EVALUATION OF TWO MANAGEMENT STRATEGIES FOR HARVESTED EMERGENT VEGETATION ON IMMATURE MOSQUITO ABUNDANCE AND WATER QUALITY

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ABSTRACT. Emergent macrophytes play critical roles in water treatment processes of free-water surface constructed treatment wetlands. Management strategies for plant biomass affect wetland function and mosquito populations. Sinking of harvested macrophyte biomass is thought to provide organic carbon that enhances denitrifying bacteria important for nutrient removal while concomitantly reducing harborage for mosquitoes. The effects of sinking versus floating dried plant biomass (California bulrush [Schoenoplectus californicus]) on immature mosquito abundance and water quality (nutrient levels, oxygen demand, and physicochemical variables) were examined in mesocosms (28-m² ponds or 1.4-m² wading pools) under different flow regimes in 4 studies. The numbers of mosquito larvae in earthen ponds with floating vegetation were greater than in ponds with sunken vegetation on most dates but did not differ significantly between the 2 vegetation treatments in experiments using wading pools. Differences of the abundance of Anopheles larvae between the 2 vegetation management treatments were larger than for Culex larvae when naturally occurring larval mosquito predators were present. At high turnover rates (>2 pond volumes/day), water quality did not differ significantly between the vegetation management treatments and the water supply. At low turnover rates (approximately 2–6% of water volume/day), water quality differed significantly between the 2 vegetation management treatments and the water supply. Sinking vegetation can enhance the effectiveness of mosquito control but, depending on water management practices, may raise the concentrations of water quality constituents in discharges that are regulated under the Clean Water Act.

KEY WORDS Anopheles, bulrush, Culex, vegetation management, water quality

INTRODUCTION

The availability and treatment of water have been important issues historically and will take on even greater significance as the human population of more than 7 billion continues to expand and places even greater demands on water resources. In 2018, 80% of global sewage was untreated and 2 billion people lacked reliable, safe, potable water (Fairbrother et al. 2019). The high densities of humans living in cities (United Nations 2018), as well as in the surrounding suburbs, concentrate consumption of resources, produce diverse waste streams, and create conditions favorable for mosquitoes and other vectors that challenge the development of sustainable environmental and public health solutions in the face of a rapidly changing environment.

As scientists strive to define research priorities; to meet the demands for water treatment, water recycling, and other basic resources; and to develop new technologies to address the increasing levels and diversity of pollutant loads, coordination across disciplines is needed to understand the impact of the diversity of chemicals released by human activities and to assess and remediate adverse ecological or health outcomes of an ever larger human population worldwide (Europe: Van den Brink et al. 2018; Latin America: Furley et al. 2018; North America: Fairbrother et al. 2019). Comparable research priorities remain undefined in Africa and Asia where large human populations are transitioning to higher levels of per capita resource consumption and vector-borne diseases are especially pernicious. One of the key issues identified by Fairbrother et al. (2019) is: What environmental and human health risks should be managed and monitored in water reuse? Whereas mosquito control practitioners have been overlooked in the cross-discipline discussions, they are aware of the public health issues created by green technologies (e.g., septic ditches [Marten et al. 2000], free-water surface [FWS] treatment wetlands [Walton 2003, 2012], combined sewage outflows [Lund et al. 2014], catch basins [Metzger 2004, Harbison et al. 2019], rain barrel storage [Trewin et al. 2019], etc.) that maintain standing water in landscapes.

There is renewed interest to incorporate constructed wetland technology as part of watershed water management strategies for water quality improvement, supplemental water storage, and flood control as a component of “un-engineering” strategies to provide ecological security for urban areas worldwide (Gies 2018). The addition of hardscape associated with urbanization has reduced the ability of urban landscapes to accommodate flooding associated with large storms. Urbanization has caused more than $100 billion in economic losses from flooding in 62% of cities in China between 2011 and 2014 (Gies 2018) and, in the USA, has resulted in a 10% increase in surface runoff between 2001 and
Two vegetation management practices were studied: submerged versus floating dried bulrush (*Schoenoplectus californicus* (C.A. Meyer) Palla). The submerged vegetation treatment consisted of 9.1 kg of dried (air-dried approximately 9 months) bulrush placed on the water surface with same screen used previously and held below the water surface of each wading pool by 2 cinder blocks. Screen and 2 cinder blocks were placed on the bottom of pools in the floating vegetation treatment. In each experiment, a treatment was assigned to one of the pools in each row using a random number generator. Each treatment was replicated 3 times.

Studies of the 2 vegetation management practices were repeated on the following dates: September 15 through December 9, 2011 (autumn 2011). Prior to each of the 2011 studies, the pools were drained to a depth of approximately 2.54 cm and refilled. The sediment was not replaced. Approximately 1 wk before the start of the 3 studies, a 3-liter water sample was taken from each pool and combined into a composite sample. The composite sample was well mixed by hand and a 3-liter sample was placed into each wading pool. A similar 2nd inoculum was made at the start of each experiment. The 2 vegetation management treatments were reassigned among the replicate pools for each experiment.

**Physicochemical and water quality factors**

Water temperatures were measured using a maximum–minimum recording thermometer ( Marks-on Scientific, Inc., Del Mar, CA) positioned verti-
cally in a pond or wading pool (F3). Water temperatures were recorded weekly. Surface water temperatures also were measured continuously at 0.5-
h intervals with an electronic sensor (HOBO Water Temperature Data Logger, Model U22-001; Onset Computer Co., Bourne, MA) in a pond (G4) or
wading pool (F4).

Physicochemical and water quality variables were measured weekly. Specific conductance, pH, water temperature, and dissolved oxygen (DO) concentra-
tion in the 6 experimental units and the inflow sample (studies using wading pools) were recorded at the time of sampling (0815–1000 h) using an electronic sensor array (ICM Water Analyzers; Perstorp Analytical, Wilsonville, OR). A composite sample consisting of 100 ml water from each of the 6 inflows was taken to quantify the quality of water entering the ponds. For the studies using wading pools, inflow rates were comparatively low (i.e., replacing water lost to evaporation) and water was not flowing in the early morning when samples were collected. Therefore, an inflow water sample was taken from the float valve at pond G1 which was being used as nursery pond for bulrush.

Nutrient concentrations and other water quality variables were measured calorimetrically using TNT-series prepackaged tests and a spectrophotometer (model 2800; Hach Chemical Co., Loveland, CO). Total phosphorus (TP; as phosphate), chemical oxygen demand (COD; mercury-free method), ammonium nitrogen (NH₄-N), nitrate nitrogen (NO₃-N), nitrite nitrogen (NO₂-N), and total nitrogen (TN) concentrations were measured following protocols specified by the manufacturer (Hach Chemical Co.). Organic nitrogen concentration was estimated as the difference between TN and the sum of the 3 forms of inorganic nitrogen.

During the spring 2010 study, 5-day biochemical oxygen demand (BOD₅) and total suspended solids (TSS) in ponds were measured following American Public Health Association (APHA 1995) protocols 5210B and 2540D, respectively. An ion-specific electrode (no. 9708; ThermoOrion, Inc., Waltham, MA) was used to measure changes in the DO concentration over the 5-day incubation in a darkened incubator at 20 ± 1°C. Known volumes of water were filtered onto preweighed, oven-dried (103–105°C) glass-fiber filters (934-AH; Whatman Inc., Florham Park, NJ). The filters containing suspended material were dried overnight (103–105°C) and placed in a desiccator to cool to room temperature before reweighing.

Turnover rates for water volume (per day) in the spring 2010 study were estimated by dividing pond volume by the mean inflow rate for each pond. Inflow rates of water were quantified weekly by determining the time required to collect a known volume of water from the spigot associated with each float valve. Triplicate 350-ml samples were collected from the water supply to each pond. Water depth was measured at 32 equidistant stations in each pond on June 17, 2010.

Evaporation rates were estimated by 2 methods: a water evaporation equation (Engineering ToolBox 2004) and a modified ETo (evapotranspiration rate over irrigated turfgrass) calculation (AZMET 2018). Daily water evaporation rates (gd) were estimated using the following equation:

\[ gd = \Theta d (x_8 - x) \times 24, \]

where \( \Theta \) is the evaporation coefficient (kg/m²/h) = (25 + 19ν); \( ν \) is mean daily wind speed (m/sec) above the water surface; \( A \) is the surface area of the experimental unit (m²; pond or pool); \( x_8 \) is the maximum saturation humidity ratio (kg/kg) that was estimated using the regression \( x_8 = 0.0039 \times e^{(0.065x \times \text{mean daily water temperature})} \), \( R^2 = 0.99 \); and \( x \) is the humidity ratio in the air (kg/kg) based on the mean daily air temperature and mean daily RH, and was estimated using a Mollier diagram (Engineering ToolBox 2004). Daily means (midnight to midnight) for the meteorological variables were calculated using the hourly measurements for each variable and obtained from California Irrigation Management Information System (CIMIS; California Irrigation Management Information System, California Department of Water Resources, Sacramento, CA) station 44 on the University of California Riverside Agricultural Experiment Station. The CIMIS sensor array was <0.2 km from the Aquatic Research Facility.

A 2nd estimate of evaporation rate was obtained using the daily ETo calculated from hourly ETo estimates summed over 24 h (midnight to midnight). Hourly ETo was calculated using relevant meteorological measurements from the CIMIS station’s sensor array and the CIMIS Penman equation (CIMIS 2018). Mean daily water temperature over the same time interval was calculated using 0.5-h measurements from the HOBO sensor. The Brown correction (ETo/0.6; AZMET 2018) was applied to daily ETo calculated by CIMIS to estimate daily evaporation rate across the water surface of the experimental units.

**Mosquitoes and nontarget invertebrates**

Four 350-ml dip samples were taken weekly near the corners of each pond to monitor the abundance of mosquitoes and nonculicid invertebrates during the spring 2010 study. For the 3 studies using wading pools, 3 samples were taken from each pool using a 350-ml dipper. Dip samples were taken between 1000 and 1100 h. The dip samples from each experimental unit were combined using a concentrator cup (mesh opening: 53 μm). Specimens were preserved in alcohol (final concentration was approximately 50%). In the laboratory, immature mosquitoes were categorized into 3 subpopulations: 1st and 2nd instars, 3rd and 4th instars, and pupae. Late
Nonculicid invertebrates were separated into microinvertebrates (zooplankton) and macroinvertebrates (aquatic insects). Microinvertebrates were separated into cladocerans, copepods, and ostracods. Macroinvertebrates were keyed to at least the family level using the keys of Merritt et al. (2008).

**Statistical analysis**

Statistical comparisons for each water quality variable among the 2 vegetation management treatments and the inflow water were made using a repeated-measures analysis of variance (RMANOVA; SYSTAT Version 9.01H; SPSS, Inc., Chicago, IL). Post hoc comparisons among the treatments were made following a statistically significant between-subjects error term using Tukey’s HSD. Statistical comparisons between the 2 vegetation management treatments based on natural log–transformed mean larval mosquito abundance per dip for each experimental unit were made using RMANOVA. Mosquito pupae and predators of immature mosquitoes were rarely collected; univariate statistical comparisons of abundance were therefore not carried out.

Aquatic invertebrate community composition and its response to vegetation management treatment and to environmental variables were examined by constrained ordination using CANOCO (ver. 4.5; ter Braak and Smilauer 2002). The response of the invertebrate community in ordination space (linear versus unimodal) was examined first using detrended correspondence analysis (DCA) on log (x + 1)–transformed abundance for each taxon. Mosquito species, families of nonculicid insects, and suborders of zooplankton (i.e., Cladocera, Copepoda, Ostracoda) were the taxonomic variables. When >1 *Culex* species were present, early larval instars (1st and 2nd) and pupae were assigned to mosquito species based on the relative abundance of species determined from 3rd and 4th instars.

Canonical correspondence analysis (CCA) was used subsequently to DCA to extract the variation in species community composition explained by the environmental variables (nutrient concentrations and physicochemical variables) and to determine the best fit model of species composition based on the environmental variables measured in the study. Vegetation management treatment and each of the 4 experiments were specified as nominal variables. Global permutation tests (499 permutations) of the 1st 2 axes and the trace were carried out. The best fit model of species composition based on the relative abundance of species determined from 3rd and 4th instars.

The differences of specific conductance between the pond study and wading pool studies are indicative of the high turnover in pond volume and the low turnover of water in the wading pools. Specific conductance in the wading pools was 39–90% higher.
than the water supply (Table 3) and did not differ significantly between the 2 vegetation treatments. Specific conductance (approximately 500 μS/cm) was similar for the water supply of the wading pool studies and in the earthen ponds.

Evaporation rates ranged from 2.2% to 6.7% of water volume/day and differed between the 2 models (Table 5). For 3 of the 4 studies, the evaporation rate based on the engineering equation was about twice that of the ETo model. In contrast to a turnover rate of >1 volume/day for the ponds where infiltration and evaporation removed water from the experimental units, evaporation was the only water loss from the plastic wading pools. The turnover rate for water volume of the wading pools ranged from 19.2 to 40 days.

**Mosquitoes**

**Pond study:** Immature mosquitoes in ponds containing floating dried vegetation were 3–5 times more abundant than in ponds containing sunken vegetation on most dates during spring 2010 (mean difference between the 2 treatments = 3.5-fold; \( F_{1,4} = 7.90, P < 0.048; \) Fig. 3A). Predaceous tadpole shrimp, *Triops newberryi* (Packard), were collected and observed between weeks 3 and 8 of the experiment. The numbers of *Culex* spp. and *Anopheles hermsi* (Williston) larvae in the sunken vegetation treatment were reduced as compared with that in pools containing floating vegetation after week 3 (Fig. 3A). The abundance of *Culex* larvae in the sunken vegetation treatment rebounded beginning in week 6 as the numbers of tadpole shrimp declined in the ponds, but abundance of *Culex* larvae remained lower than in the floating vegetation treatment for the remainder of the experiment.

*Anopheles* larvae were affected by vegetation management treatment more than were *Culex* larvae. The abundance of *An. hermsi* larvae (all instars) in ponds with sunken vegetation was only 7% that in ponds with floating vegetation. The differences in the abundance of *Culex* spp. larvae (all instars) between the 2 vegetation management treatments were considerably smaller: abundance of larvae in ponds with sunken vegetation was about one-third (33%) that in ponds with floating vegetation.

The immature mosquitoes were dominated by *Culex tarsalis* Coquillett (proportion of 3rd and 4th instars collected: 0.65) during the experiment. *Anopheles hermsi* larvae (3rd and 4th instars) comprised 33% of the total numbers of mosquito larvae collected. *Culiseta inornata* (Williston) was rarely collected (approximately 1% of 3rd and 4th instars) and a single late instar of *Cx. quinquefasciatus* Say was collected.

The relative abundance of *Cx. tarsalis* and *An. hermsi* larvae in ponds with floating vegetation was more similar than in the ponds containing sunken vegetation where the relative abundance of both species differed by 8-fold. *Culex tarsalis* and *An.
hermsi were 58% and 40%, respectively, of late instars collected from ponds with floating vegetation. Culex tarsalis was 89% and An. hermsi was 11% of late instars collected from ponds with sunken vegetation.

Wading pool studies: Immature mosquito abundance in the 2 vegetation management treatments did not differ significantly in the 3 studies in wading pools (F-tests, \( P > 0.05 \)). During autumn 2010, immature mosquito abundance was reduced markedly (<3 larvae/dip) in 5 of the 6 wading pools when predaceous tadpole shrimp were collected throughout the study (Fig. 3B). Immature mosquito abundance ranged between 30 and 100 larvae/dip in pool E4 where tadpole shrimp were not collected (Fig. 3B). The mean number of larvae collected in dipper samples (≤10 larvae/dip) did not differ appreciably during the 2 studies in 2011 (Fig. 3C, 3D) even though 4.3 times (669 versus 157) as many larvae were collected during autumn 2011 compared with spring 2011.

Culex quinquefasciatus increased in relative abundance in the wading pool studies as compared with the pond study, especially during autumn. Nearly half (49.6%) of the late-stage larvae collected during autumn 2010 were Cx. quinquefasciatus. The other half (49.9%) of the collections of late-stage larvae were Cx. tarsalis. Anopheles hermsi and Cs. inornata were collected rarely (<0.3%) in autumn 2010. During autumn 2011, Cx. quinquefasciatus and Cx. tarsalis comprised 94% and 6%, respectively, of the late-stage larvae collected.

Table 2. Water quality for 2 vegetation management practices carried out in 28-m² earthen ponds and 1.4-m² plastic wading pools at the University of California Riverside Aquatic Research Facility, Riverside, CA.

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>COD (mg/liter)</th>
<th>BOD₅ (mg/liter)</th>
<th>TSS (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring 2010 (ponds)</td>
<td>Floating 40.5 ± 1.9 a</td>
<td>3.76 ± 0.35 a</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Submerged 38.2 ± 1.5 a</td>
<td>3.95 ± 0.35 a</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inflow 37.3 ± 1.7 a</td>
<td>4.47 ± 0.74 a</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Autumn 2010 (wading pools)</td>
<td>Floating 120.0 ± 8.5 b</td>
<td>15.93 ± 2.32 b</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Submerged 168.9 ± 8.4 b</td>
<td>28.79 ± 3.22 b</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inflow 38.7 ± 1.7 a</td>
<td>6.55 ± 1.52 a</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Spring 2011 (wading pools)</td>
<td>Floating 93.2 ± 5.8 b</td>
<td>—</td>
<td>6.61 ± 1.68 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Submerged 92.6 ± 4.5 b</td>
<td>—</td>
<td>2.19 ± 0.84 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inflow 28.4 ± 1.8 a</td>
<td>—</td>
<td>1.45 a</td>
<td></td>
</tr>
<tr>
<td>Autumn 2011 (wading pools)</td>
<td>Floating 146.2 ± 6.3 c</td>
<td>—</td>
<td>10.40 ± 2.35 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Submerged 111.2 ± 2.3 b</td>
<td>—</td>
<td>3.36 ± 0.77 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inflow 28.7 ± 1.1 a</td>
<td>—</td>
<td>0.83 ± 0.21 a</td>
<td></td>
</tr>
</tbody>
</table>

1 BOD₅, 5-day biochemical oxygen demand; COD, chemical oxygen demand; TSS, total suspended solids.
2 Mean ± SE. Letters indicate a statistically significant difference among treatments for each water quality variable within a study (\( P < 0.05 \)).
3 June 14.

Table 3. Physicochemical conditions (mean ± SE, \( N = 6 \)) during studies of 2 vegetation management practices carried out in 28-m² earthen ponds and 1.4-m² plastic wading pools at the University of California Riverside Aquatic Research Facility, Riverside, CA.

<table>
<thead>
<tr>
<th>Study</th>
<th>Variable</th>
<th>Pond or pool</th>
<th>Inflow¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring 2010 (ponds)</td>
<td>Mean temperature (°C) 18.1 ± 0.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Dissolved oxygen (mg/liter) 7.8 ± 0.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Specific conductance (µS/cm) 499.4 ± 1.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>pH 7.7 ± 0.04</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Autumn 2010 (wading pools)</td>
<td>Mean temperature (°C) 16.1 ± 2.3</td>
<td>19.3 ± 3.3</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Dissolved oxygen (mg/liter) 5.2 ± 0.4</td>
<td>8.3 ± 1.0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Specific conductance (µS/cm) 797.0 ± 29.9</td>
<td>503.0 ± 16.0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>pH 7.9 ± 0.1</td>
<td>8.1 ± 0.3</td>
<td>—</td>
</tr>
<tr>
<td>Spring 2011 (wading pools)</td>
<td>Mean temperature (°C) 16.4 ± 0.6</td>
<td>19.8 ± 1.6</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Dissolved oxygen (mg/liter) 4.4 ± 0.6</td>
<td>8.4 ± 1.3</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Specific conductance (µS/cm) 639.0 ± 45.7</td>
<td>461.0 ± 34.8</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>pH 7.5 ± 0.1</td>
<td>7.9 ± 0.1</td>
<td>—</td>
</tr>
<tr>
<td>Autumn 2011 (wading pools)</td>
<td>Mean temperature (°C) 18.0 ± 1.3</td>
<td>21.5 ± 2.6</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Dissolved oxygen (mg/liter) 1.5 ± 0.5</td>
<td>6.4 ± 2.2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Specific conductance (µS/cm) 988.0 ± 63.0</td>
<td>521.1 ± 22.5</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>pH 7.5 ± 0.1</td>
<td>7.7 ± 0.1</td>
<td>—</td>
</tr>
</tbody>
</table>

¹ Mean ± SD (autumn studies: \( N = 8 \); spring 2011: \( N = 5 \).
During spring 2011, more than half (54%) of the late-stage larvae collected were *Cx. tarsalis*. *Culex quinquefasciatus* was 26% of the late-stage larvae in samples. *Anopheles hermsi* represented 8% and *Cs. inornata* was 1% of the late instars collected. Ten percent of the late instars collected were identified as *Culex* spp.

**Aquatic invertebrate community**

The beta diversity in the data set (the length of the longest axis in the DCA) was 2.8, indicating that the unimodal ordination model was appropriate. The close relationship between species composition and the environmental variables was indicated by the strong correlation of each of the 1st 2 axes of the DCA with the environmental data (*r* = 0.76 and 0.59, respectively for axis 1 and axis 2). In the ordination constrained by the variation explained by the environmental variables (CCA), these axes explained 65% of variance in the species–environment relation and 19% of the variance in species data. Species–environment correlations for the 2 axes increased to 0.85 and 0.79, respectively for axis 1 and axis 2, in the constrained ordination.

The quantitative environmental variables retained in the reduced model are illustrated with arrows in the CCA ordination (Fig. 4). The canonical eigenvalues accounted for 42% of the variation in species composition, and the environmental variables in the reduced model accounted for >95% of this variation. The association of *Cx. quinquefasciatus* with comparatively enriched conditions in the wading pools is suggested by its relationship with high levels of COD, conductivity, and TP (Fig. 4). In contrast, *Cx. tarsalis*, *Cs. inornata*, and *An. hermsi* exhibited stronger associations with conditions found in the larger ponds where water quality was better than in the wading pools, denitrification was comparatively reduced (higher levels of NO3-N), and DO concentration was comparatively high (Fig. 4).

Vegetation management practices (Trt) were a minor but significant component of the reduced model, but overall had little effect on the species composition as compared with differences among the 4 experiments and predation by *Triops*. Tadpole shrimp predation had a pronounced effect by reducing larval mosquito abundance when physical structure in the water column was reduced (i.e., cut vegetation was sunk).

### DISCUSSION

Aquatic vegetation fulfills important functions in treatment wetlands, but balancing the many positive functions of macrophytes with the negative consequences of dense stands of vegetation is critical for water quality improvement, habitat supplementation, and mosquito control in treatment wetlands. Thullen et al. (2005) summarized the findings of numerous studies documenting the roles of aquatic plants in treatment wetlands. Besides aesthetics and providing wildlife habitat, aquatic plants act as physical filters that reduce water movement promoting sedimentation and adsorb constituents that reduce water quality, uptake nutrients and heavy metals, provide a substrate for microbiota and macroinvertebrates, reduce daily temperature fluctuations in the water column and substrate, contribute carbon and create anaerobic zones for denitrification, add oxygen to root zones where mineralization and nitrification occur, and enhance denitrification by translocating nitrates from the water column into anaerobic zones within the sediments.

Plant detritus can provide a requisite carbon source for denitrifying bacteria and enhance nitrogen removal through denitrification (Thullen et al. 2005, Kadlec and Wallace 2009). Whereas organic carbon levels in most treatment wetlands are probably adequate to fuel denitrification without supplementing natural sources of carbon, carbon can be limiting for denitrification in wetlands receiving low levels of BOD (input of less than approximately 5–9 g BOD/g NO3-N) and under high BOD loads where oxidized nitrogen is at low concentrations (Kadlec and Wallace 2009). Smith et al. (2000) found that nitrification within a treatment wetland receiving ammonia-rich municipal wastewater was enhanced by the reduction of emergent macrophyte biomass and an increase in the area of interspersed deep open water. After drying the wetland and burning the dead plant biomass, reducing the wetland surface area supporting dense stands of emergent macrophytes by either increasing zones of deep water (depth >1.5 m) or using raised planting beds (hummocks) enhanced denitrification rates (Smith et al. 2000) and reduced mosquito production (Thullen et al. 2002; Walton et al. 2012, 2013).

Inundation of harvested plant biomass is an alternative management strategy used by some managers of treatment wetlands to enhance denitrification (Keiper et al. 2003, Walton and Jiannino 2005). Inundation of cut and dried emergent vegetation that floats on the water surface can provide both harborage (e.g., protection from predation) and enrichment of food supplies for mosquito larvae (Berkelhammer and Bradley 1989) as well as increase the likelihood of transmission of mosquito-borne pathogens (Mackay et al. 2016). The abundance of mosquito larvae in treatment wetlands containing floating cut vegetation (Keiper et al. 2003, Walton and Jiannino 2005) and stormwater wetlands...
containing inundated vegetation that had been mowed (Mackay et al. 2016) was greater than in wetlands where vegetation had not been managed. Areal mosquito production from wetlands containing floating cut vegetation increased up to 100,000-fold from 0.49 to 0.65 individuals/m²/wk under normal operations (Keiper et al. 1999, 2003) to between 750–1,000 individuals/m²/wk and >50,000 individuals/m²/wk (based on dipper samples; Keiper et al. 2003, Walton and Jiannino 2005, Walton 2019). This management practice is discouraged for mosquito control (Keiper et al. 2003, Walton and Jiannino 2005).

Sinking dried emergent plant biomass in gabions might enhance nitrogen removal in constructed treatment wetlands where denitrification is limited by carbon supply while concomitantly reducing harborage for larval mosquitoes. Although we did not measure denitrification per se, the concentration of NO₃-N was reduced as compared with that in the inflow water, but the reduction of nitrate nitrogen in mesocosms with sunken vegetation was not enhanced as compared with that in mesocosms containing floating vegetation. The ratio of NO₃-N/BOD in the water supply was low, ranging from 1.2 to 2.1, with external NO₃-N loading rates ranging between 0.02–0.05 kg NO₃-N/day during spring 2010 and 13.2–32.6 mg NO₃-N/day during the 3 wading pool studies. Sinking harvested plant biomass might enhance the effectiveness of mosquito control but, depending on water management practices, enrich the resources used by mosquito larvae and raise the concentrations of water quality constituents in discharges that are regulated under the Clean Water Act (CWA).

Technology-based effluent limitations of publicly owned treatment works (POTW) under the CWA set minimum levels of effluent quality attainable based on the capabilities of the technologies available to control pollutant discharges into the waters of the

Fig. 1. Concentration of 3 water quality variables (mean ± SD) in the water supply (inflow) and in 2 vegetation management practices in 1.4-m² wading pools during autumn 2010 (left panels) and autumn 2011 (right panels). The dashed line is the lower limit of the range of effluent limits for chemical oxygen demand (COD; 90–120 mg/liter). NO₃-N, nitrate nitrogen; TP, total phosphorus.

Fig. 2. The 5-day biochemical oxygen demand (BOD₅; mean ± SE) in 2 vegetation management treatments in wading pools and in the water supply during autumn 2010. The dashed line is the 30-day average BOD₅ effluent limit for Reach 4 of the Santa Ana River (SAR). The BOD₅ effluent limit for the 7-day average in Reach 4 of the SAR and the federal 30-day average is 30 mg/liter.
USA. The secondary treatment standards for municipal wastewaters are based on an evaluation of performance data for POTWs practicing a combination of physical and biological treatment to remove conventional pollutants such as biodegradable organics and suspended solids (USEPA 2010). To be in compliance with the CWA, POTWs must meet 30-day and 7-day averages for the concentrations (30 mg/liter and 45 mg/liter, respectively, of BOD$_5$ and/or TSS), attain removal rates of not less than 85% of influent loading for these water quality constituents, and not discharge waters with pH outside the limits defined by the range 6–9. The secondary treatment standards are slightly higher (i.e., higher concentrations and lower removal efficiency) for technologies such as trickling filters and waste stabilization ponds (USEPA 2010). The states have some leeway to adjust the effluent compliance standards appropriate for the water treatment technology being used and local conditions (e.g., 30-day and 7-day averages for BOD$_5$ are 20 mg/liter and 30 mg/liter, respectively, for Reach 4 of the Santa Ana River; CRWQCB 2013). The effluent limits for “nonconventional” pollutants such as nutrients (forms of nitrogen and phosphorus), COD, etc. differ among ecoregions and are generally under the purview of the states that have different water quality standards criteria for DO and algal biomass. Treatment efficiency and discharge levels will be influenced by flow rate and its impact on turnover time of the water volume. Because permitted discharges are based typically on mass, the discharge limit for a potential pollutant is determined by the product of the treatment plant design flow, the mass to volume conversion factor, and the concentration-based limitation (USEPA 2010).

The addition of constructed treatment wetlands to treatment trains often reduces the concentrations of nutrients, pathogenic microbes, and suspended particulate matter in municipal effluent and agricultural

![Graph showing immature mosquito abundance](image)

Fig. 3. Immature mosquito abundance (mean ± SD) in 28-m$^2$ ponds or 1.4-m$^2$ wading pools in 2 vegetation management practices during 4 experiments: (A) spring 2010, (B) autumn 2010, (C) spring 2011, and (D) autumn 2011. The filled bar in panels A and B indicates the period when predaceous tadpole shrimp were present in the water column. Tadpole shrimp were not observed in wading pool E4 in panel B.

### Table 5. Evaporation rates from experimental units during 4 studies of 2 vegetation management practices.

<table>
<thead>
<tr>
<th>Study</th>
<th>Engineering equation</th>
<th>Modified ETo$^1$</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liters/day</td>
<td>Percent (volume)</td>
<td>Liters/day</td>
</tr>
<tr>
<td>Spring 2010</td>
<td>427.4 ± 71.3</td>
<td>4.1 ± 0.01</td>
<td>230.0 ± 48.8</td>
</tr>
<tr>
<td>Autumn 2010</td>
<td>17.1 ± 7.6</td>
<td>6.8 ± 3.0</td>
<td>6.3 ± 4.1</td>
</tr>
<tr>
<td>Spring 2011</td>
<td>17.1 ± 5.3</td>
<td>6.8 ± 2.1</td>
<td>13.3 ± 3.3</td>
</tr>
<tr>
<td>Autumn 2011</td>
<td>15.3 ± 6.3</td>
<td>6.1 ± 2.5</td>
<td>7.3 ± 3.0</td>
</tr>
</tbody>
</table>

$^1$ ETo, evapotranspiration rate over irrigated turfgrass.
Fig. 4. Ordination (canonical correspondence analysis) diagram illustrating the variation in the abundances of invertebrate taxa and environmental variables in 4 experiments of 2 vegetation management practices. The nominal explanatory variables are indicated as centroids (Δ) in the ordination diagram. The centroids for the abundance of common taxa are illustrated as Δ. Coleop is predaceous coleopteran larvae. COD, chemical oxygen demand; Cond, conductivity; DO, dissolved oxygen; NO₂⁻N, nitrite nitrogen; NO₃⁻N, nitrate nitrogen; TN, total nitrogen; TP, total phosphorus.

Nutrient uptake by macrophytes during the early- season growth phase is greater than during autumn as later during wetland development (Smith et al. 2000). Annual growth cycles and interannual changes of nutrient uptake by plants undergoing senescence (Reddy and DeBusk 1987, Reddy and DeLaune 2008). Soluble compounds including labile organic substances, nutrients (nitrogen, phosphorus), and cations (i.e., calcium, magnesium, potassium, sodium) rapidly leach out of inundated senescent macrophyte biomass (Nykvist 1959, Barko et al. 1991, Sartoris et al. 2000). Under anoxic conditions particulate nitrogen mineralizes and forms NH₄⁻N (Sartoris et al. 2000), which is a nutrient and volatilizes as ammonia in accordance to a pH-governed equilibrium. Mosquito populations increase over time as coverage and density of emergent vegetation increase (Walton et al. 1998, Thullen et al. 2002, Walton 2012). Harvesting emergent vegetation to remove nitrogen is labor intensive, expensive, and generally discouraged by wetlands engineers (Kadlec and Wallace 2009).

Several aspects of the experimental design merit consideration of the relationship of our findings to mosquito control in constructed treatment wetlands. The scale of experiments and mesocosm artifacts (e.g., wall effects, hydrological factors, redox potential, etc.) must be considered when cautiously generalizing the results from mesocosm studies to large field-scale wetlands (Ahn and Mitsch 2002). Irrespective of the vegetation management strategy, the decaying vegetation in the studies using wading pools reduced water quality, and the concentration of water quality constituents occasionally exceeded effluent limitations under the CWA. The BOD₅ and TSS CWA effluent limits were exceeded occasionally in some of the experiments. Likewise, COD effluent limits (range of National Pollutant Discharge Elimination System limitations: 90–120 mg COD/liter; USEPA 2000) were exceeded on most dates. The relative abundance of Cx. quinquefasciatus in the immature mosquito community increased when water quality was comparatively poor in the small wading pools. The concentrations of the potential pollutants would have been lower had higher water flows through the mesocosms diluted the water volume.

Evaporation was the only hydraulic loss process in the wading pools and accounted for a small turnover in the water volume (3.2–6.7% per day) and long residence times (>20 days). The lower evaporation rates calculated using the modified ET₀ model were probably better estimates of the evaporation rates from the mesocosms than were those calculated using the engineering model because the mesocosms were partially shaded and the evaporation rates were similar to the long-term monthly averages of evapotranspiration for the ecoregion (CIMIS data set) recommended for design of treatment wetlands (Kadlec and Wallace 2009). The porous substrate of the earthen ponds created high turnover rates (approximately 2–6 volumes per day), and the 2 vegetation management strategies had no demonstrable effect on water quality.

Whereas, hydraulic residence times were shorter (earthen pond studies) and longer (wading pool
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Vegetation management for bulrush is preferred to sinking plant biomass; both of these approaches can enhance mosquito control as compared with inundating cut vegetation. (2) Sinking plant biomass enhanced mortality of larval mosquitoes caused by some predators, but larval mosquito abundance was not always reduced by sinking plant biomass. Decaying vegetation created habitats favorable for mosquitoes regardless of whether or not the vegetation was sunk. (3) Depending on wetland size, design, and operational practices (i.e., hydraulic loading rates), cutting vegetation for mosquito control without removing plant biomass could, besides enhancing mosquito production, cause the concentrations of some water quality constituents to exceed pollutant limitations for effluent under the CWA and effluent standards set by some states.

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AZMET [The Arizona Meteorological Network]. 2018. Evapotranspiration: relationship to pan evaporation and


