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SCIENTIFIC NOTE

DISTRIBUTION OF *CULEX* SPECIES IN VEGETATION BANDS OF A CONSTRUCTED WETLAND UNDERGOING INTEGRATED MOSQUITO MANAGEMENT

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ABSTRACT. The distribution and abundance of emerging *Culex* spp. were assessed within narrow (width: 3 m) and wide (width: 20 m) bands of California bulrush (*Schoenoplectus californicus*) and in the open water adjacent to emergent vegetation in 2 marshes of an ammonia-dominated wastewater treatment wetland in southern California. Emerging mosquitoes were collected along transects perpendicular to the path of water flow at 3 distances (1.5, 5, and 10 m) from the vegetation–open water interface in the wide bands of emergent vegetation, at the center of narrow bands of emergent vegetation, and at 1.5 m from the edge of emergent vegetation in the open water. The width of vegetation bands (3 vs. 20 m) influenced the effectiveness of integrated mosquito management practices, especially the application of mosquito control agents. Mosquito production from the 2 marshes also differed up to 14-fold, suggesting that the distance between the shorelines (62 vs. 74 m) of each marsh also influenced the efficacy of mosquito control agents applied from the shore and boats. Hot spots of mosquito production (75–424 female *Culex*/m^2/day) were found within the wide bands of bulrush. During summer, the relative abundance of *Culex stigmatosoma* among emerging mosquitoes increased from the periphery to the center of wide bands of emergent vegetation. *Culex erythrothorax* emergence rates were comparatively similar among the transects in the wide bands of emergent vegetation. *Culex tarsalis* adults increased in number from the periphery to the center of wide bands of bulrush and, in May, were >95% of emerged mosquitoes.

KEY WORDS Constructed wetlands, bulrush, emergent vegetation, integrated mosquito management, *Culex*

Design and management practices are important components of integrated mosquito management (IMM) programs for constructed treatment wetlands (Knight et al. 2003, Walton 2012). Wetland vegetation provides important ecological functions for water quality improvement (Kadlec and Wallace 2008), but emergent macrophytes have several drawbacks for mosquito control in constructed treatment wetlands. Dense stands of emergent vegetation and mats of decaying macrophytes impede the penetration to the water surface by most of the current formulations of biorational mosquito control agents, limit the effectiveness of larvivorous fishes, and reduce the mortality of mosquito immatures caused by insect predators (Knight et al. 2003, Walton et al. 2012).

Islands or contiguous bands of vegetation oriented perpendicularly to the path of water flow and separated by open water are among the planting schemes used in free-water-surface constructed wetlands (Thullen et al. 2002, Kadlec et al. 2010, Keefe et al. 2010). Thullen et al. (2005) recommended the use of isolated raised planting beds (hummocks) surrounded by deep water to limit the growth of emergent vegetation, enhance decomposition of senescent macrophytes, and improve water quality performance of surface-flow wastewater-treatment wetlands receiving ammonia-dominated effluent.

Walton et al. (2012) investigated the impact of narrow versus wide raised planting beds of emergent vegetation on water quality, the distribution of larvivorous fishes and predaceous insects, and mosquito production in a constructed wetland treating ammonia-rich municipal effluent that had undergone secondary wastewater treatment. They found that mosquito production was lowest in the open water adjacent to bands of emergent vegetation and was often greater in the center of wide bands of California bulrush (*Schoenoplectus californicus* (C.A. Mey.) Palla) than in the narrow bands of bulrush. However, Walton et al. (2012) did not discuss the relative contribution of particular mosquito species to adult production and did not provide the spatial details of mosquito emergence rates under the IMM program. This note describes these important details of mosquito production from the 2 planting regimes.

Mosquito production was studied at a 9.9-ha surface-flow constructed treatment wetland used

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to treat ammonia-dominated secondary municipal effluent at the Hemet-San Jacinto Regional Wastewater Reclamation Facility (HSJRWRF) in San Jacinto, CA (33°47′52″N, 117°01′19″W) during 2005 and 2006. A narrow (width parallel to water flow ~3 m wide) and wide (width parallel to water flow ~20 m) marsh separated by a deepwater zone (~12 m wide; water depth ≥1.5 m; Fig. 1) were studied in 2 inlet marshes (Inlet Marsh 1 and Inlet Marsh 5; Sartoris et al. 2000) of the wetland. After reconfiguration of the wetland to improve water quality performance and reduce mosquito production, approximately 70% of the wetland surface area was open water; deepwater zones were present on the upstream (inflow) and downstream (outflow) sides of all the marshes. Water depth in the marshes varied seasonally from approximately 0.4 m during summer to 1 m during winter.

Eight transects were positioned in each of the 2 inlet marshes (Fig. 1). Five transects were established in each wide band of California bulrush. Transects of 3 trapping sites were positioned at 1.5 m and 5 m from the open-water zones on the upstream and downstream sides of the wide band of emergent vegetation. A transect of 6 trapping sites was located at 10 m from the open water on each side of the wide band of emergent vegetation. A transect of 6 trapping sites also was run along the center of a narrow band of emergent vegetation. Transects of 3 trapping sites were positioned in the open water at 1.5 m from the vegetation–open water interface on the upstream and downstream sides of the wide band of emergent vegetation.

Emerging adult mosquitoes were collected using 0.25-m^2 floating emergence traps (Walton et al. 1999). Sixty emergence traps (30 per marsh) were deployed for 4 days on 4 sampling dates: July and September 2005 and February and May 2006. Emergent vegetation was cut above the water surface to facilitate placement of each emergence trap and to maintain the physical structure below the water surface. After 4 days, the collection jars were returned to the laboratory, frozen, and mosquitoes were enumerated at 25–50× magnification using a stereo dissecting microscope. Mosquitoes were identified to species using Meyer and Durso (1998). Mosquitoes were not collected in February and only 8 mosquitoes were collected in September; these data will not be considered further.

Control measures against larval and adult mosquitoes at HSJRWRF were carried out by the Riverside County Department of Environmental Health using truck-mounted and backpack-mounted application technologies. Larvicides included VectoBac® AS (aqueous solution) or G (granules) of *Bacillus thuringiensis* subsp. *israelensis* de Barjac and VectoLex® CG (corncob granules impregnated with *Lysinibacillus* (formerly *Bacillus*) *sphaericus* Neide (Valent BioSciences Corp., Libertyville, IL). Permanone® (Bayer Environmental Science, Research Triangle Park, NC) was used against adult mosquitoes. The dates of the larvicide and adulticide treatments from April 2005 until May 2006 can be found in Walton et al. (2012). Of relevance to the mosquito production discussed here, adulticide and larvicides (Vectobac G and Vectolex CG) were applied 3 days before deploying emergence traps in July and 7.5 months before the May samples.

The statistical significance of the effects of transect location, marsh, and their interaction on mosquito production were tested using a general linear model analysis of variance (SYSTAT v. 9; SPSS 1998). The number of mosquitoes in each trap collection was $\ln(X+1)$ transformed prior to statistical analysis. The pattern of mosquito emergence in each marsh was depicted as a 2-dimensional contour plot by kriging (PLOT: SYSTAT v. 9; SPSS 1998) mosquito production across the sample grid. Tension in the model was set so that only adjacent samples influenced the interpolation of mosquito production between sampling sites; mosquito production hot spots could occur and a large-scale spatial trend for mosquito production was therefore assumed not to be present.

The width of emergent vegetation bands along the direction of water flow (3 m vs. 20 m), as well as the distance between the shoreline of each marsh (i.e., width of the marshes perpendicular to the direction of water flow), influenced the efficacy of the IMM practices. Mosquito production from the 20-m bands of emergent vegetation was greater than from open water during the peak period of annual mosquito abundance in early summer and differed between the 2 marshes (marsh: $F_{1,49} = 3.790$; $P = 0.057$; position: $F_{4,49} = 3.818$; $P < 0.009$; marsh × position: $F_{4,49} = 0.231$; $P > 0.9$). Between 6- and 14-fold more mosquitoes were collected from Inlet Marsh 1 compared to Inlet Marsh 5. Inlet Marsh 1 was approximately 12 m wider than Inlet Marsh 5 (width perpendicular to water flow: 74 vs. 62 m). Within each marsh, mosquito production from the 3-m bands of emergent vegetation was intermediate between mosquito emergence along the transects within the 20-m bands of bulrush and the open water, but did not differ significantly from the low mosquito production from open water (Walton et al. 2012).

As compared to the wide bands of bulrush, mosquito production from the narrow bands of emergent vegetation and the open water was effectively reduced or eliminated in May. During May, adult mosquitoes were not collected in traps above open water and in the narrow band of vegetation in Inlet Marsh 5. Moreover, the number of mosquitoes emerging from open water and in the narrow band of vegetation in Inlet

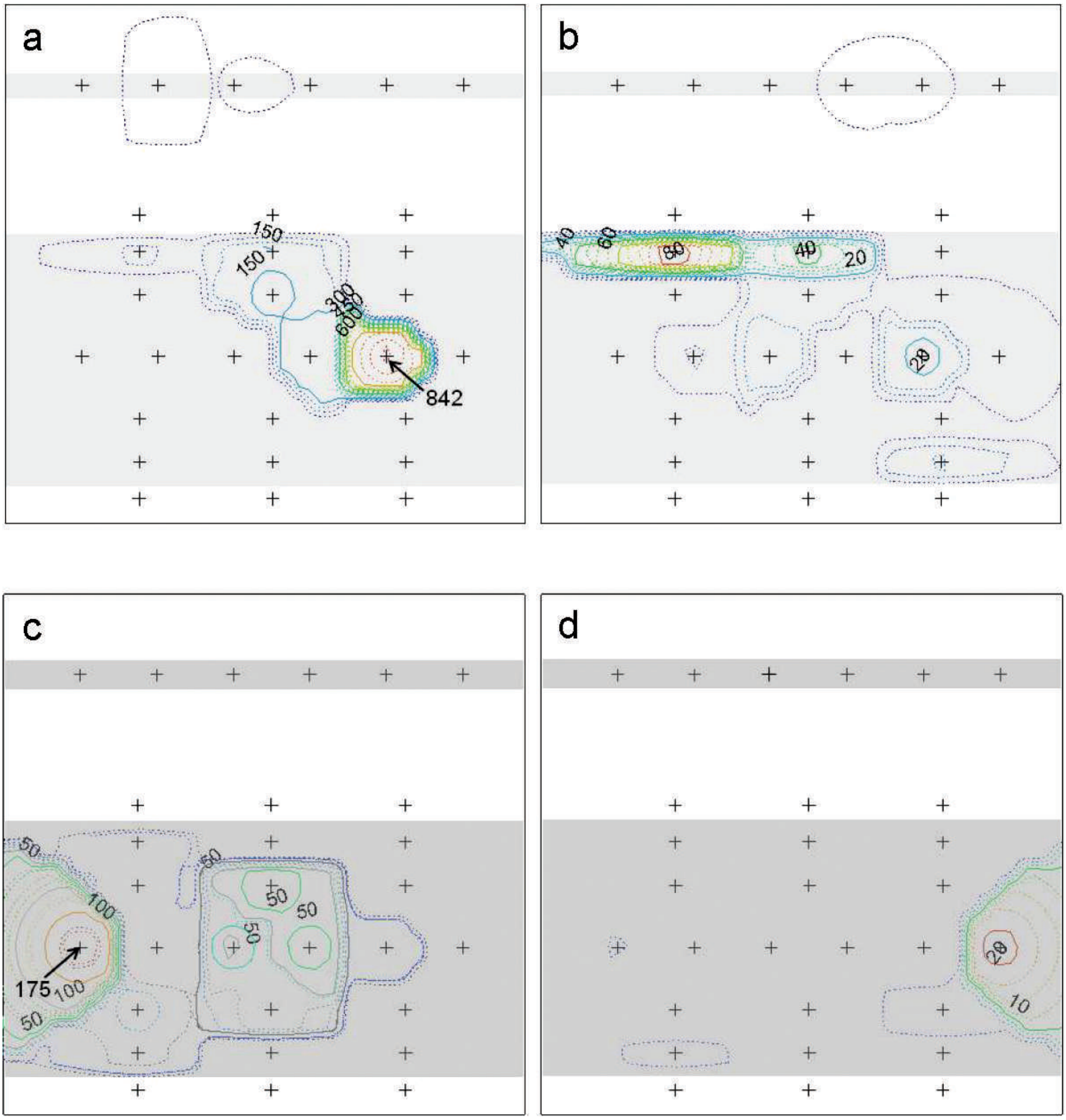

Fig. 1. *Culex* adult production (individuals/m²/day) from 2 marshes at the Hemet-San Jacinto Regional Wastewater Reclamation Facility demonstration constructed wetland: (a) Inlet Marsh 1, July 2005; (b) Inlet Marsh 5, July 2005; (c) Inlet Marsh 1, May 2006; and (d) Inlet Marsh 5, May 2006. Gray shading depicts a zone of emergent vegetation between zones of deep water. The narrow band of vegetation is downstream from the wide band of vegetation. "+" is an emergence trap. The dotted lines represent contours with values equally spaced between either zero and the solid line for lowest value indicated in each panel or between the solid contour lines for the values in each panel.

Marsh 1 did not differ significantly from 0 individuals/m²/day (Walton et al. 2012). For transect positions where mosquitoes were collected, mosquito production differed between the 2 marshes and among transect positions within the marshes (marsh: $F_{1,30} = 30.620$; $P < 0.0005$; position: $F_{2,30} = 3.354$; $P < 0.05$; marsh × position: $F_{2,30} = 1.358$; $P > 0.27$). In the 20-m-wide bands of emergent vegetation of both marshes, mosquito production increased directly with distance from the open water–vegetation interface.

Hot spots of mosquito production were observed, especially in the wide band of emergent vegetation. Mosquito production was concentrated near the center of the wide band of emergent vegetation in Inlet Marsh 1 (Fig. 1a: 842 individuals/m²/day; 424 female *Culex*/m²/day) and towards the center of the downstream side of the vegetated area in July. Mosquito production from Inlet Marsh 5 was concentrated along the periphery in the center of the wide band of bulrush in July (Fig. 1b). In May, mosquito production was concentrated along the periphery of the center as well as centrally within the wide band of emergent vegetation in Inlet Marsh 1 (Fig. 1c). Mosquito production was effectively reduced in Inlet Marsh 5 except at the periphery on one side of the center of the wide band of emergent vegetation (Fig. 1d). Walton (2009) found that about 50% of emerged mosquitoes were collected in jars of the emergence traps during a 4-day trapping period; mosquito production therefore could have been about twice that depicted in Fig. 1.

An inability to distribute larvicidal agents uniformly into the dense emergent vegetation presumably contributed to the spatial differences of mosquito production. Bacterial larvicides applied by truck-mounted and backpack-mounted application technologies cannot always effectively penetrate dense vegetation in the center of 20-m-wide bands of emergent vegetation. The effective distance of application for granular formulations of bacterial larvicides by the equipment used during this study is only about 4.5–6 m (15–20 ft). Trees (e.g., willows [*Salix* sp.]) growing along the periphery of the marshes also could have interfered with the application of mosquito control agents. Nevertheless, weekly or biweekly applications of larvicides and ultra-low volume applications of chemical adulticides reduced the annual maximum of host-seeking populations by more than an order of magnitude relative to the large adult mosquito populations observed before an IMM program was implemented at this constructed treatment wetland (<1,350 individuals/trap/night vs. >33,000 individuals/trap/night; Walton et al. 1998, 2012).

All emerging mosquitoes were in the genus *Culex*. The 3 prevalent species collected during July were *Culex tarsalis* Coquillett, *Cx. stigmatosoma* Dyar, and *Cx. erythrothorax* Dyar. *Culex tarsalis* was >80% of the few mosquitoes collected from open water and comprised about 50% of the adults emerging from the narrow bands of emergent vegetation and along transects near open water in the wide band of bulrush during July (Fig. 2). The relative abundance of *Cx. stigmatosoma* increased towards the center of the wide bands of bulrush, comprising about 60% of adult mosquitoes collected along the transects in the center of wide vegetated bands during July. The relative abundance of the tule mosquito, *Cx. erythrothorax*, was largest in vegetation near open water (42%) during July and declined towards the center of the wide band of bulrush. However, as compared to *Cx. tarsalis* and *Cx. stigmatosoma* production, which increased with distance from the vegetation–open water interface, the number

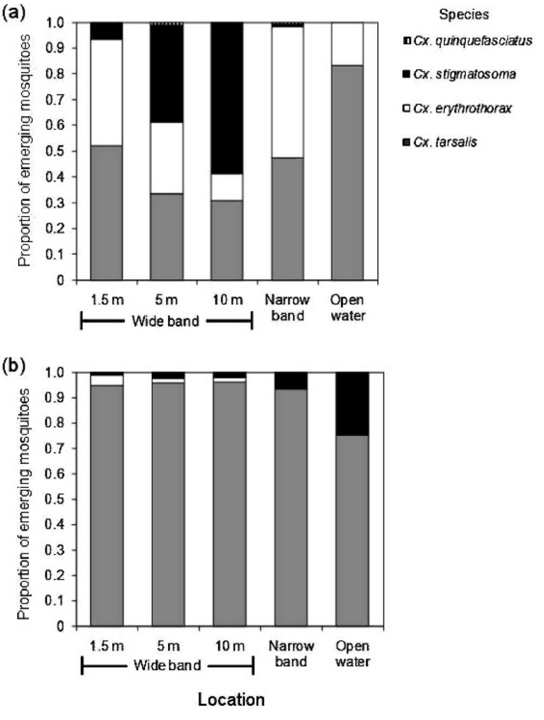

Fig. 2. The relative abundance of *Culex* adults produced along transects at 5 positions in 2 marshes at the Hemet-San Jacinto Regional Wastewater Reclamation Facility demonstration wetland: (a) July 2005, (b) May 2006.

of *Cx. erythrothorax* emerging per unit area was relatively similar across the transects in the wide band of vegetation. *Culex quinquefasciatus* Say was rarely collected (<1% of emerging mosquitoes). In contrast to July, the relative abundance of *Cx. tarsalis* in emergence trap collections from emergent vegetation was >90% during May (Fig. 2).

In addition to difficulties applying effective dosages of larval mosquito control agents to the wide bands of bulrush, mosquito abundance could have been enhanced by several factors. Smaller predator populations coupled with lower prey detection rates in dense emergent vegetation (Thullen et al. 2002, Walton et al. 2012) could have reduced larval mosquito mortality as compared to sites in the narrow band of vegetation and in open water. Mats of decaying vegetation that attracted ovipositing mosquitoes (Sanford et al. 2003) and enhanced nutrition of mosquito larvae (Berkelhamer and Bradley 1989) also could have enhanced mosquito production in the wide bands of vegetation.

The greater relative abundance of *Cx. stigmatosoma* towards the center compared to the periphery of wide bulrush stands might have been related to a profusion of decaying plant material in summer 2005. Natural lodging of dead vegetation and prior nest building by water birds (primarily coots [*Fulica americana* Gmelin]) contributed to mats of decaying vegetation in the center of wide bands of emergent vegetation during the low water levels maintained during summer. The sinking of guano-laden nest material of recently abandoned nests following fledging of young birds would have contributed nutrients that enhanced autochthonous production of larval mosquito resources. Survival and growth of *Cx. stigmatosoma* larvae in hypereutrophic conditions is greater than for *Cx. tarsalis* (Gordillo and Walton 2010).

Reducing the area of planting beds from expansive shallow marshes (e.g., 80–100% of the wetland surface area) to either islands (hummocks) surrounded by deep water (depth >1.5 m) or contiguous narrow bands (<10 m in width) of emergent vegetation oriented perpendicular to water flow between deepwater zones should help to reduce mosquito production from free-water-surface constructed wetlands treating ammonia-dominated wastewater. Source reduction will reduce the area of the wetland surface conducive to mosquito production and enhance the penetration of emergent vegetation by mosquito control agents, such as bacterial larvicides and insect growth regulators. Source reduction can also promote environmental conditions that enhance the population sizes of important predators of immature mosquitoes such as notonectids (Thullen et al. 2002, Walton 2012) as well as augment rates of nitrification of wastewater and decomposition of downed emergent vegetation (Thullen et al. 2008).

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