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 physiochemical 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 ever 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 [Martén 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...
2011 (Chen et al. 2017). Not only has urbanization exacerbated flooding in urban and suburban areas, conversion of natural areas capable of absorbing stormwater into urban hardscapes and gray infrastructure has contributed to water shortages as areas providing recharge to subterranean aquifers have been reduced (Gies 2018). In order to allow natural hydrologic and ecologic processes to fulfill a greater role in water management strategies, standing water that is conducive to mosquito production can occur as a component of some green technologies.

Emergent macrophytes play critical roles in water treatment processes of constructed treatment wetlands (Thullen et al. 2005, Kadlec and Wallace 2009) and management strategies for plant biomass affect wetland function and mosquito populations (Thullen et al. 2002, Knight et al. 2003, Walton 2012, Mackay et al. 2016). Reduction of coverage by emergent macrophytes can be needed in FWS constructed treatment wetlands to improve water quality performance and reduce mosquito production, especially in wetlands treating municipal wastewaters (Sartoris et al. 2000, Smith et al. 2000, Thullen et al. 2002). Inundation of harvested plant biomass is contraindicated for mosquito control (Walton and Jiannino 2005, Walton 2019). Sinking of harvested macrophyte biomass is thought to retain organic carbon that enhances denitrifying bacteria important for nutrient removal while concomitantly reducing harborage for mosquitoes. The consequences of sinking versus floating dried plant biomass on mosquito abundance and water quality parameters (nutrient levels, oxygen demand, and physicochemical variables) were examined in 2 types of mesocosms under different flow regimens.

MATERIALS AND METHODS

Study site

Four studies in 2 types of mesocosms (earthen ponds or plastic wading pools) were completed at the Aquatic Research Facility on the Agricultural Experiment Station at University of California, Riverside. An 8-wk study was carried out in earthen ponds (4 m × 7 m) from April 13 to June 17, 2010. Six ponds in the northwestern block of ponds at the facility were used in the study (ponds E3, E4, F3, F4, G3, and G4). The ponds were devoid of emergent vegetation. Water was supplied continuously through a single pipeline from a reservoir fed by the Gage Canal, and water depth in the ponds was maintained at 0.124 m (±0.011; mean ± SE, N = 6) by float valves.

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 below 14-gauge galvanized steel welded wire garden fence coated with green polyvinyl chloride (Everbilt; The Home Depot, Atlanta, GA; mesh opening: 5.0 × 7.3 cm) in each pond. Eight cinder blocks were used to keep the fencing submerged in each pond. The floating vegetation treatment consisted of 9.1 kg dried bulrush placed on the water surface above fencing and cinder blocks that had been situated on the bottom of each replicate pond. One pond in each of the 3 rows (E–G) was assigned to one of the vegetation management treatments using a random number generator. Each treatment was replicated 3 times.

The 3 studies that followed the aforementioned study in spring 2010 were carried out in plastic wading pools. A wading pool (internal diam: 1.34 m; Sizzlin’ Cool®; Toys R Us Inc., Wayne, NJ) was positioned below the float valve for the water supply to each pond used in spring 2010. Water depth was 0.14 m. Dried sediment from each pond and 1 shovelful of damp sediment from another pond at the facility (C1; Offill and Walton 1999) were added to each wading pool to a depth of approximately 2.5 cm.

For the autumn 2010 and spring 2011 studies, dried bulrush culms were collected from the stockpile used in spring 2010. For the autumn 2011 study, bulrush was collected from the Prado Wetlands (Norco, CA; Walton et al. 2016) on August 17 and dried for approximately 30 days. Dried bulrush culms were cut to a length of approximately 0.25 m, and 0.46 kg of dried biomass was added to each pool in all studies. Bulrush in the submerged vegetation treatment was submerged with the 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 November 17, 2010 (autumn 2010); April 21 through June 14, 2011 (spring 2011); and 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) concentration 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 (1 h at 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 weighing.

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:

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

where \( \Theta \) is the evaporation coefficient (kg/m²/h) = (25 + 19v); \( v \) is mean daily wind speed (m/sec) above the water surface; \( A \) is the surface area of the experimental unit (m²; pond or pool); \( x_s \) is the maximum saturation humidity ratio (kg/kg) that was estimated using the regression \( x_s = 0.0039 \times e^{0.065x} \) (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
instars were identified to species using Meyer and Durso (1999).

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 was determined from 3rd and 4th instars.

**RESULTS**

**Physicochemical and water quality factors**

The concentrations of nutrients (nitrogen and phosphorus; Table 1) and other water quality variables (COD and BOD₅; Table 2) did not differ significantly between earthen ponds containing floating or sunken dried emergent vegetation during the spring 2010 study. The DO concentration in the earthen ponds was high (1.5–5 times higher) compared with that in the subsequent studies in wading pools (Table 3). The COD and BOD₅ in the ponds were similar to that observed in water supply (Table 2) and were indicative of the high turnover rate of pond water volume, which ranged between 1.8 and 6.3 pond volumes/day (Table 4). Water volume in the ponds ranged between 2.8 and 4.4 m³.

The concentrations of nutrients and other water quality variables in the water column often did not differ significantly between wading pools containing floating or sunken dried bulrush during the 3 studies after spring 2010 (Tables 1 and 2). Mean NO₃-N concentration in the water column of pools containing vegetation was 24–25% and 10–15% of NO₃-N in the inflow water during the autumn 2010 and both 2011 studies, respectively (Table 1). The concentration of NO₃-N in the water column of pools containing either floating or submerged vegetation was significantly less than that in the water supply in all studies (Fig. 1; autumn 2010: \(F_{2,4} = 74.79, P < 0.001\); spring 2011: \(F_{2,4} = 782.39, P < 0.0005\); autumn 2011: \(F_{2,4} = 379.09, P < 0.0005\)). The addition of dried emergent vegetation increased TN levels and COD as compared with the levels in the water supply (autumn 2011: TN: \(F_{2,4} = 37.07, P < 0.003\); COD: \(F_{2,4} = 205.5, P < 0.0008\)). Total phosphorus concentration in the wading pools was greater than in the water supply (Table 1) and reached a maximum at 2–3 wk and then declined across the experiments (Fig. 1). Water quality of the supply water did not differ significantly between the spring and autumn (F-tests; \(P > 0.05\)).

Bacterial degradation of the dried bulrush biomass added to wading pools decreased DO concentration in the water column. The mean DO in the wading pools was 23–62% of that in the water supply (Table 2); however, DO did not differ appreciably between the 2 vegetation management treatments. Mean COD either increased or remained >100 mg/liter across the experiments (Fig. 1). During the autumn 2010 wading pool study, BOD₅ increased initially and then fluctuated at >10 mg/liter across time (Fig. 2).

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
Table 1. Water quality for the water supply and 2 vegetation management practices carried out in earthen ponds and plastic wading pools at the University of California Riverside Aquatic Research Facility, Riverside, CA. 1

| Study | Treatment | NO3-N | NO2-N | NH4-N | Organic N | TN  | TP  |
|-------|-----------|-------|-------|-------|-----------|-----|-----|
| Spring 2010: earthen ponds | Floating | 2.54 ± 0.26 b | 0.21 ± 0.02 d | 0.03 ± 0.03 c | 0.94 ± 0.04 a | 3.41 ± 0.37 a | 0.01 ± 0.01 d |
| | Infiltrated | 2.48 ± 0.23 d | 0.23 ± 0.03 a | 0.05 ± 0.05 a | 0.97 ± 0.04 a | 3.46 ± 0.14 a | 0.03 ± 0.02 a |
| Autumn 2010: earthen ponds | Floating | 2.88 ± 0.17 b | 0.32 ± 0.09 b | 0.05 ± 0.05 a | 0.92 ± 0.03 a | 3.78 ± 0.14 a | 0.06 ± 0.02 b |
| | Infiltrated | 2.73 ± 0.12 a | 0.23 ± 0.03 c | 0.05 ± 0.05 a | 0.99 ± 0.04 a | 3.75 ± 0.21 a | 0.05 ± 0.01 c |
| Spring 2011: wading pools | Floating | 2.485 ± 0.21 a | 0.21 ± 0.02 a | 0.04 ± 0.04 a | 0.94 ± 0.04 a | 3.23 ± 0.12 a | 0.09 ± 0.03 d |
| | Infiltrated | 2.405 ± 0.20 a | 0.22 ± 0.03 a | 0.05 ± 0.05 a | 0.98 ± 0.03 a | 3.22 ± 0.10 a | 0.07 ± 0.02 a |
| Autumn 2011: wading pools | Floating | 1.406 ± 0.14 a | 0.14 ± 0.02 a | 0.03 ± 0.03 a | 0.89 ± 0.03 a | 2.63 ± 0.07 a | 0.06 ± 0.02 a |
| | Infiltrated | 1.425 ± 0.15 a | 0.14 ± 0.02 a | 0.03 ± 0.03 a | 0.90 ± 0.03 a | 2.69 ± 0.16 a | 0.07 ± 0.02 a |

1 NH4-N, ammonium nitrogen; NO3-N, nitrate nitrogen; NO2-N, nitrite nitrogen; TN, total nitrogen; TP, total phosphorus.
2 Mean ± SE. Letters indicate a statistically significant difference among treatments for each water quality variable within a study (P < 0.05).
3 Phosphate.

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; F1,4 = 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 Barr and Guttavani 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%) of 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 (Willistain) 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 ($\leq$10 larvae/dip) in 5 of the 6 wading pools when predaceous tadpole shrimp were collected throughout the study (Fig. 3B). Immature mosquito abundance ranged between 30 and 100 larvae/dip in pool E4 where tadpole shrimp were not collected (Fig. 3B). The mean number of larvae collected in dipper samples ($\leq$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 $C_\text{x. quinquefasciatus}$. The other half (49.9%) of the collections of late-stage larvae were $C_\text{x. tarsalis}$. Anopheles hermsi and $C_\text{s. inornata}$ were collected rarely ($\leq$0.3%) in autumn 2010. During autumn 2011, $C_\text{x. quinquefasciatus}$ and $C_\text{x. tarsalis}$ comprised 94% and 6%, respectively, of the late-stage larvae collected.

Table 2. Water quality for 2 vegetation management practices carried out in 28-m$^2$ earthen ponds and 1.4-m$^2$ plastic wading pools at the University of California Riverside Aquatic Research Facility, Riverside, CA.$^1$

| Study                  | Treatment     | COD      | BOD$_5$   | TSS       |
|------------------------|---------------|----------|-----------|-----------|
| Spring 2010 (ponds)    | Floating      | 40.5 ± 1.9 a | 3.76 ± 0.35 a | —         |
|                        | Submerged     | 38.2 ± 1.5 a | 3.95 ± 0.35 a | —         |
|                        | Inflow        | 37.3 ± 1.7 a | 4.47 ± 0.74 a | —         |
| Autumn 2010 (wading pools) | Floating       | 120.0 ± 8.5 b | 15.93 ± 2.32 b | —         |
|                        | Submerged     | 168.9 ± 8.4 b | 28.79 ± 3.22 b | —         |
|                        | Inflow        | 38.7 ± 1.7 a | 6.55 ± 1.52 a | —         |
| Spring 2011 (wading pools) | Floating       | 93.2 ± 5.8 b  | —          | 6.61 ± 1.68 a |
|                        | Submerged     | 92.6 ± 4.5 b  | —          | 2.19 ± 0.84 a |
|                        | Inflow        | 28.4 ± 1.8 a  | —          | 1.45$^3$ |
| Autumn 2011 (wading pools) | Floating       | 146.2 ± 6.3 c | —          | 10.40 ± 2.35 c  |
|                        | Submerged     | 111.2 ± 2.3 b | —          | 3.36 ± 0.77 b  |
|                        | Inflow        | 28.7 ± 1.1 a  | —          | 0.83 ± 0.21 a  |

1 BOD$_5$, 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-m$^2$ earthen ponds and 1.4-m$^2$ plastic wading pools at the University of California Riverside Aquatic Research Facility, Riverside, CA.

| Study                  | Variable             | Pond or pool | Inflow$^1$ |
|------------------------|----------------------|--------------|------------|
| Spring 2010 (ponds)    | Mean temperature ($^\circ$C) | 18.1 ± 0.1 | —          |
|                        | Dissolved oxygen (mg/liter) | 7.8 ± 0.9 | —          |
|                        | Specific conductance ($\mu$S/cm) | 499.4 ± 1.8 | —          |
|                        | pH                   | 7.7 ± 0.04 | —          |
| Autumn 2010 (wading pools) | Mean temperature ($^\circ$C) | 16.1 ± 2.3 | 19.3 ± 3.3 |
|                        | Dissolved oxygen (mg/liter) | 5.2 ± 0.4 | 8.3 ± 1.0  |
|                        | Specific conductance ($\mu$S/cm) | 797.0 ± 29.9 | 503.0 ± 16.0 |
|                        | pH                   | 7.9 ± 0.1  | 8.1 ± 0.3  |
| Spring 2011 (wading pools) | Mean temperature ($^\circ$C) | 16.4 ± 0.6 | 19.8 ± 1.6 |
|                        | Dissolved oxygen (mg/liter) | 4.4 ± 0.6 | 8.4 ± 1.3  |
|                        | Specific conductance ($\mu$S/cm) | 639.0 ± 45.7 | 461.0 ± 34.8 |
|                        | pH                   | 7.5 ± 0.1  | 7.9 ± 0.1  |
| Autumn 2011 (wading pools) | Mean temperature ($^\circ$C) | 18.0 ± 1.3 | 21.5 ± 2.6 |
|                        | Dissolved oxygen (mg/liter) | 1.5 ± 0.5 | 6.4 ± 2.2  |
|                        | Specific conductance ($\mu$S/cm) | 988.0 ± 63.0 | 521.1 ± 22.5 |
|                        | pH                   | 7.5 ± 0.1  | 7.7 ± 0.1  |

1 Mean ± SD (autumn studies: $N = 8$; spring 2011: $N = 5$).
Table 4. Turnover rate of water volume in 4-m × 7-m earthen ponds during the spring 2010 experiment.

| Pond | Water volume (m$^3$) | Turnover rate (per day) |
|------|---------------------|------------------------|
| E3   | 3.47                | 2.5                    |
| E4   | 3.88                | 2.2                    |
| F3   | 2.76                | 2.8                    |
| F4   | 4.44                | 1.8                    |
| G3   | 2.75                | 6.3                    |
| G4   | 2.77                | 3.1                    |

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 NO$_3$-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 NO$_3$-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 Jianiino 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 Jianiino 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
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 BOD5 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 BOD5 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

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

| Study       | Engineering equation | Modified ETo$^1$ |
|-------------|-----------------------|-----------------|
|             | Liters/day | Percent (volume) | Liters/day | Percent (volume) |
| Spring 2010 | 427.4 ± 71.3 | 4.1 ± 0.01 | 230.0 ± 48.8 | 2.2 ± 0.5 |
| Autumn 2010 | 17.1 ± 7.6   | 6.8 ± 3.0   | 6.3 ± 4.1   | 3.2 ± 2.1   |
| Spring 2011 | 17.1 ± 5.3   | 6.8 ± 2.1   | 13.3 ± 3.3  | 6.7 ± 1.7   |
| Autumn 2011 | 15.3 ± 6.3   | 6.1 ± 2.5   | 7.3 ± 3.0   | 3.7 ± 1.5   |

$^1$ ETo, evapotranspiration rate over irrigated turfgrass.
Nutrient uptake by macrophytes during the early-season growth phase is greater than during autumn as there are higher nutrient concentrations in the water column and longer mass (approximately 2–6 volumes per day), and the 2 experiments had no demonstrated effect on water quality. Algal production in nutrient-rich open-water zones (Kadlec and Wallace 2009) and detritus derived from periphyton and macrophyte biomass affect effluent TSS concentrations. The response of aquatic organisms to failures of compliance with CWA effluent limits (range of National Pollutant Discharge Elimination System limitations: 90–120 mg COD/liter; USEPA 2000) were exceeded on most dates. Elimination System limitations: 90–120 mg COD/liter; USEPA 2000) were exceeded on most dates. The relative abundance of *Culex quinquefasciatus* in the immature mosquito community increased when 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 *Culex 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 ETo 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
studies) than those typically found in operational constructed treatment wetlands (3.5–14 days; Sartoris et al. 2000, see table 18.1 in Kadlec and Wallace 2009; 0.9–20 days, O’Geen and Bianchi 2015), the amount of dried macrophyte biomass in the mesocosms was small as compared with that found in operational treatment wetlands. The dried plant biomass in each experimental unit in all studies was equivalent to only about 15 S. californicus stems/m². The mean stem (culm) densities of mature bulrush stands in the vegetated zones of constructed wetlands treating municipal effluent in southern California are >200 stems/m² (231 stems/m², Sartoris et al. 2000; 250 stems/m², Thullen et al. 2002). The maximum stem density recorded by Thullen et al. (2002) was 992 stems/m². Some of these wetlands contained deep open-water zones; therefore, mean bulrush stem densities standardized for both total area (vegetated shallow zones and unvegetated open water in the Hemet/San Jacinto constructed multipurpose demonstration wetland: 1997, 1st design: 184 stems/m²; 2001, 2nd design: 116 stems/m²; Daniels et al. 2010) and volume (1997: 160 stems/m³; 2001: 73 stems/m³) are lower. Besides vegetation density, differences in the natural mortality cycles of macrophytes (annual dieback of the all aboveground biomass [e.g., Schoenoplectus acutus (Muhl. ex J.M. Bigelow) A. Löve & D. Löve, S. maritimus (L.) Palla] versus partial dieback of mature stands [e.g., S. californicus]) and treatment wetland design and operation (i.e., the ratio of zones supporting vegetation to open water, layout of vegetated zones, hydraulic loading rate, etc.) will influence the extent that internal loading will affect the concentrations of water quality constituents. Moreover, drying cut plant biomass for >1 month before inundation reduced its attractiveness to egg-laying mosquitoes (Sanford et al. 2003). The lengthy drying period for the bulrush used in our experiments presumably reduced its propensity to attract mosquito oviposition.

Sinking plant biomass enhanced the impact of tadpole shrimp predation on the abundance of mosquito larvae, especially Anopheles larvae. The occurrence of tadpole shrimp in the 2010 experiments was not intended. Individuals hatched from desiccation-resistant eggs in the substrate of the ponds and the substrate transferred into the wading pools. Benthic foraging activities of tadpole shrimp create bioturbation but T. newberryi also forages at the water surface (Tietze and Mulla 1991), principally at night. The differences of the relative abundance of Anopheles larvae in the larval mosquito populations between the 2 vegetation management treatments suggest that anopheline larvae which reside closer to the air–water interface than do Culex larvae were especially susceptible to the carnivorous shrimp. Triops has been shown to be an effective biological control agent against Culex spp. (Tietze and Mulla 1991) and Psorophora spp. (Su and Mulla 2002) in flood-irrigated agriculture where the flood-water life history of Triops is well suited. Interestingly, the few insect predators that colonized the wading pools in the 2011 experiments, when tadpole shrimp were not present, did not differentially affect mosquito abundance in the 2 vegetation management treatments.

Our findings have implications for source reduction practices that are often an essential component of integrated mosquito management programs for constructed treatment wetlands. Not surprisingly, decaying plant biomass reduced water quality as compared with that of the water supply and water management (i.e., turnover time) had marked consequences on the concentrations of important water quality constituents. Important conclusions for integrated mosquito management in wetlands include (1) removing cut plant biomass from treatment wetlands 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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