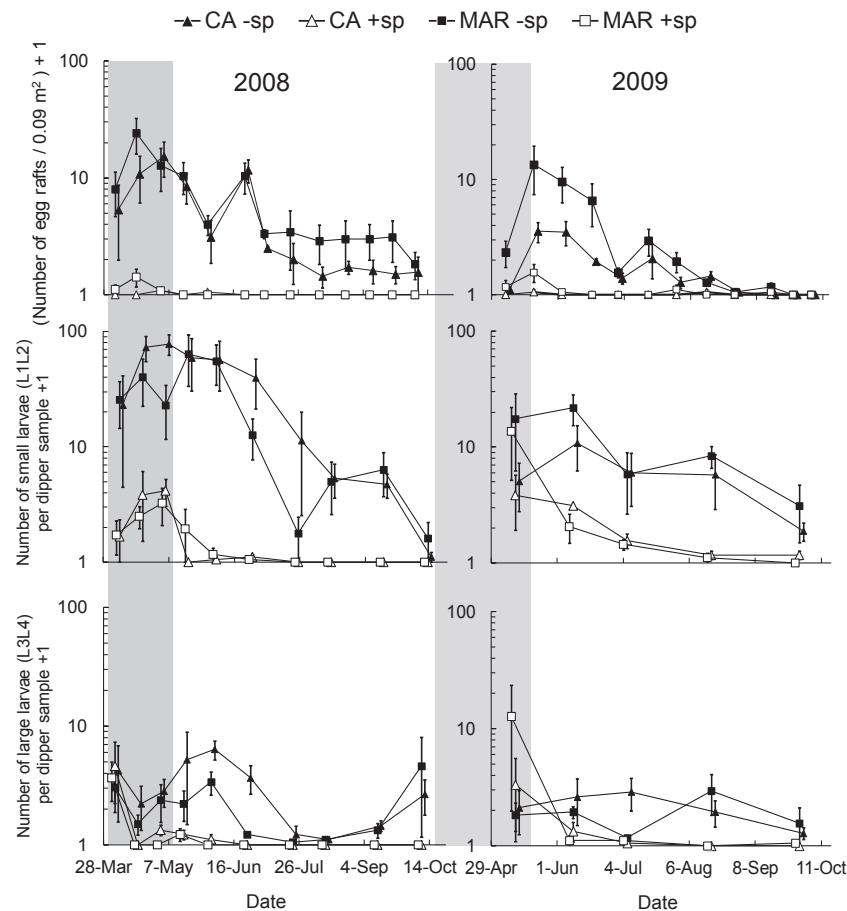


Figure 2. Mean (± SE) abundance of *Culex* spp. egg rafts (a), small larvae (b: 1st and 2nd instars) and large larvae (c: 3rd and 4th instars). Trends are illustrated between sprinkler treatment (+ sp) and control (- sp) ponds within *S. californicus* (CA) and *S. maritimus* (MAR) plots. The shaded areas indicate the durations of sprinkler malfunction in one pond (April, 2008) and inactivity in all ponds (October, 2008 – May 2009).

sprinklers, *S. maritimus* plots contained significantly more egg rafts (64% of total) than *S. californicus* plots (2008: $F_{1,30} = 4.88$, $P < 0.04$; 2009: $F_{1,30} = 17.98$, $P < 0.01$). Egg laying by mosquitoes differed among ponds (2008: $F_{2,30} = 5.97$, $P < 0.01$; 2009: $F_{2,30} = 8.82$, $P < 0.01$); however, the trends for oviposition into the two bulrush species were consistent across ponds within each year of the study (bulrush ' pond: 2008: $F_{2,30} = 0.94$, $P = 0.40$; 2009: $F_{2,30} = 1.68$, $P = 0.20$). The number of egg rafts in *S. maritimus* plots was significantly greater than in *S. californicus* plots during summer 2008 and spring 2009 ($P \leq 0.05$). Egg raft abundance in the second year of the study declined from the first year of the study by about 6.5-fold in *S. californicus* plots and 2.4-fold in *S. maritimus* plots.

In contrast to trends for egg raft abundance in plots without sprinklers, greater numbers of large (3rd and 4th instars) mosquito larvae were present in *S. californicus* plots than in *S. maritimus* plots during spring and early summer (Figure 2). Whereas, the abundance of small larvae was greater in *S. californicus* plots during 2008 ($F_{1,30} = 5.84$, $P = 0.02$) but was greater in *S. maritimus* plots during 2009 ($F_{1,30} = 5.07$, $P = 0.03$), the abundance of large mosquito larvae in *S. californicus* plots was greater than in *S. maritimus* plots in spring and early summer of both years (2008: $F_{1,30} = 8.90$, $P = 0.006$; 2009: bulrush \times pond: $F_{2,30} = 3.35$, $P = 0.05$; June and July, 2009: $F_{2,30} = 14.69$, $P = 0.0006$). About 2-fold more large larvae were present in *S. californicus* plots than in *S. maritimus* plots during 2008. Larval mosquito abundance declined during summer. Spatial variation of mosquito abundance among locations along transects (sprinkler-side, center, perimeter-side) in bulrush plots or between locations relative to the pond inflow for either egg rafts (MANOVAs: $P > 0.14$) or immatures (MANOVAs: $P > 0.28$) was not statistically significant.

Crepuscular sprinkler treatments reduced mosquito production (Figure 2). The numbers of egg rafts (<1% of total) and immature mosquitoes (<10% of total) in bulrush plots in the sprinkler treatment were significantly lower than in bulrush plots without operational sprinklers. Mosquito reductions were sustained at similar levels across both study years (Table 1: ANOVAS, $P < 0.05$). In general, mosquito populations increased in bulrush mesocosms when sprinklers were inactive or malfunctioning and declined within 2 weeks after sprinkler operation was re-established (see shading in Figure 2). Mosquito production from wetland plots did not differ between bulrush species and spatially within the bulrush plots in the sprinkler treatment (KW ANOVAS by date; $P > 0.05$).

Bulrushes

Schoenoplectus maritimus and *S. californicus* differed in height, density, mortality, and percent cover across seasons and in response to the sprinkler treatments during year 1 (Figure 3). *Schoenoplectus maritimus* was 70 cm shorter and 60% more dense, on average, than *S. californicus* across all sample dates (Table 2: MANOVA: $P < 0.001$). Stem mortality was minimal in bulrush stands (4% of total stems) and similar for both species (KW-ANOVA: $P > 0.050$) until October, 2008 when *S. maritimus* senesced (Figure 4). Standing dead stem

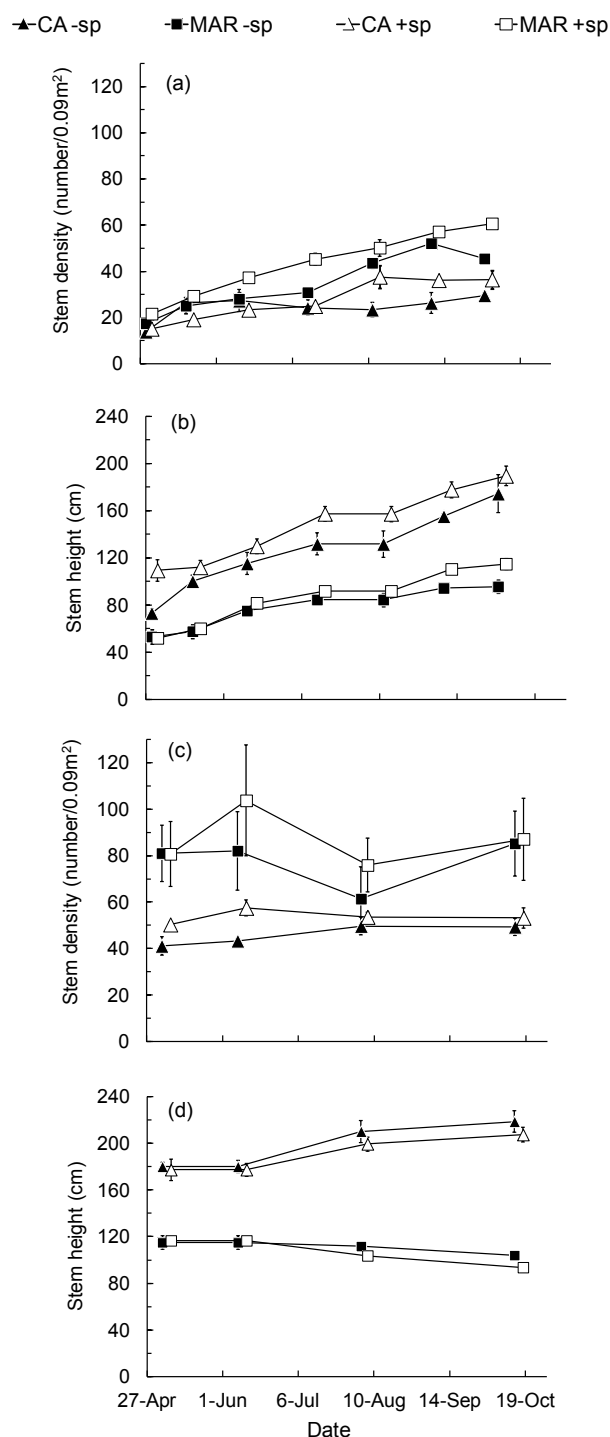


Figure 3. Mean (\pm SE) stem density (2008 (a); 2009 (c)) and height (2008 (b); 2009 (d)) of *S. californicus* (CA) and *S. maritimus* (MAR) with (+ sp) and without (- sp) sprinkler applications. Stem height differences between bulrush species are significantly different ($P < 0.001$) on all dates. Points are offset to facilitate illustration.

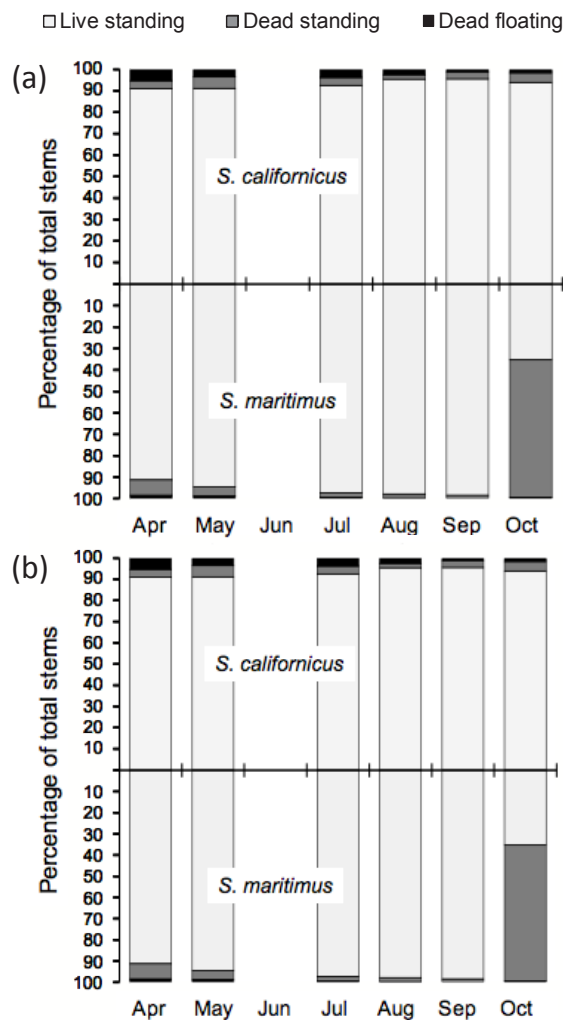


Figure 4. The relative abundance of live and senescent (dead standing or floating) stems of California (*S. californicus*) and alkali (*S. maritimus*) bulrushes during 2008 (a) and 2009 (b).

counts of *S. maritimus* increased 60%, while *S. californicus* mortality was constant. Dead floating stems of *S. maritimus* increased from <1% to 28% of total dead stem counts in subsequent surveys during 2009. Overall, the proportion of total dead to live stems was significantly higher for *S. maritimus* than *S. californicus* throughout 2009 (MANOVA: $P < 0.001$).

The percent cover for each species was similar (mean = 10% of pool surface area) immediately after transplantation in November, 2007. However, coverage diverged during 2008, especially during the first month of sampling (April-May), when *S. maritimus* covered (mean = 41%) nearly twice as much surface area of pools than *S. californicus* (mean = 24%). By autumn 2008, vegetation covered approximately 50% of the wetland mesocosm surfaces in three of the bulrush-sprinkler treatments; percent cover in the *S. californicus* treatment without sprinklers was only about 34%.

Sprinkler applications enhanced the growth of bulrush in 2008 but not in 2009 when water levels the ponds were maintained above the wading pools. Bulrush stem densities

in plots under sprinklers were on average 15% higher than wetland plots without sprinkler applications (Table 2: MANOVA: $P \leq 0.036$). *Schoenoplectus californicus* receiving sprinkler applications was on average 14 cm taller than in plots without sprinklers in 2008 (MANOVA: $P < 0.001$); however, plant height did not differ between the sprinkler treatments in 2009 (MANOVA: $P = 0.11$). *Schoenoplectus californicus* survivorship was positively related to the presence of sprinklers, especially during the first growing season when dead stems in plots under sprinklers were nearly 3-fold fewer than in plots without sprinklers. Sprinkler effects on alkali bulrush appeared minimal before senescence; however, post-senescence, the number of dead floating stems was greater in plots under sprinklers (Table 2: 2009 – KW-ANOVA: $P < 0.01$).

Spatial variability of bulrush along pool transects was exemplified by a 40% increase in total stem density at pool edges compared to pool centers (Table 2: MANOVA: $P = 0.011$). This difference was more distinct in *S. maritimus* (71% higher at edge) than *S. californicus* (23% higher at edge) plots. Stem heights were not statistically different among quadrat locations; however, the average height differential was noticeably greater at pool centers compared to pool edges in *S. californicus* (+ 22 cm) but not *S. maritimus* (+ 3 cm). Bulrush height and density did not differ between pools in relation to proximity to the inflows to ponds.

Invertebrates

CCA axes 1 and 2 explained 79% of the variation between invertebrates and the environment and 22% of the variation among invertebrate groups (Figure 5a). The insect predator community was primarily (98% of total numbers) composed of damselflies (Zygoptera: Coenagrionidae), dragonflies (Epirocta, Anisoptera: Libellulidae and Aeschnidae), and surface-dwelling hemipterans (Hemiptera: Veliidae and Hebridae). Damselflies were the most numerous taxon (63% of total abundance) and the trends for damselfly nymph abundance in bulrushes with sprinklers differed from those without sprinklers (Figures 5b and 5c; bulrush \times sprinkler: 2008: $F_{1,56} = 8.78$, $P < 0.004$; 2009: $F_{1,68} = 9.51$, $P < 0.003$). Without sprinklers, damselflies were three- to five-fold more abundant in *S. maritimus* than in *S. californicus* (2008: $F_{1,34} = 118.52$, $P < 0.0005$; 2009: $F_{1,34} = 65.58$, $P < 0.0005$). In general, predators were 60% more abundant in *S. maritimus* than *S. californicus*. Predaceous insects declined with sprinkler activity during year 1 of the study; 66% more predators were present in wetlands without sprinklers. Abundances did not differ appreciably between the sprinkler treatments during 2009.

Immature mosquitoes constituted more than 25% of the non-predaceous insects and a strong negative association to sprinkler applications is evident in the CCA plot (Figure 5a). The remaining non-predatory insect taxa were largely midges (Diptera: Chironomidae), mayflies (Ephemeroptera: Baetidae, *Callibaetis* sp.), and caddisflies (Trichoptera: Hydroptilidae). In combination, these insects were consistently detected at greater levels in *S. californicus* than *S. maritimus* and varied in relation to the sprinkler treatment. For example, midges, the most common group (greater than 70% of total numbers),

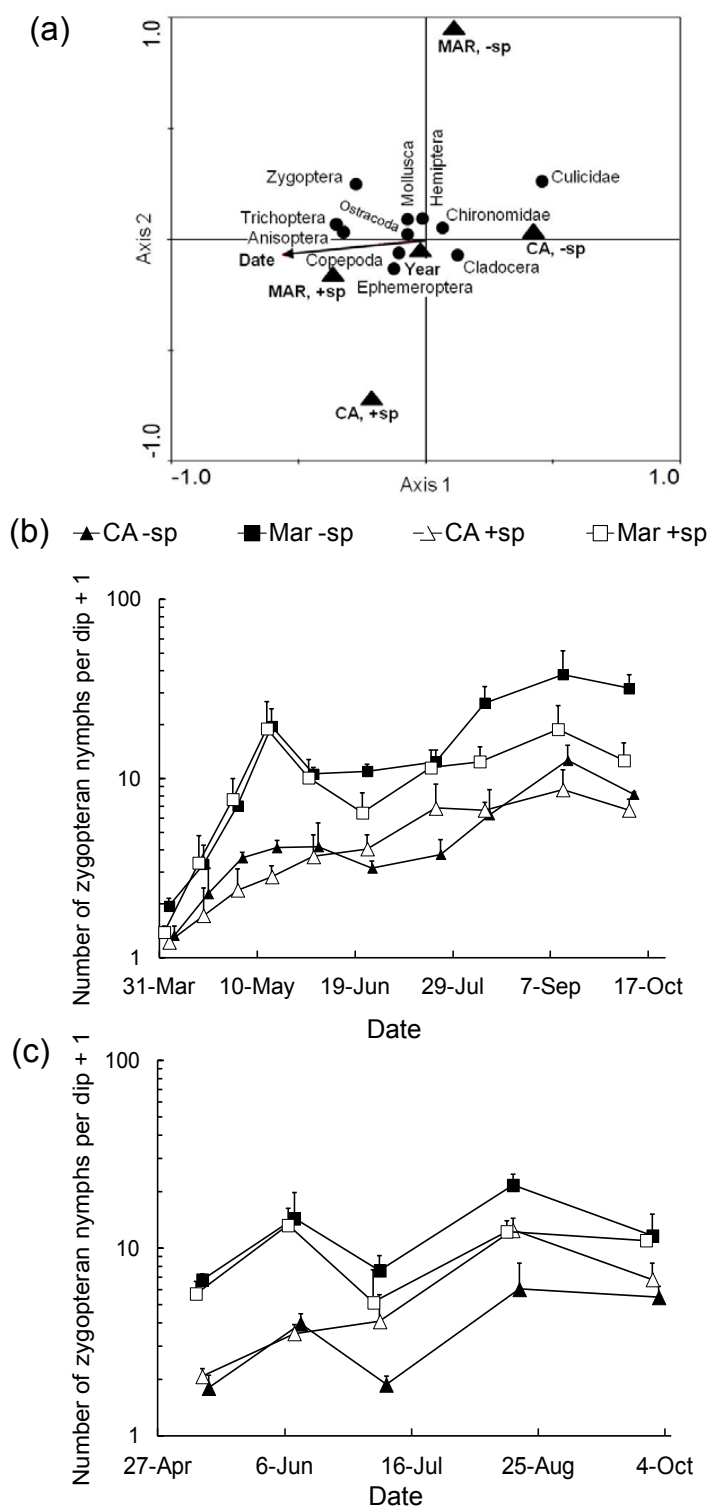


Figure 5. Canonical Correspondence Analysis of the invertebrate community (11 taxa) in relation to sampling date and five nominal environmental factors (a) and the abundance (mean \pm SE) of zygopteran nymphs in four treatments during 2008 (b) and 2009 (c). MAR = *S. maritimus*; CA = *S. californicus*; +sp = with sprinklers; -sp = without sprinklers. Points in panels b and c are offset to facilitate illustration.

were four-fold more abundant in California bulrush without sprinklers compared to *S. maritimus* under sprinklers during 2008. Despite greater densities of *S. californicus* in year 2, midge abundance declined. The mayflies displayed the largest difference in abundance between bulrush species and were, on average, more than five times more likely to be found in *S. californicus* than in *S. maritimus*.

The most numerous invertebrate groups belonged to the Class Crustacea, with more than 91% of all dipper specimens made up of three orders: Cladocera, Copepoda, and Ostracoda. Cladocerans and copepods, which comprised 86% of all crustaceans, averaged 38% greater numbers in California bulrush wetlands compared to alkali bulrush wetlands. In general, crustaceans appeared to benefit from the effects of sprinkler applications during both study years and were 33% more abundant in sprinkler treatment plots compared to plots without sprinklers.

DISCUSSION

Different growth traits of *S. maritimus* and *S. californicus* contributed to differences of mosquito abundance in the absence of sprinkler applications. *Culex tarsalis* uses a combination of visual, olfactory and gustatory behaviors during oviposition site selection (Beehler et al. 1993, Isoe et al. 1995), and is expected to be sensitive to differences in the physical and chemical conditions between the bulrush species. Stem density of emergent macrophytes is generally associated directly with mosquito production (Walton and Workman 1998, Jiannino and Walton 2004). Stem densities of *S. maritimus* were greater than for *S. californicus* and the numbers of *Culex* egg rafts present in wetland mesocosms containing *S. maritimus* were greater than in mesocosms containing *S. californicus*. The pliable *S. maritimus* stems also were more vulnerable to collapse by disturbances than were stems of *S. californicus*. Mortality related to senescence that occurred predominantly in *S. maritimus* increased the rate at which biomass re-entered the water column to stimulate vegetative decay and release of chemicals that enhance egg-laying by mosquitoes (Isoe and Millar 1995, Sanford et al. 2003).

Despite greater egg raft counts in *S. maritimus* plots, the abundance of mosquito immatures (3rd and 4th instars) in *S. maritimus* wetlands was less than in *S. californicus* plots. Reduced prey detection rates by mosquito predators and enhanced resource availability within dense stands of *S. californicus* contribute to mosquito production (Berkelhamer and Bradley 1989, Walton and Workman 1998, Jiannino and Walton 2005). In our study, the higher abundance of predaceous damselfly naiads in *S. maritimus* plots was associated with lower immature mosquito abundance, even though the stem densities of *S. maritimus* were greater than for *S. californicus*. Emergence rates of adult mosquitoes are directly related to the abundance of large larvae (Popko 2005); based on the abundance of 3rd and 4th instars, mosquito production from *S. californicus* plots was greater (2008: 2-fold; 2009: 1.3-fold) than that from *S. maritimus* plots. Comparatively greater predator abundance, especially

damselfly nymphs (Zygoptera: Coenagrionidae), coupled with lower numbers of mosquitoes and other non-predaceous taxa, (e.g., microcrustaceans and chironomid midges) is concordant with a conclusion that top-down regulation of larval mosquito populations in *S. maritimus* plots was greater than in *S. californicus* plots. Moreover, top-down effects on larval mosquito populations can be even greater in some regions of wetlands. Some important predators of mosquitoes in wetlands, such as backswimmers (Hemiptera: Notonectidae) and larvivorous fish which are known to be effective mosquito predators at the interface of open water and vegetation (Walton and Workman 1998, Peck and Walton 2008, Walton et al. 2012), were very rare or absent, respectively, in the wetland mesocosms.

Sprinklers reduced mosquito abundance by 99% of that from wetlands without sprinklers. This reduction was comparable to that observed for sprinklers in a system treating wastewater with floating vegetation (Epibane et al. 1993). The failure of sprinkler systems to markedly reduce mosquito oviposition in *S. californicus* in operational constructed wetlands is likely caused by insufficient height of the sprinkler heads relative to the 3 to 4 m height of bulrush (personal observation), by incomplete coverage by sprinkler water of the area supporting emergent macrophytes and by high plant densities (densities up to 800 stems/m²: Thullen et al. 2002, Popko 2005). Smaller *S. maritimus* was expected to allow for greater sprinkler penetration and mosquito oviposition deterrence than in the larger *S. californicus*. A uniform spatial distribution of mosquito egg rafts in wetland plots subjected to sprinkler treatments and the presence of sprinkler water in the plastic washtubs positioned on the outside of bulrush plots indicate bulrush growth did not reduce sprinkler efficacy in our experiments. Plant height in our study may have been a less important determinant of sprinkler efficacy than in operational constructed wetlands since sprinkler stands were about 1 m higher than *S. californicus* plants (mean stem height ~2 m) and water was not impeded significantly by the plants.

Growth of both bulrush species might have been restricted by the limited depth and area of the wading pools and by nutrient limitation of bulrushes during the first year of the study. Soil depths of 15 cm probably limited vertical and horizontal growth of the root and rhizome systems of both species, although to different degrees. The roots of large bulrushes can extend to nearly 1 m in depth (60 to 90 cm: Campbell and Ogden 1999) in wetlands and were likely stunted vertically to a greater extent than the comparatively shallow (down to 0.25 m in depth) web-like roots of *S. maritimus*. The root/rhizome systems of *S. californicus* filled the wading pools during the first year of the study and occluded the holes intended to provide water exchange between the wading pools and the pond. The horizontal rhizomous growth of *S. maritimus* was nearly twice as fast as *S. californicus* and was likely affected by area constraints of the wading pools more than *S. californicus*. Daily sprinkler washes raised water levels above the edge of the wading pools and created conditions that stimulated growth and resulted in healthier bulrush stands with greater densities and heights for both species during year 1. The mean *S. californicus* density

Table 2. ANOVAs of bulrush stem density and height related to differences in sprinkler treatment and bulrush species.

Year	Treatment	Stem Density						Stem Height	
		Total		Live		Dead			
		Test statistic	P-value	Test statistic	P-value	Test statistic	P-value	Test statistic	P-value
2008	Sprinkler	$F_{1,68} = 6.78$	0.01	$F_{1,68} = 4.32$	0.04	$\dagger U_1 > 806$	≤ 0.05	$F_{1,68} = 25.66$	<0.001
	Bulrush	$F_{1,68} = 32.78$	<0.001	$F_{1,68} = 10.79$	<0.01	$\dagger U_1 < 788$	≥ 0.10	$F_{1,68} = 279.26$	<0.001
	Sprinkler × Bulrush	$F_{1,68} = 0.24$	0.63	$F_{1,68} = 0.95$	0.33	—		$F_{1,68} = 3.73$	0.06
2009	Sprinkler	$F_{1,68} = 4.56$	<0.04	$F_{1,68} = 5.86$	<0.02	$F_{1,68} = 10.39$	0.002	$F_{1,68} = 2.61$	0.11
	Bulrush	$F_{1,68} = 45.79$	<0.001	$F_{1,68} = 49.66$	<0.001	$F_{1,68} = 211.78$	<0.001	$F_{1,68} = 590.58$	<0.001
	Sprinkler × Bulrush	$F_{1,68} = 0.08$	0.77	$F_{1,68} = 15.38$	<0.001	$F_{1,68} = 0.007$	0.93	$F_{1,68} < 0.03$	0.88

[†]Kruskal-Wallis ANOVA from April to August, 2008.

was similar to that (200–450 culms/m²; Thullen et al. 2002, Popko 2005) observed in constructed wetlands but plant height was roughly a meter less than what is characteristic of mature stands. Mortality rates of *S. californicus* in mesocosms with a sprinkler treatment during the first growing season was lower than in mesocosms without operational sprinklers; but a similar trend was not apparent in *S. maritimus*. Low nutrient levels (NH₄-N: <0.045 mg/liter; NO₃-N: <4.35 mg/liter; total phosphorus (PO₄⁻³): <0.07 mg/liter; Walton, unpublished data) are characteristic of the reservoir water. Restricted water movement inside the wading pools probably caused nutrient limitation in wetland mesocosms in ponds without operational sprinklers during year 1.

Sprinklers effectively limited production of *Cx. tarsalis*, *Cx. stigmatosoma* and *An. hermsi*. The latter two species were present at variable levels lower than *Cx. tarsalis* during the 2-year study. *Culex tarsalis* larvae flourish in comparatively low nutrient habitats and adults are a key bridge vector of arboviruses such as the West Nile virus among vertebrate hosts in rural settings (Goddard et al. 2002). *Culex stigmatosoma* prefers to lay eggs on moderately polluted waters and plays an important role in maintaining reservoir levels of arboviruses in avian populations (Goddard et al. 2002). *Anopheles hermsi* is the key vector of human malaria in southern California (Meyer and Durso 1998). In general, malaria mosquitoes (*Anopheles* spp.) lay eggs singly either from the air or in contact with surfaces. They require a similar amount of time as *Culex* spp. mosquitoes to complete oviposition (average ~30 min) and have been observed to oviposit crepuscularly (Clements 1999). Sprinkler disruption of *Culex* spp. and *Anopheles* spp. was therefore not an unexpected outcome given known similarities in the onset and duration of oviposition in both

mosquito genera.

Crepuscular operation of sprinklers was sufficient to markedly reduce egg laying by mosquitoes in this study. Ebipane et al. (1993) found that sprinklers operated throughout the night reduced the abundance of immature mosquitoes, primarily *Culex quinquefasciatus* Say, by >94% in wastewater ponds with floating macrophytes. The peak oviposition period of *Cx. quinquefasciatus* starts at sunset and lasts for at least 2 h (Schreiber et al. 1989, Beehler et al. 1993); however, low levels of oviposition have been recorded outside of peak hours, which can account for 20% or more of the total egg raft production (Beehler et al. 1993). Laboratory experiments under controlled light conditions revealed that oviposition by *Cx. tarsalis* peaks at dusk and dawn (Logen and Harwood 1965). Sprinkler operation was limited to the predominant crepuscular periods of mosquito oviposition for our experiment because of potential flooding from sprinklers run continuously from before dusk until after dawn.

Schoenoplectus maritimus should be conducive to IMM strategies designed to reduce the use of chemical pesticides (Knight et al. 2003, Walton 2012). *Schoenoplectus maritimus* and closely related species (i.e., *Schoenoplectus robustus* (Pursh) M.T. Strong) generally prefer shallow (<0.30 m) water (Kantrud 1996, Deegan et al. 2005, Howard and Rafferty 2006) and, depending on the configuration of the constructed treatment wetland, may not require the labor-intensive, costly methods used in large-scale wetlands to manage large emergent macrophytes (Knight et al. 2003). The pliability and small stature of mature *S. maritimus* (height <1.5 m) should increase penetration of emergent vegetation by ground-based applications of mosquito-specific biorational control agents. Moreover, top-down effects which reduce the abundance

of immature mosquitoes were enhanced in wetland plots containing small-stature macrophytes. Walton and Mulla (1991) found that the abundance of nymphs of endophytically egg-laying odonates (i.e., *Enallagma civile* (Hagen) and *Ishnura barberi* Currie) in mesocosms containing spikerush (*Eleocharis* sp.) was greater than mesocosms lacking small-stature emergent macrophytes. The abundance of predaceous damselfly nymphs in *S. maritimus* wetlands was 3-5 times greater than in *S. californicus* plots and, despite enhanced mosquito oviposition in *S. maritimus* plots, larval mosquito populations were reduced relative to those in mesocosms containing *S. californicus*.

Based on its comparative ease of management and growth characteristics, *S. maritimus* is a potential alternative to larger emergent species such as *S. californicus* in shallow multipurpose wastewater wetlands. The rapid growth of *S. maritimus* might necessitate annual management to prevent excess biomass from accumulating in wetland waters and creating mosquito habitat, but relatively inexpensive management techniques such as mowing could be employed to thin emergent growth. Raising water levels during winter could be used to inundate and sink dead plant biomass during periods of low mosquito blood-feeding activity in climate zones with mild winters such as southern California. In wetlands treating wastewater, a rapid rate of coverage and high stem density would ensure vegetated zones are available during the growing season and provide sufficient surface area for the attachment of microorganisms to aid in treatment processes and conditions that reduce particulate matter (Kadlec and Wallace 2008). The sustainable IMM practices explored in this project increase the utility of constructed wetlands that provide essential water treatment and reclamation services while reducing the need to use chemical pesticides.

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