Effects of nutrients on mosquitoes and an emergent macrophyte, *Schoenoplectus maritimus,* for use in treatment wetlands

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ABSTRACT: *Schoenoplectus maritimus* (alkali bulrush) has desirable attributes, such as a short growth habit (height of mature stands < 1.5 m) and annual senescence, for a potential alternative to tall (height > 3 m) emergent macrophytes in shallow constructed treatment wetlands treating ammonium-dominated wastewater. The effects of different ammonium nitrogen (NH_4-N) levels on alkali bulrush growth and its ability to take up nutrients from the wastewater, as well as on mosquito production, across the range of NH_4-N found in constructed wetlands of southern California are unknown. We evaluated the effects of enrichment with NH_4-N on mosquito production and on the nutrient uptake and growth of alkali bulrush in two studies. Overall, significantly greater numbers (> 50%) of immature mosquitoes (mainly *Culex tarsalis*) were found in mesocosms enriched with NH_4-N than in mesocosms receiving ambient (<0.3 mg/liter) NH_4-N. High NH_4-N enrichment (up to 60 mg/liter) did not adversely impact the height and stem density of *S. maritimus,* although a significant decrease in biomass was observed at the highest enrichment level. Nitrogen uptake by alkali bulrush increased directly with NH_4-N enrichment, whereas carbon was conserved in the above-ground biomass across the enrichment gradient. Alkali bulrush is recommended for use as part of integrated mosquito management programs for moderately enriched, multipurpose, constructed treatment wetlands that improve water quality as well as provide wetland habitat for waterfowl. ***Journal of Vector Ecology*** **39 (1): 1-13. 2014.**

Keyword Index: *Culex*, ammonium nitrogen, alkali bulrush, nitrogen, carbon, mesocosms.

INTRODUCTION

The management of emergent macrophytes in constructed wetlands is a significant concern for wetland managers. Macrophytes fulfill several important functions for water quality improvement, such as enhancing nitrification by increasing oxygen concentration in the sediments of treatment wetlands and enhancing removal of toxic compounds such as ammonia and pathogens from wastewater (Brix 1997, Stottmeister et al. 2003). The macrophytes used in treatment wetlands include California bulrush [*Schoenoplectus californicus* (C.A. Meyer) Palla], cattail (*Typha* spp.) or common reed (*Phragmites australis* (Cav.) Trin ex. Steud); with *Typha* being the most commonly planted species worldwide (Kadlec and Wallace 2009). Paradoxically, these perennial emergent macrophytes grow very large (> 3 m high) and dense (up to 800 stems/m^2; Thullen et al. 2002) in nutrient-rich habitats and consequently impede mosquito control agents such as aerial application of granular forms of mosquito biopesticides (Walton 2003). Furthermore, these macrophytes form mats of decaying matter that are known to increase food resources for larval mosquitoes (Berkelhamer and Bradley 1989, Walton and Jiannino 2005).

Nutrient-elevated sewage is often associated with enhanced mosquito production (Chaves et al. 2009, Walton 2012). Besides increasing the production of macrophytes that can produce detrital resources for mosquito larvae, nutrient enrichment increases epiphytic and planktonic algal populations and other microbial assemblages that are food resources for mosquito larvae (Victor and Reuben 2002, Johnson et al. 2010). Among the dominant nutrients in effluent waters, nitrogenous compounds are known to increase mosquito abundance (Sanford et al. 2005).

Nitrogenous nutrients are also known to influence the growth and survival of emergent macrophytes. Ammonium (NH_4^+) is a predominant form of nitrogen in flooded wetland soils and an initial form of nitrogen fertilizer readily taken up by plants (Mitsch et al. 2001); however, ammonium can inhibit growth (Britto and Kronzucker 2002). An increase in growth of *Schoenoplectus* (= *Scirpus*) *acutus* (Muhl. ex Bigelow) Á. Löve & D. Löve var. *acutus* was observed with the addition of ammonium ranging between 30-50 mg/liter; whereas, a significant biomass reduction was observed at high (> 60 mg/liter) ammonium nitrogen concentrations (Hill et al. 1997). Excess total ammonia (NH_3 + NH_4^+) is thought to interfere with core functions of plant physiology, such as the inhibition of plant respiration (Santamaria et al. 1994).

Schoenoplectus maritimus (L.) Lye (Cyperaceae), commonly known as alkali (or cosmopolitan) bulrush, is a relatively short (< 1.5 m high) bulrush and has been recommended for use in treatment wetlands (Tilley 2012). Synonyms include *Scirpus maritimus* L. and *Bolboschoenus maritimus* (L.) Palla (Clevering et al. 1995, Kantrud 1996). Its life cycle in northern California and the Pacific Northwest includes dormancy in the winter, new shoot growth from corms in March and April, flowering in May, peak growth rate in June and July, peak shoot mass in August or September, and senescence in October (Miller et al. 2009). Alkali bulrush produces larger achenes and more carbohydrate-rich corms than most bulrushes and was ranked second to sea purslane (*Sesuvium portulacastrum* (L.) L.; Aizoaceae) as waterfowl food in northern California (Kantrud 1996, Miller et al. 2009).

The ability of *S. maritimus* to thrive in anaerobic aquatic substrate (Clevering et al. 1995) is a desirable attribute of this bulrush. The tuber of this species was reported to remain viable even after three months of anaerobic conditions (Clevering et al. 1995). Below-ground structures (roots, rhizomes, and corms) of alkali bulrush occur within 0.2 m of the surface and produce a large surface area that harbors beneficial microbes involved in degradation of nutrients (Tilley 2012). In addition, *S. maritimus* is reported to enhance the removal of fecal pathogens from sewage effluent and phenols from industrial wastes (Seidel 1971). Information on the effects of different nitrogen levels on alkali bulrush growth and its ability to take up nutrients from the wastewater is lacking. Moreover, the production of mosquitoes and other associated invertebrates from alkali bulrush within ammonium-dominated wastewater treatment wetlands is unknown.

The objectives of this study were to determine the effects of ammonium nitrogen enrichment on growth, biomass, and nutrient uptake of *S. maritimus*, and on the abundances of mosquitoes and aquatic invertebrates in wetland mesocosms. We tested the null hypotheses that enrichment with ammonium does not have an impact on alkali bulrush and on the abundance of invertebrates, including mosquitoes.

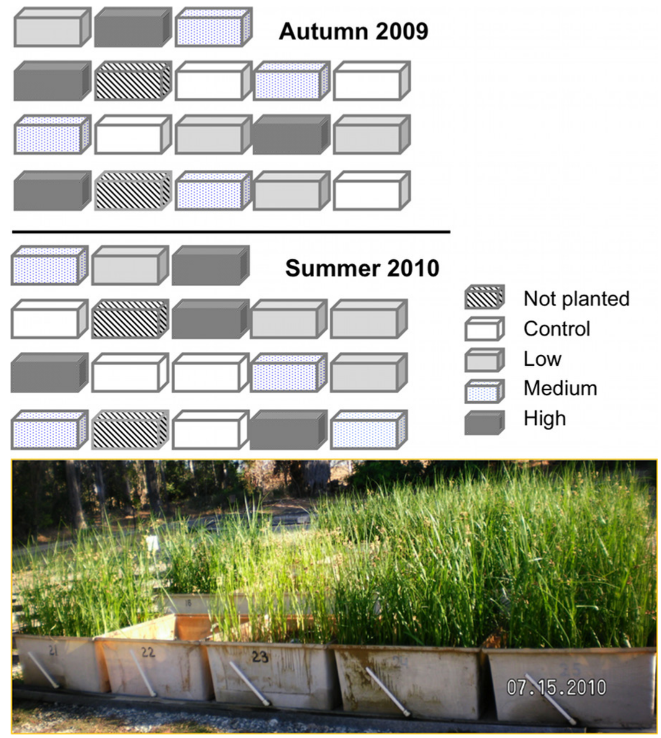

Figure 1. Schematic diagram of the experimental set up of the two studies (autumn 2009 and summer 2010). Treatments were assigned in completely randomized design.

MATERIALS AND METHODS

Study site and experimental design

Two independent studies (autumn 2009 and summer 2010) were conducted in fiberglass mesocosms (area= 1 m^2; volume = 0.5 m^3) at the Aquatic and Vector Control Research Facility of University of California, Riverside, U.S.A. (Figure 1). Soil mix (plaster sand mixed with peat moss; 0.17 m deep) and five uniform (average dry mass = 1.2 ± 0.15 g) seedlings of alkali bulrush were transplanted to each mesocosm. The seedlings were selected from a stock population established from seeds collected from natural stands in Riverside County, CA, in 2007. Transplantation was carried out during two periods of the annual cycle of alkali bulrush: late summer at peak shoot mass or early spring prior to peak growth rate. Seedlings were transplanted on 5 August 2009 and 22 March 2010 for the autumn (September-December, 2009) and summer (June-September, 2010) experiments, respectively. Replacement of seedlings from the nursery pond was conducted immediately for seedlings that failed to establish.

Water was supplied to the mesocosms from an irrigation reservoir. The water depth was kept constant during the studies at approximately 0.17 m except during the initial phase of plant establishment when the water depth was 0.1 m. A PVC pipe [(½ inch (1.27 cm) diameter] inside each mesocosm was connected to an outside stand pipe for drainage and control of the water level in the tubs. The average inflow and outflow rates from the mesocosms were 2.2 ± 0.44 (SE, n = 16) and 1.4 ± 0.5 (SE, n = 16) ml/sec, respectively.

Three ammonium nitrogen (NH_4-N) treatments (low: 15 mg/liter, medium: 30 mg/liter, and high: 50 mg/liter) were applied weekly to the mesocosms in the form of granular ammonium sulfate ($(NH_4)_2SO_4$; 21% nitrogen and 24% sulfur; Lilly Miller Brands, Walnut Creek, CA) for six consecutive weeks in autumn 2009 and for 11 consecutive weeks during summer 2010. These concentrations span the range of average NH_4-N concentration in the water column of treatment wetlands processing secondary- and tertiary-treated municipal effluent in southern California (Sartoris et al. 2000, Thullen et al. 2002, Sanford et al. 2005, Popko et al. 2009). The ambient NH_4-N concentration in the irrigation water entering the mesocosms was < 0.3 mg/liter (autumn 2009: 0.23 ± 0.07 mg/liter; summer 2010: 0.03 ± 0.002 mg/liter; SE, n = 4). The three enrichment treatments and an untreated control were assigned to the mesocosms in a completely randomized manner and replicated four times (n = 16 mesocosms).

Physicochemical variables

Water samples were taken in 500 ml dark plastic bottles 24 h and 7 d after enrichment with NH_4-N and transported to the laboratory on ice. Water also was collected from the control mesocosms. Ammonium-, nitrate- and nitrite-nitrogen concentrations were analyzed colorimetrically using a Hach DR™ 2800 spectrophotometer (TNT Plus tests, Hach Chemical Co., Loveland, CO). Nitrite and nitrate were not measured in autumn 2009. Water temperature was recorded using Taylor maximum-minimum thermometers in 2009, while in 2010 it was recorded every 0.5 h using a water temperature data logger (HOBO Temp Pro v2 (U22-001), Onset Computer Inc., Bourne, MA) in 2010.

Mosquitoes and invertebrates

A 350 ml plastic dipper was used to assess larval mosquito abundance in the mesocosms. Three dip samples were collected weekly from each mesocosm and filtered in a concentrator cup (screen mesh opening: 53 μm). However, during the first two weeks in the autumn experiment, only one dip sample per mesocosm was taken. The samples were then transferred into 20 ml plastic vials, preserved with 95% ethanol and transported to the laboratory for identification (Meyer and Durso 1998) and enumeration of the mosquitoes and other invertebrates (Merritt et al. 2008). Zooplankton was identified using Pennak (1989). Dip sampling was carried out for six weeks between 16 September and 11 November 2009 during the autumn experiment and for 10 weeks between 15 June and14 September 2010 during the summer experiment. Mosquito sampling ceased after the population declined both in autumn and summer experiments.

Plant parameter measurements

Plant growth parameters (stem density, height, number of inflorescences) were assessed monthly during both experiments. Plant density was estimated by counting the number of stems in three quadrats (size = 0.25 m^2) in the mesocosms. Height above the soil substrate for five flowered stems was measured from each mesocosm. Above-ground (leaves, stems, and flowers) and below-ground (roots, rhizomes, and corms) parts of five senesced stems per mesocosm were sampled in December for the autumn 2009 experiment and in September for the summer 2010 experiment. Samples were oven-dried at 50° C for five days and weighed in the laboratory. The oven–dried plant samples were then ground into a fine powder and analyzed for elemental carbon and nitrogen using a Thermo-Finnigan Model EA1112 flash elemental analyzer (Thermo Finnigan LLC, San Jose, CA). The percentage concentration of elemental nitrogen and carbon in the plant tissues was determined in three replicate 10 mg subsamples of the ground plant tissues.

Statistical analyses

The data for the autumn and summer experiments were analyzed separately using JMP version 10 (SAS Institute, Cary, NC). Repeated-measures ANOVA was carried out to discern the effects of ammonium nitrogen enrichment on plant parameters (culm density and height), mosquito production, and physicochemical parameters across time. Wilks' Lambda was used to test the significance of treatment effects and time interactions. Normality was assessed using a Sphericity test and, if the test was significant, adjusted *F*-values (Univar Geisser- Greenhouse Epsilon) were used instead of Wilks' Lambda to determine the significance of the treatment effects and time interactions. Numbers of mosquitoes were $\log_{10}(x+1)$ transformed before analysis. The effects of nitrogen enrichment on plant biomass, percent nitrogen, and carbon content of the plant tissue were analyzed using one-way ANOVA. Mosquito counts from the first two weeks of the autumn 2009 experiment were analyzed separately because the sampling regimen for these two weeks was different. If the treatment effect was significant, Tukey's HSD test was conducted to assess the significance of differences among the means.

RESULTS

Water physiochemical characteristics

In 2009, the average NH_4-N concentrations in the water column at 24 h after enrichment were between 86 to 93% of the nominal enrichment treatments of 15, 30, and 50 mg/liter and differed significantly among the treatments (Tables 1 and 2). In summer 2010, the mean NH_4-N concentration at 24 h after enrichment for the two lower enrichment treatments was about 85% of the target levels; whereas, the NH_4-N concentration in the High treatment exceeded the target of 50 mg/liter by about 18% (Table 1). The water column NH_4-N concentration differed significantly among the treatments (Table 2). Seven days after enrichment, NH_4-N was considerably reduced, but in the high treated mesocosms the concentration was still signifcantly greater than the concentration in the medium, low, and control mesocosms (Table 1; 2009: $F_{3,12}$ = 3.96, P<0.05; 2010: $F_{3,11}$ = 3.2, P=0.05).

The nitrite-nitrogen (NO_2-N) concentration differed

Table 1. Mean (± SE) ammonium nitrogen concentration in mesocosms 24 h and 7 d after weekly ammonium sulfate applications during autumn 2009 and summer 2010.

Year	Treatment	Mean (± SE) concentration (mg/liter)			
		N	24 h	N	7 d
2009	Control	19	0.19 ± 0.08[a]	21	0.27 ± 0.12[a]
	Low	20	14.0 ± 2.18[b]	20	0.89 ± 0.39[a]
	Medium	22	26.6 ± 3.58[c]	20	2.34 ± 1.04[a]
	High	24	42.8 ± 4.33[d]	20	5.35 ± 1.78[b]
2010	Control	44	0.03 ± 0.002[a]	44	0.03 ± 0.002[a]
	Low	44	12.6 ± 1.09[b]	43	0.50 ± 0.13[a]
	Medium	44	25.8 ± 2.70[c]	44	1.90 ± 0.82[a]
	High	42	59.0 ± 4.72[d]	42	10.9 ± 3.23[b]

[a-d] For each column, different letters within each year differ by P < 0.05 (Tukey test).

Table 2. Repeated-measures ANOVA of enrichment effect on nitrogen species 24 h after enrichments.

Year	Sources	Ammonium-N			Nitrite-N			Nitrate-N		
		F	df	*P*	F	df	*P*	F	df	*P*
2009	Between groups									
	Treatment	15.8	3,12	0.0002						
	Within groups									
	Date	41.8	3,40	0.0001						
	Date x treatments	8.8	10,40	0.0001						
2010	Between groups									
	Treatment	12.8	3,9	0.0014	6.73	3,12	0.006	4.75	3,12	0.021
	Within groups									
	Date	19.5	3,24	0.0001	5.80	6,75	0.0001	4.63	5,56	0.002
	Date x treatments	4.8	8,24	0.0013	1.29	19,75	0.218	1.12	14,56	0.357

Table 3. Mean (± SE) nitrate-nitrogen and nitrite-nitrogen concentration (mg/liter) in mesocosms 24 h and 7 d after weekly applications of ammonium sulfate during summer 2010.

Treatments	Nitrate			Nitrite			
	N	24 h	7 d	N	24 h	N	7 d
Control	44	0.94 ± 0.11^{a}	0.66 ± 0.08^{a}	43	0.03 ± 0.004^{a}	44	0.03 ± 0.005^{a}
Low	44	1.28 ± 0.10^{b}	1.21 ± 0.08^{a}	43	0.05 ± 0.01^{b}	43	0.04 ± 0.006^{a}
Medium	44	1.70 ± 0.11^{c}	1.37 ± 0.09^{a}	43	0.13 ± 0.03^{bc}	42	0.07 ± 0.01^{a}
High	44	1.66 ± 0.12^{bc}	1.36 ± 0.10^{b}	44	0.31 ± 0.06^{c}	43	0.29 ± 0.06^{b}

[a-d]For each column, different letters differ by $P < 0.05$ (Tukey test).

significantly among the enrichment treatments (Table 3; 24 h: $F_{3,9}$ = 6.7, P<0.05; 7 d: $F_{3,9}$ = 4.4, P<0.05). The High and Medium enrichment levels resulted in significantly greater NO_2-N levels than were recorded in the Low enrichment and control mesocosms (Tables 2 and 3). Similarly, there was also a significant difference in nitrate-nitrogen (NO_3-N) levels among the four treatments (24 h: $F_{3,12}$ = 4.8, P<0.05; 7 d: $F_{3,12}$ = 8.2, P<0.05; Tables 2 and 3). There was a peak in NO_3-N level in the middle of August that coincided with the peak nitrite level, suggesting the presence of more nitrification during that period.

Water temperature in the experimental mesocosms varied among months during the study periods. The maxium temperatures were recorded in August (28.6 ± 0.78 °C (SE); n = 12) and September (29.0 ± 0.88 °C; n = 10) during the 2009 experiment, and in July (25.3 ± 0.38 °C; n = 31) and August (24.8.± 0.28 °C; n = 31) during the 2010 experiment.

Table 4. Repeated-measures ANOVA of the effect of four ammonium nitrogen levels on the abundance of immature mosquitoes (larvae and pupae) in mesocosms planted with *Schoenoplectus maritimus*.

Year	Sources	F	df	P
	Between groups			
	Treatment	7.0	3, 44	0.0006
2009	Within groups			
	Date	77.6	3,42	0.0001
	Date x treatments	2.9	9, 102	0.0046
	Between groups			
	Treatment	7.6	3, 38	0.0004
2010	Within groups			
	Date	23.1	9, 30	0.0001
	Date x treatments	2.5	27, 88	0.0008

Mosquitoes and other invertebrates

The immature (larvae and pupae) mosquitoes found in the experimental mesocosms were *Culex tarsalis* Coquillett and *Anopheles hermsi* Barr and Guptavanij with the former being the dominant mosquito species found during both experiments. The mean of the total number of immature mosquitoes per dip sample varied significantly among the four enrichment treatments and by sampling date during both studies (P < 0.01; Control < Low, Medium, High NH_4-N treatments). However, repeated measures ANOVA showed that the treatment effect on mosquito counts during the first two weeks in the 2009 experiment was not significantly different among treatments (Date x treatment effect, $F_{3,12}$ = 1.4, P > 0.05). Less than 16% of the total immature mosquitoes were produced in control treatments while about 27%, 32%, and 25% of the total mosquitoes were produced in mesocosms that received low, medium, and high NH_4-N enrichments, respectively, in autumn 2009. Immature mosquito abundance in the enriched mesocosms was greater (> 50%) than in the control mesocosms at three weeks after the initial application of ammonium nitrogen in the autumn experiment (P < 0.001; Figure 2). However, NH_4-N ranging from 15 mg/liter to 50 mg/liter did not influence the total number of immature mosquitoes in the mesocosms during autumn 2009.

In summer 2010, the mean numbers of immature mosquitoes produced from the four treatments were also significantly different (Table 4). Mosquitoes from control mesocosms accounted for nearly 17% of the total, which was comparable to mesocosms that received low NH_4-N (16%) enrichment. Mesocosms that received medium and high NH_4-N enrichments produced about 28% and 39%, respectively, of the total immature mosquitoes sampled during summer 2010.

Sampling date also significantly influenced the number of immature mosquitoes collected per dip sample, with more mosquitoes produced during early weeks of enrichments (Figure 2). The abundance of the mosquito population

Table 5. Repeated-measures ANOVA of the effect of four ammonium nitrogen levels on culm height and density of *Schoenoplectus maritimus*.

Year	Sources	Height			Culm density		
		F	df	P	F	df	P
2009	Between groups						
	Treatment	5.53	3, 74	0.002	0.64	3,12	0.603
	Within groups						
	Date	28.8	2, 136	0.100	39.5	2,22	0.001
	Date x treatments	0.64	5, 136	0.954	1.35	5,22	0.194
2010	Between groups						
	Treatment	71.5	3, 76	0.001	12.7	3,12	0.005
	Within groups						
	Date	332	4, 73	0.001	390	7,84	0.001
	Date x treatments	11.6	12, 193	0.001	14.4	21,84	0.001

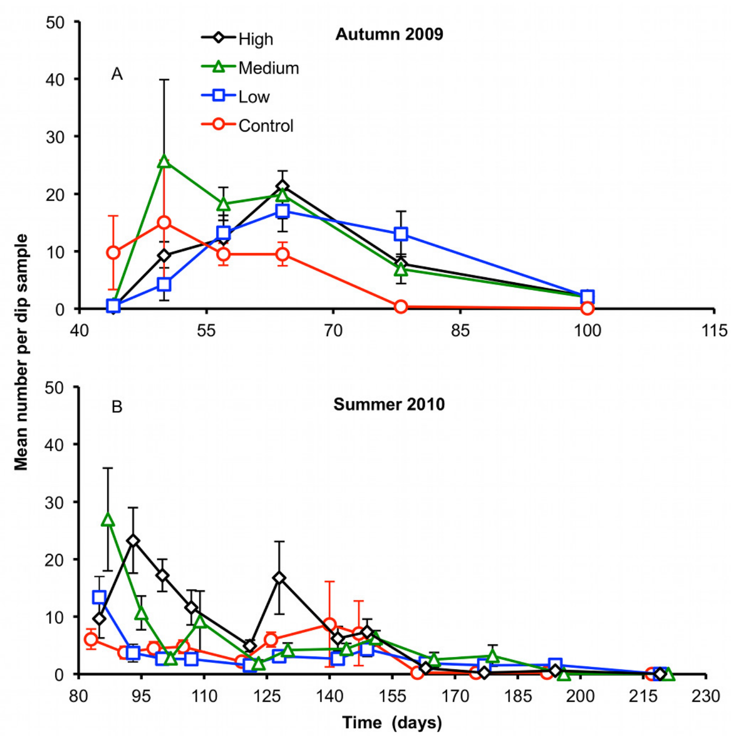

Figure 2. Immature (larvae and pupae) *Culex* mosquito abundance (mean ± SE, n = 4) in 0.17-m^3 mesocosms containing *Schoenoplectus maritimus* and four levels of ammonium nitrogen during autumn 2009 (A) and summer 2010 (B).

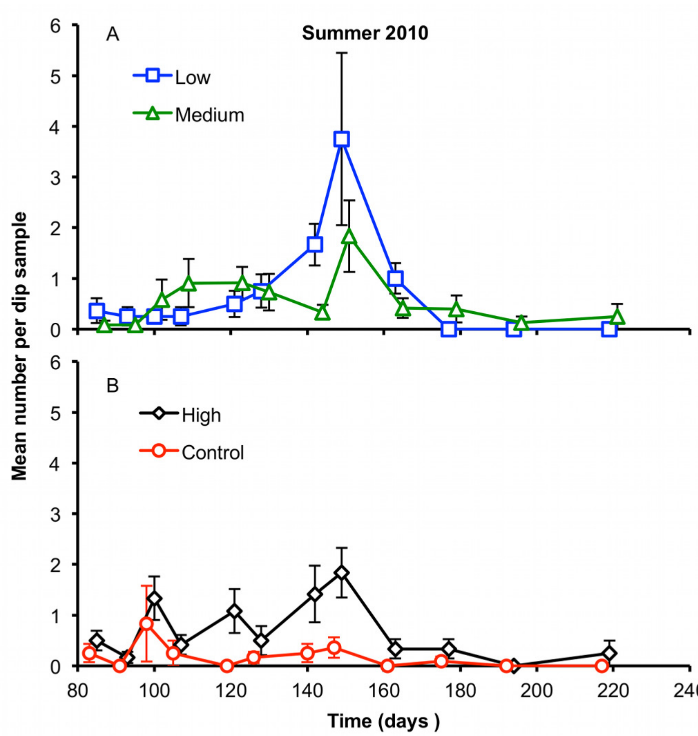

Figure 3. Abundance of immature *Anopheles hermsi* (mean ± SE, n = 4) in 0.17-m^3 mesocosms containing *Schoenoplectus maritimus* and low and medium (C) or high and control (D) levels of ammonium nitrogen during summer 2010.

plummeted towards the end of the 2009 experiment in response to cool temperatures at the end of the mosquito production season. The abundance of *An. hermsi* averaged less than one individual per dipper sample for the 2009 study and was not separately analyzed. During summer 2010, the treatment effects on immature mosquito abundance were also significantly different from three days after the initial enrichment continuously for five weeks, after which the treatment effect was no longer significant (Figure 2). *Culex* mosquitoes were more abundant than *An. hermsi* in the study mesocosms. *Anopheles* dominated late in the summer during the 2010 experiment (Figure 3).

The abundance of the immature stages of the predator community (the majority of which were in the order Odonata) was not significantly different among the four NH_4-N treatments in both experiments. In the 2009 experiment, the average number of naiads collected was less than one individual (mean ± SE: 0.05 ± 0.02; n = 96) per dip sample; whereas, in the summer 2010 experiment, the mean number of naiads per dip sample was considerably greater (2.1 ± 0.14; n = 192) but was not significantly different ($P > 0.05$, $F_{3, 44} = 0.59$) among the four treatments. Naiads first appeared in the dip samples three weeks following the initial enrichment of mesocosms (data not shown). The predators tended to colonize the mesocosms following the primary colonizers such as mosquitoes and crustaceans (dominantly cladocerans). The decline of the mosquito population coincided with an increase in predator abundance.

The total number of crustaceans (copepods, cladocerans, and ostracods) per dip sample did not differ significantly among the enrichment treatments ($P > 0.05$; data not shown). Cladocerans predominated (~88% of the total abundance) in the mesocosms. Both Cladocera and Ostracoda increased significantly three weeks after the first enrichment, whereas Copepoda abundance stayed constant throughout autumn 2009. In summer 2010, Cladocera was present in a proportion (> 90%) higher than the two other crustacean groups and was negatively affected by the highest ammonium nitrogen enrichment.

The abundance of chironomid larvae ranged between 0.5-1 individuals per dip sample and did not differ significantly among the four enrichment treatments (data not shown). Chironomids are adapted to benthic substrates and were not expected to occur in large numbers on the plant surfaces and in the water column. Benthic samples were not taken in this experiment.

Growth characteristics of *S. maritimus*

Culm height, density, and inflorescences

Culm height was greatest in the mesocosms enriched at low levels of NH_4-N, and differed significantly among the four treatments in autumn 2009 ($P < 0.05$) (Figure 4A; Table 5). The mean culm heights in all the enriched mesocosms were significantly higher than the control treatments in summer 2010, with the greatest mean culm heights in the mesocosms enriched with low and medium levels of NH_4-N (Figure 4B).

The mean culm density did not differ significantly among the four enrichment levels ($P > 0.05$) during autumn 2009 (Figure 4C). However, the mean culm densities in enriched mesocosms were significantly greater than the control mesocosms during 2010 with the maximum density attained in July (Figure 4D).

The mean number of flowers did not differ among treatments in both experiments; although low and medium enrichment treatments tended to produce slightly higher number of flowers in July and August, 2010 ($P > 0.05$).

Biomass

The above-ground (Figure 6A) mean dry mass of five senesced *S. maritimus* plants (stems and inflorescence) was not significantly different among treatments in autumn 2009 ($P > 0.05$). However, the mean dry weight of below-ground plant structures (Figure 5A) was significantly different among the treatments in autumn 2009 ($F_{3, 12} = 4.41$; $P = 0.026$). The above-ground mean dry weight of *S. maritimus* was not significantly different among treatments ($P > 0.05$) in July, 2010 (data not shown) but was significantly different among enrichment treatments in September, 2010 ($F_{3,12} = 7.58$; $P = 0.024$; Figure 5B). No statistically significant difference was found in the weights of below-ground structures among the treatments in September, 2010. The mean total dry weight (above- and below-ground masses combined) of the plants grown in the four treatments was significantly different in both year studies. Mesocosms enriched at the high treatment level yielded a significantly lower dry biomass compared to the low and medium enrichment levels (Figure 5).

Elemental nitrogen and carbon

The mean percentage of nitrogen in above-ground biomass differed significantly among treatments both in autumn 2009 ($F_{3, 44} = 41.8$, $P < 0.001$) and summer 2010 ($F_{3,92} = 11.5$, $P < 0.001$)(Figures 6A and 6C, respectively). Similarly, the mean percentage of nitrogen in below-ground biomass also differed significantly among treatments in autumn 2009 ($F_{3,44} = 28.1$, $P < 0.001$) and summer 2010 ($F_{3,44} = 4.5$, $P < 0.001$) (Figures 6B and 6D). The percentage of N found in above-ground plant biomass increased directly with ammonium enrichment in both studies. Overall, plants that were raised in mesocosms enriched with NH_4-N incorporated a greater percentage of nitrogen than did alkali bulrush plants in the control mesocosms (Figure 6).

Elemental carbon in the above-ground biomass did not vary significantly among the treatments in each of the two studies (Figure 6). However, mean percentage of carbon in below-ground alkali bulrush tissues differed significantly among treatments (autumn 2009: $F_{3, 44} = 3.0$, $P = 0.040$; summer 2010: $F_{3,44} = 23.9$, $P < 0.001$) (Figures 6B and 6D, respectively). The percentage of carbon in below-ground biomass of nitrogen-enriched plants was significantly greater than in the control plants (Figure 7). A greater percentage of carbon accumulated in the above-ground structures (stem, leaf, and flowers) than in the below-ground (rhizomes and corms) structures (Figure 7). Although there was variability among treatments, overall, the ratio of carbon to nitrogen (C:N; mass) was significantly greater in flowers (35.7 ± 1.38, n = 48) and roots (34.9 ± 1.40) than in stems and leaves (22.1

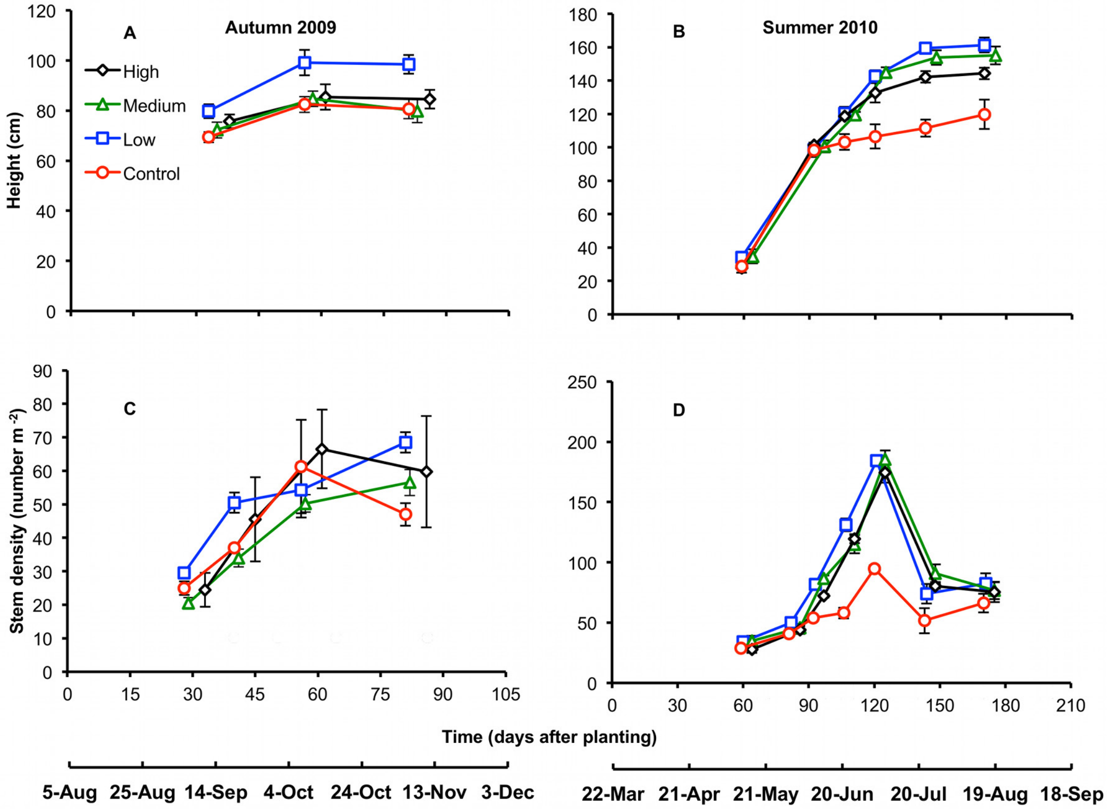

Figure 4. Mean (± SE, n = 4) height (cm) and density (number m^{-2}) of *S. maritimus* in mesocosms containing four levels of ammonium nitrogen during autumn 2009 (A and C) and summer 2010 (B and D), respectively.

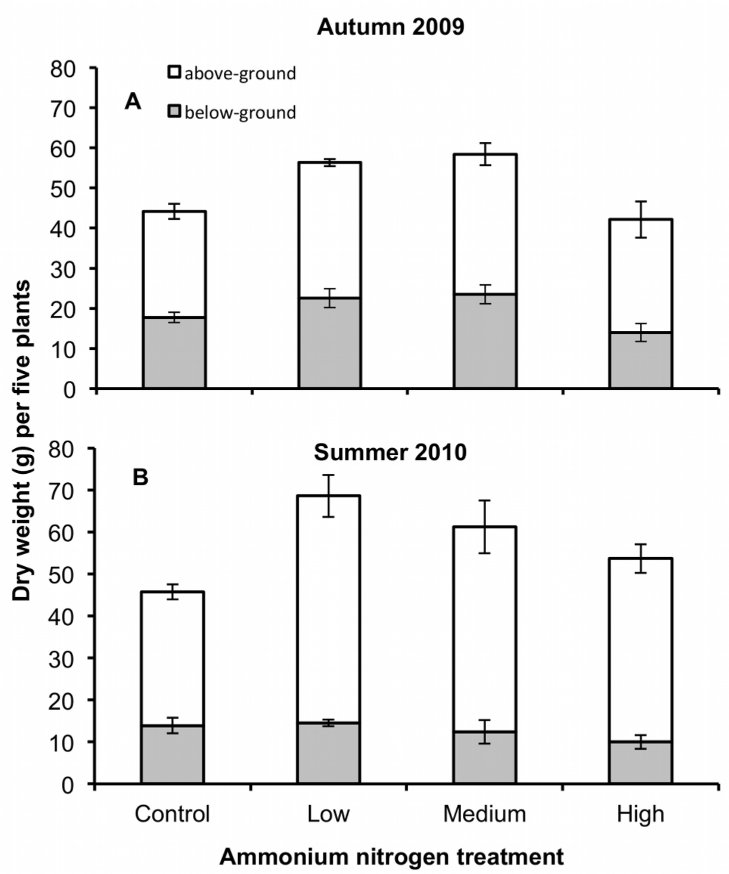

Figure 5. Dry weight (mean ± SE, n = 4) of five above-ground (light gray) and below-ground (dark gray) parts of *Schoenoplectus maritimus* culms in autumn 2009 (A) and summer 2010 (B) among four enrichment treatments.

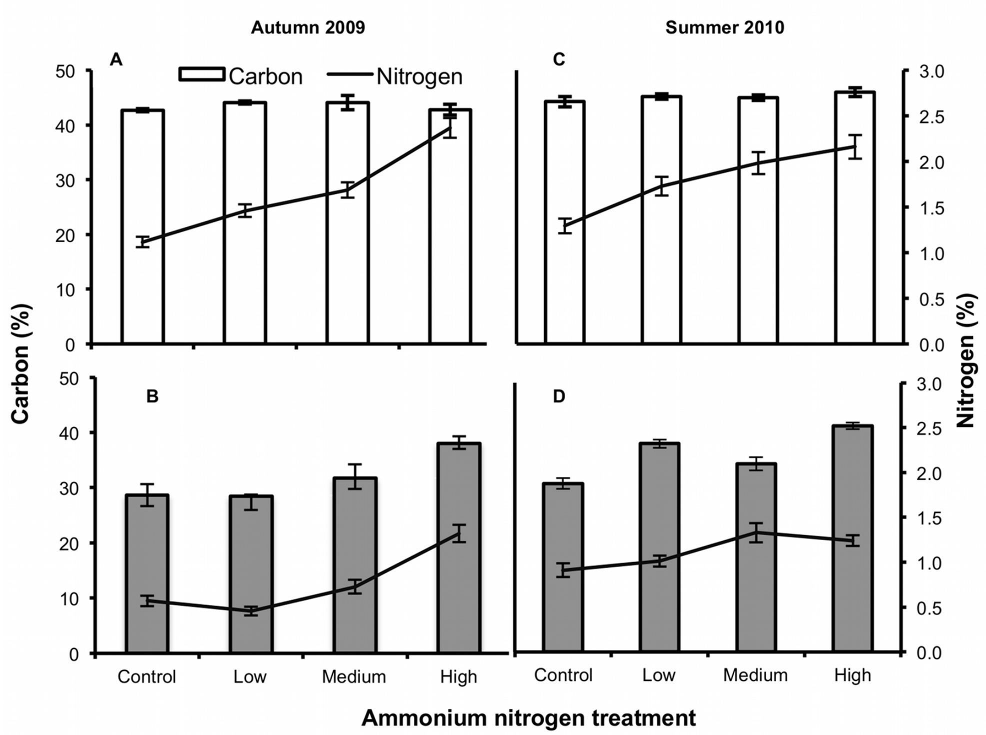

Figure 6. Percentages of carbon (mean ± SE, n = 12) and nitrogen in above- (A and C) and below-ground (B and D) plant tissues of *S. maritimus* across a gradient of ammonium nitrogen enrichment.

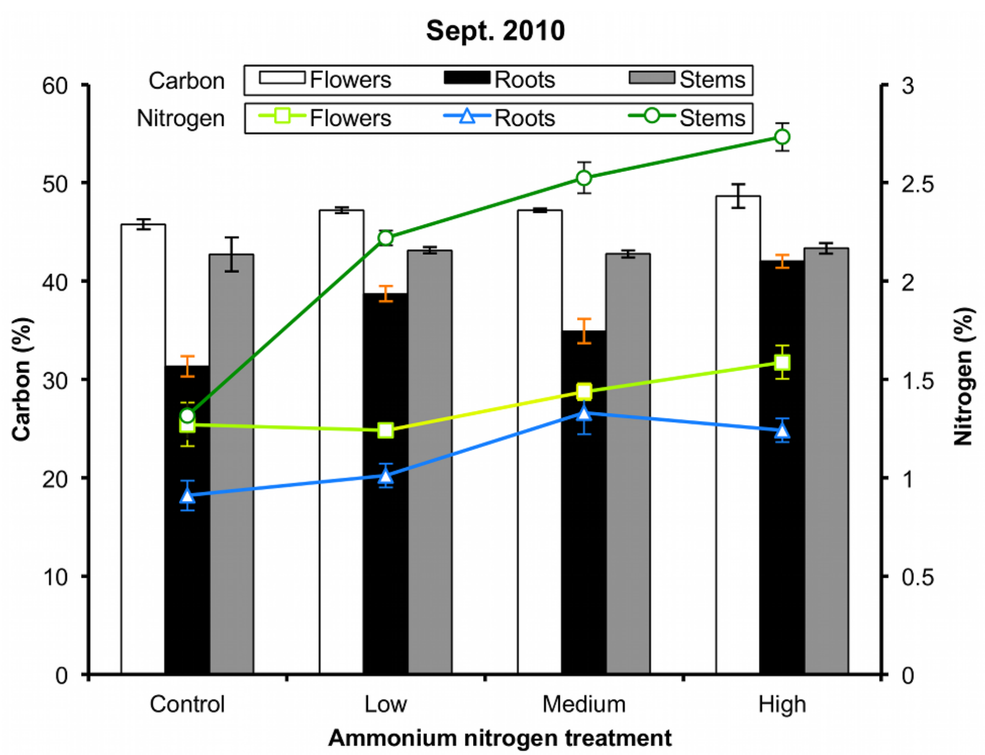

Figure 7. Carbon and nitrogen content (mean ± SE, n = 12) in three (roots, stem and leaves, and flowers) plant tissues of *S. maritimus* raised under four enrichment treatments in September 2010. Roots are composed of rhizomes and corms.

± 1.49) of the plants. Nitrogen was more concentrated in the above-ground photosynthetic structures particularly in the stems and leaves than in other parts of the plants.

DISCUSSION

Effect of nutrient enrichment on mosquitoes and other invertebrates

Mosquito production, primarily *Culex tarsalis*, increased by nearly two-fold in the enriched mesocosms and this finding is in agreement with the findings (Beattie 1932, Victor and Reuben 2000, Sunish et al. 2003, Mutero et al. 2004) that enrichment with ammonium nitrogen is associated with enhanced oviposition by mosquitoes. Ammonia also has been shown to attract host-seeking adult *Anopheles* mosquitoes (Meijerink et al. 2001). Sanford et al. (2005) observed a similar effect of nutrient enrichment on immature mosquito abundance even at a very low (<1 mg/liter) ammonium nitrogen concentration in wetlands treating river water derived primarily from tertiary-treated municipal effluent.

Nitrogenous effluents are known to change trophic cascades in wetlands by increasing detritus and microbial communities that are primary food resources of mosquito larvae. Johnson et al. (2010) linked an increase of critical nutrients such as nitrogen and phosphorus into the aquatic environment to an increased incidence of debilitating diseases vectored by mosquitoes. Nutrients leaching from agriculture and released from other industrial activities can significantly influence the composition and abundance of aquatic macrophytes, which can in turn reduce mosquito predator activities while increasing microbial communities that are food resources for mosquito larvae (Johnson et al. 2010). In addition, high levels of nitrogen compounds such as ammonia can directly inhibit survival and reproduction of mosquito predators such as fish (Walton 2003).

The abundance of immature mosquitoes in our study could have been influenced by a combination of reduced predation rates under enriched conditions as well as enhancement of mosquito production from bottom-up processes. Mosquito abundance was greater in enriched mesocosms that also had higher plant biomass and bulrush culm densities compared to the control mesocosms. High culm (stem) densities are known to reduce mosquito predator efficiency (Thullen et al. 2002). The abundance of invertebrate predators (primarily zygopterans) did not differ significantly among the enrichment treatments in our study. Because predator abundance did not change across the enrichment gradient, predation efficiency in the enriched mesocosms could have been reduced by the enhanced physical structure provided by the alkali bulrush as compared to the control mesocosms. Greater habitat complexity reduces predator efficiency and reduces prey vulnerability (Saha et al. 2009). Increased macrophyte production has been known to support large mosquito populations (de Szalay and Resh 2000). Low dissolved oxygen levels in the water column are characteristic of dense stands of emergent macrophytes in eutrophic treatment wetlands (Sartoris et al. 2000, Thullen et al. 2008, Walton et al. 2012). Mosquito predator abundance declines at low dissolved oxygen concentrations (Walton 2012) and top-down effects on mosquito populations appear to decrease with enrichment as predator abundance is decoupled from enrichment gradients in hypereutrophic constructed treatment wetlands (Peck and Walton 2008). Immature *Culex* abundance increases directly with bottom-up enrichment (Chaves et al. 2009, Walton 2012).

Although we did not quantify water column resources available to mosquito larvae across the enrichment gradient and ammonium nitrogen concentration in enriched mesocosms declined to ambient levels in the control treatment by one week after enrichment, a combination of factors could have contributed to the enhancement of mosquito populations with ammonium nitrogen enrichment. Our sampling technique —standard dip sampling mainly used for immature mosquito and zooplankton sampling— might have underestimated the abundance of odonates especially the dragonfly naiads which tend to inhabit the substrates below the water column. Substrate sampling was not suitable in this mesocosm study because it could interfere with plant growth.

Abundances of other invertebrates, such as cladoceran populations did not respond differently to nitrogen enrichment in autumn 2009; however, a significant (20%) reduction of cladoceran abundance occurred in mesocosms that received the highest enrichment level during the summer 2010. Prolonged enrichment at high levels of ammonium nitrogen was known to have a detrimental effect on *Daphnia magna* developmental stages (Yang et al. 2012). Cladocera were not identified to species in our study.

Effect of nitrogen on *S. maritimus*

An understanding of the growth response and nutrient uptake of an alternative macrophyte species under different nutrient conditions is required before utilizing the plant in constructed treatment wetlands for wastewater quality remediation. Lack of this knowledge has been the most frequently reported problem for the failure and poor survival of plants in treatement wetlands (Kadlec and Wallace 2009). In this study, low and medium levels (~15-30 mg/liter) of NH_4-N enrichment maximized culm density, stem height, and the overall plant biomass. At greater than 50 mg NH_4-N/liter, alkali bulrush biomass was significantly reduced. This is consistent with the typical relationship reported for plant growth response and nutrient enrichments reported elsewhere (Hill et al. 1997, Kadlec and Wallace 2009). For example, the growth of *S. acutus* was reduced in wetlands when ammonium nitrogen concentration exceeded 60 mg/liter. The highest NH_4-N concentration (~50 mg/liter) in our experiment suppressed *S. maritimus* biomass (Figure 5). Ammonium nitrogen is known to settle or bind to the substrate (clay) and is likely to increase toxic ammonia production in the rhizosphere of plants, which in turn affects the root physiology of the plant (Wang 1991). We observed significantly higher culm mortality and below-ground biomass reduction in the mesocoms enriched at the highest ammonium nitrogen treatment.

Transplantation of *S. maritimus* in late summer (at peak shoot mass) or early spring (prior to peak growth rate) was successful. The rate of growth of alkali bulrush decreased over time with the maximum growth attained two and three months after planting in autumn and summer experiments, respectively, after which alkali bulrush reached maturity. Both enriched and control treatments exhibited a similar growth pattern, except that plants in the control treatments were significantly shorter and less dense than enriched treatments (Figures 5B and 5D).

However, the percent nitrogen content in the alkali bulrush tissues increased directly with nitrogen enrichment (Figures 6 and 7). The concentration measured in alkali bulrush was within the range of nitrogen composition found in most emergent macrophytes used in treatment wetlands (Reddy and Delaune 2008). Emergent macrophytes generally incorporate less than 5% of nitrogen into their tissues (Reddy and Delaune 2008). The majority of nutrient removal takes place within the microbial communities that inhabit these emergent macrophytes (Stottmeister et al. 2003). The nitrogen concentration in vegetative portions of alkali bulrush ranged between 8-24 g/kg of dry weight and compares reasonably well with the amount reported for other *Scirpus* species (8-27g/kg: Reddy and Delaune 2008). In our study, alkali bulrush in the enriched mesocosms contained about 77g N/m^2 which was three times greater than the amount (24 g N/m^2) found in plants in the control mesocosms. This suggests that the mass-specific uptake rates of nutrients by alkali bulrush is comparable with the large-stature emergent macrophytes (California bulrush, cattail, etc.) used in wastewater treatment wetlands. The carbon content of alkali burush was also comparable to the carbon content in other congeners (Reddy and Delaune 2008) and even to oak leaves (Walker et al. 1997).

In this study, we observed a greater percentage of carbon and nitrogen (by mass) in flowers and roots than in the vegetative portion (stems and leaves) of the plants. However, carbon did not change across the enrichment gradient. Similar to our obervations, Santamaria et al. (1994) reported that the net photosynthesis of other submerged macrophytes did not change under different levels of enrichment which supports our observation that carbon was conserved across different enrichment levels in the above-ground biomass (Figures 6 and 7).

Because the above-ground biomass of *S. maritimus* dies off each winter and its below-ground biomass (rhizomes and tubers) can persist in the soil for several years for future regeneration, this bulrush does not likely require the costly routine management (harvesting and removal) of biomass similar to the other emergent macrophytes currently being utilized in many treatment wetlands. The annual senescence and die-back of *S. maritimus* can also be considered an important trait of a species to be utilized in constructed wetlands because it can provide detritus and carbon to the denitrifying bacteria and other microbial communities when mosquito activity is limited by cool weather conditions. Moreover, *S. maritimus* is considered a preferred diet for waterfowl (Kantrud 1996, Miller et al. 2009).

In conclusion, mosquito production was increased by ammonium nitrogen enrichment and the enrichment effect varied across time and between seasons. Mosquito production in the enriched mesocosms was enhanced soon after inundation when insect predators were comparatively rare. The differences in mosquito abundance between the enriched vs control mesocosms lessened across time as the wetland plots aged, especially during the summer. Although the abundance of immature mosquitoes was not directly related to ammonium nitrogen treatment during the autumn, immature mosquito abundance in mesocosms of all the enrichment treatments was comparatively greater than in the control mesocosms. Overall, mosquito abundance increased by nearly two-fold in the enrichment treatments (15-50 mg NH_4-N/liter). Our findings also indicated that ammonium concentration up to 60 mg/liter has no detrimental effect on survival of *S. maritimus,* although plants enriched at the high (>50 mg/liter) ammonium nitrogen regimen had lower biomass than did plants exposed to lower NH_4-N levels. We found ammonium nitrogen levels ranging between (15-30 mg/liter) to be more favorable for alkali bulrush growth than were the ambient (< 0.3 mg NH_4-N/liter) and highest NH_4-N concentrations; consequently, we can recommend that alkali bulrush be planted in constructed wetlands exhibiting a wide range of reduced nitrogen concentrations, but especially for moderately enriched treatment wetlands. However, our experiments were carried out in shallow (depth < 0.2 m) experimental mesocosms and further research on the impact of water depth on alkali bulrush survival and growth is warranted. Moreover, *S. maritimus* has a cosmopolitan distribution and investigations of populations from different habitat types across the geographic range of this species might be helpful.

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REFERENCES CITED

Beattie, M.V.G. 1932. The physicochemical factors of water in relation to mosquito breeding in Trinidad. Bull. Entomol. Res. 23: 477-496.

Berkelhamer R.C. and T.J. Bradley. 1989. Mosquito larval development in container habitats: the role of rotting *Scirpus californicus*. J. Am. Mosq. Contr. Assoc. 5: 258-260.

Britto, D.T. and H. J. Kronzucker. 2002. $NH4^+$ toxicity in higher plants: a critical review. J. Plant Physiol. 159**:** 567-584.

Brix, H. 1997. Do macrophytes play a role in constructed wetland treatment wetlands? Water Sci. Tech. 35: 11-17.

Chaves, L. F., C.L. Keogh, G.M. Vazquez-Prokopec, and U.D.

Kitron, 2009. Combined sewage overflow enhances oviposition of *Culex quinquefasciatus* (Diptera: Culicidae) in urban areas. J. Med. Entomol. 46: 220-226.

Clevering, O.A., W.V. Vierssen, and P.M. Blom.1995. Growth, photosynthesis and carbohydrate utilization in submerged *Scirpus maritimus* L. during spring growth. New Phytologist 130: 105-116.

de Szalay, F.A. and V.H. Resh. 2000. Factors influencing macroinvertebrate colonization of seasonal wetlands: responses to emergent plant cover. Freshwater Biol. 45: 295-308.

Hill, D.T., V.W.E. Payne, J.W. Rogers, and S.R. Kown. 1997. Ammonia effects on the biomass production of five-constructed wetland plant species. Bioresource Technol. 62:109-113.

Johnson P.T.J, A.R. Townsend, C.C. Cleveland, P.M. Glibert, R.W. Howarth, V.J. McKenzie, E. Rejmankova, and M.H. Ward. 2010. Linking environmental nutrient enrichment and disease emergence in humans and wildlife. Ecol. Applic. 20: 16-29.

Kadlec, R.H. and S.D. Wallace. 2009. *Treatment Wetlands.* 2nd ed. CRC press. Boca Raton. FL. Online. http://www.environetbase.com/books/7115/l1526fm.pdf.

Kantrud, H.A. 1996. The Alkali (*Scirpus maritimus* L.) and Saltmarsh (*S. robustus* Pursh) Bulrushes: A Literature Review. National Biological Service, Information and Technology Report 6. Jamestown, N.D.: Northern Prairie Wildlife Research Center online. http://www.npwrc.usgs.gov/resource/plans/bulrush/index.htm (version 16Jul97).

Meijerink, J., M.A.H. Braks, and J.J.A. Van Loon. 2001. Olfactory receptors on the antennae of the malaria mosquito *Anopheles gambiae* are sensitive to ammonia and other sweat-borne components. J. Insect Physiol. 47: 455-464.

Merritt, R.W., K.W. Cummins, and M.B. Berg (eds.). 2008. *An Introduction to the Aquatic Insects of North America*, 4th ed. Kendall Hunt, IA.

Meyer, R.P. and S.L. Durso. 1998. Identification of the mosquitoes of California. Mosq. Vector Contr. Assoc.. Calif., Sacramento, CA.

Miller, M.R., E.G. Burns, B.E. Wickland, and J.M. Eadie. 2009. Diet and body mass of wintering ducks in adjacent brackish and freshwater habitats. Waterbirds 32: 374-378.

Mitsch, W.J., J.W. Day Jr., J.W. Gilliam, P.M. Groffman, D.L. Hey, G.W. Randall, and N.G. Wang. 2001. Reducing nitrogen loading to the Gulf of Mexico from the Mississippi River Basin: Strategies to counter a persistent ecological problem: Ecotechnology-the use of natural ecosystems to solve environmental problems-should be a part of efforts to shrink the zone of hypoxia in the Gulf of Mexico. Bioscience 51: 373-388.

Mutero, C.M., P.N. Ng'ang'a, P. Wekoyela, J. Githure, and F. Konradsen. 2004. Ammonium sulphate fertiliser increases larval populations of *Anopheles arabiensis* and culicine mosquitoes in rice fields. Acta Trop. 89: 187-192.

Peck, G.W. and W.E. Walton. 2008. Effect of mosquitofish (*Gambusia affinis*) and sestonic food abundance on the invertebrate community within a constructed treatment wetland. Freshwater Biol. 53: 2220-2233.

Pennak, R.W. 1989. *Fresh-water Invertebrates of the United States, Protozoa to Mollusca*. 3rd ed. John Wiley & Sons, Inc. NY. 628 pp.

Popko, D.A., M.R. Sanford, and W.E. Walton. 2009. The influence of water quality and vegetation on mosquitofish in mosquito control programs in wastewater wetlands. Proc. Papers Mosq. Vector Contr. Assoc. Calif. 77: 230-237.

Reddy, K.R. and R.D. Delaune. 2008. *Biogeochemistry of Wetlands: Science and Applications*. 2nd ed. CRC Press. Boca Raton, U.S.A. 774 pp.

Saha, N., G. Aditya, and G.K. Saha. 2009. Habitat complexity reduces prey vulnerability: an experimental analysis using aquatic insect predators and immature dipteran prey. J. Asia-Pacific Entomol. 12: 233-239.

Sanford, M.R., K. Chan, and W.E. Walton. 2005. Effects of inorganic nitrogen enrichment on mosquitoes (Diptera: Culicidae) and the associated aquatic community in constructed treatment wetlands. J. Med. Entomol. 42: 766-776.

Santamaria L., C. Dias, and M. Hootsmans. 1994. The influence of ammonia on the growth and photosynthesis of *Ruppia drepanensis* Tineo from Doñana National Park (SW Spain). Hydrobiologia 275: 219-231.

Sartoris, J.J., J.S. Thullen, L.B. Barber, and D.E. Salas. 2000. Investigation of nitrogen transformations in a southern California constructed wastewater treatment wetland. Ecol. Eng. 14: 49–65.

Seidel, K. 1971. Macrophytes as functional elements in the environment of man. Hidrobiologia Bucuresti 12: 121-130.

Stottmeister, U., A. Wießner, P. Kuschk, U. Kappelmeyer, M. Kästner, O. Bederski, R.A. Müller, and H. Moormann. 2003. Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotech. Adv. 22: 93-117.

Sunish, I. P., R. Rajendran, and R. Reuben. 2003. The role of urea in the oviposition behaviour of Japanese encephalitis vectors in rice fields of south India. Mem. Inst. Oswaldo Cruz 98: 789-791.

Thullen, J.S., S.M. Nelson, B.S. Cade, and J.J. Sartoris. 2008. Macrophyte decomposition in a surface-flow ammonia-dominated constructed wetland: rates associated with environmental and biotic variables. Ecol. Eng. 18: 281–290.

Thullen, J.S., J.J. Sartoris, and W.E. Walton. 2002. Effects of vegetation management in constructed wetland treatment cells on water quality and mosquito production. Ecol. Eng. 18: 441–457.

Tilley, D. 2012. Plant guide for cosmopolitan bulrush (*Schoenoplectus maritimus*). USDA-Natural Resources Conservation Service, Idaho Plant Materials Center. Aberdeen, ID. http://plants.usda.gov/plantguide/pdf/pg_scma8.pdf.

Victor, T.J. and R. Reuben. 2000. Effects of organic and inorganic fertilizers on mosquito populations in rice fields of southern India. Med. Vet. Entomol. 14: 361-368.

Walker, E.D., R.W. Merritt, M.G. Kaufman, M.P. Ayres, and M.H. Riedel.1997. Effects of variation in quality of leaf detritus on growth of the eastern tree-hole mosquito, *Aedes triseriatus* (Diptera: Culicidae). Can. J. Zool. 75: 706-718.

Walton, W.E. 2003. Managing mosquitoes in surface-flow constructed treatment wetlands. University of California, Division of Agriculture and Natural Resources, Davis, CA. Publ. No. 8117, 11 pp.

Walton, W.E. 2012. Design and management of free water surface constructed wetlands to minimize mosquito production. Wetlands Ecol. Manag. 20: 173-195.

Walton, W.E. and J.A. Jiannino. 2005. Vegetation management to stimulate denitrification increases mosquito abundance in multipurpose constructed treatment wetlands. J. Am. Mosq. Contr. Assoc. 21: 22-27.

Walton, W.E., D.A. Popko, A.R. Van Dam, A. Merrill, J. Lythgoe, and B. Hess. 2012. Width of planting beds for emergent vegetation influences mosquito production from a constructed wetland in California (USA). Ecol. Eng. 42: 150-159.

Walton, W.E., P.D. Workman, L.A. Randall, J.A. Jiannino, and Y.A. Offill. 1998. Effectiveness of control measures against mosquitoes at a constructed wetland in southern California. J. Vector Ecol. 23: 149-160.

Wang, W. 1991. Ammonia toxicity to macrophytes (common duckweed and rice) using static and renewal methods. Environ. Toxicol. Chem. 10: 1173-1177.

Yang, Z., K.Y. Lü, D.J. Chen, and S. Montagnes. 2012. The interactive effects of ammonia and microcystin on life-history traits of the cladoceran *Daphnia magna*: synergistic or antagonistic? PLoS ONE 7:e32285.