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EMERGENCE PATTERNS OF *CULEX* MOSQUITOES AT AN EXPERIMENTAL CONSTRUCTED TREATMENT WETLAND IN SOUTHERN CALIFORNIA

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ABSTRACT. The emergence patterns of mosquitoes inhabiting a 0.1-ha experimental wetland in southern California were monitored using emergence traps during the late summer and autumn of 1996. *Culex erythrothorax* was the largest contributor to emerging populations, comprising 94% of the total emerged adults with an average emergence rate of 59 adults/day/m^2. None of the *Culex* species exhibited a pattern of emergence associated with water depth (range: 5–60 cm). *Culex quinquefasciatus* and *Cx. tarsalis* did not show a pattern of emergence associated with the inflow–outflow gradient; however, *Cx. erythrothorax* emerged in higher numbers along a transect at the middle of the wetland than from near the inflow and outflow. Additionally, the number of emerged *Cx. erythrothorax* was positively correlated with the density of vegetation below emergence traps. The comparatively large number of adults emerging from the middle of the wetland was most likely caused by a trade-off between an increasing gradient of resource abundance and a decreasing gradient of toxic compounds from the inflow to the outflow of the small wetland.

KEY WORDS *Culex erythrothorax,* adult emergence, constructed treatment wetlands, *Schoenoplectus,* bulrush

INTRODUCTION

Constructed treatment wetlands are thought to provide a cost-effective alternative to conventional water reclamation efforts and are likely to play an increasing role in water reclamation strategies in the arid regions of the United States (McCarthy 1997). Such wetlands can be used to remove nutrients from secondary-treated sewage effluent, to provide habitat for wildlife, and to serve as a site for public education on issues related to water and wildlife conservation. Additionally, constructed wetlands are being used to mitigate the loss of natural wetlands due to past development and are often included as an amenity in housing developments. Host-seeking mosquitoes can be extremely abundant (>10,000 females/trap/night) at large (≥10-ha) constructed treatment wetlands that use emergent vegetation to remove nutrients from effluent containing a high concentration of ammonium nitrogen (Walton et al. 1998). The increasing construction of artificial wetlands and their proximity to human habitation can pose a significant abatement dilemma for agencies charged with control of disease-transmitting and pestiferous insect species.

Previous studies (Walton et al. 1996a, Walton and Workman 1998) at a constructed treatment wetlands research complex in San Jacinto, CA, found that the relative abundance of *Culex* species in dip samples differed appreciably from that in carbon dioxide-baited traps. In particular, the tule mosquito, *Culex erythrothorax* Dyar, was underrepresented in larval surveys as compared to its relative abundance in the host-seeking population. *Culex erythrothorax* was absent from dip samples taken in open water near the inlet and outlet weirs of experimental marshes where larvae of congeners (*Culex quinquefasciatus* Say, *Culex stigmatosoma* Dyar, and *Culex tarsalis* Coquillett) were occasionally aggregated. Moreover, *Cx. erythrothorax* was rare (<5% of 3rd–4th instars) in dip samples taken in vegetated zones along the perimeter of experimental marshes (Walton and Workman 1998). Yet, adult abundance indicated that the wetlands produced large numbers of the tule mosquito. *Culex erythrothorax* was the most abundant adult host-seeking mosquito and was often present in numbers up to 100 times that of the next most abundant species, *Cx. tarsalis,* during the summer months (Walton and Workman 1998). By late autumn, *Cx. erythrothorax* was the only host-seeking *Culex* present.

Spatial differences in mosquito production might contribute significantly to the discrepancy of species relative abundance observed in larval versus adult samples. The tule mosquito utilizes seeps, freshwater impoundments, and other habitats containing emergent vegetation, particularly bulrush and cattail, as developmental sites in the western United States (Chapman 1962, Bohart and Washino 1978). Because *Cx. erythrothorax* typically is associated with large, usually deep marshy areas, larvae often are not collected by standard sampling methods (Nielsen 1996). Disturbance created while sampling in dense emergent vegetation hinders the collection of immature mosquitoes. If *Cx. erythrothorax* larvae occur predominantly in densely vegetated, deep zones of marshes, then dips taken along the periphery of vegetated wetlands might fail to collect a representative sample of *Cx. erythrothorax* larval abundance in a wetland.

The objective of this study was to examine the adult emergence patterns of mosquitoes inhabiting a small constructed treatment wetland. The rates and spatial distribution of adult emergence were measured in vegetated regions of the wetland. The numbers of *Culex* emerging were examined for associations with trap position, water depth, and vegetation density.

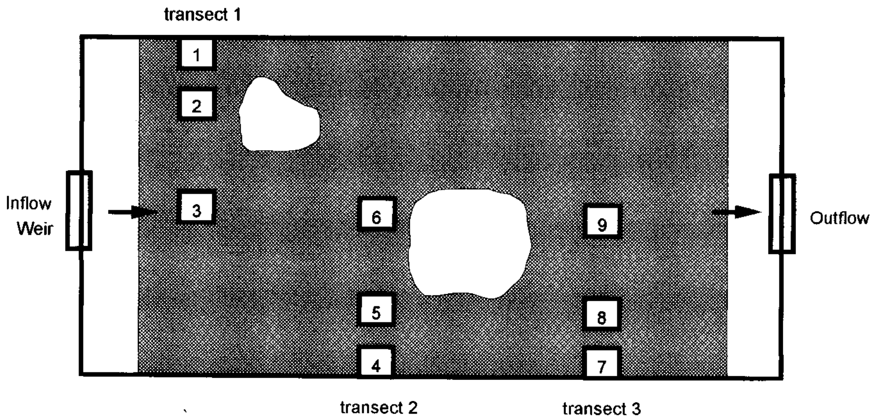

Fig. 1. The arrangement of emergence traps in the 0.1-ha (14 × 69-m) experimental wetland (research cell) at the Multipurpose Wetlands Research Facility in San Jacinto, CA. Cross-hatching represents vegetation. To facilitate illustration, vegetation is not shown within the emergence traps 1–9. Arrows indicate direction of water flow.

MATERIALS AND METHODS

Study site: This study was carried out in a 0.1-ha (14 × 69-m) experimental wetland (research cell) from August to October 1996 at the Multipurpose Wetlands Research Facility operated by the Eastern Municipal Water District (EMWD) in San Jacinto, CA. Bulrush (*Schoenoplectus californicus* (Meyer) Soják) was present throughout the marsh at a median density of 330 culms/m^2 with the exception of a narrow strip of open water (approximately 3 m) at the inlet and outlet weirs (Fig. 1). Dieback in 1995 left some gaps in bulrush stands.

The wetland was supplied with secondary-treated sewage effluent (average flow of 32 liters/min) from a nearby treatment plant. As the sewage effluent flowed through the marsh chemical oxygen demand, total Kjeldahl nitrogen, and total inorganic nitrogen decreased, whereas a corresponding increase occurred in biochemical oxygen demand, total suspended solids (TSS), total organic carbon (TOC), and turbidity between the inlet and outlet areas of the wetland (1993 data: U.S. Bureau of Reclamation [USBR], National Biological Survey [NBS], and EMWD 1994).

Emerging mosquitoes were collected using 9 0.25-m^2 emergence traps. Emergence trap frames were constructed with 1.3-cm-diameter PVC tubing and fitted with fiberglass mesh window screening. Emerged mosquitoes were collected in a wide-mouth (473-ml) Mason jar fitted with a plastic funnel placed at the apex of the emergence traps. The collection jars were collected and replaced weekly.

The spatial distribution of mosquito emergence was examined by placing 3 emergence traps in each of 3 transects along the inflow–outflow gradient in the research cell (Fig. 1). In each transect, traps were placed over bulrush at the shore (5-cm maximum depth), in shallow water (30-cm maximum depth), and in deep water (60-cm maximum depth). The vegetation in each quadrat was counted and then cut to approximately 3 cm above the water surface to allow for trap placement while not disturbing the integrity of the vegetation at or below the surface of the water. To prevent traps from being tipped over by growing vegetation, the bulrush was clipped above the water line twice weekly. All traps were situated to ensure that the bottom margin of the trap remained below the water surface.

Samples were returned to the laboratory, killed by freezing, and counted under 25× magnification. Mosquitoes were identified to species (Bohart and Washino 1978) and sexed.

Data analysis: Adult mosquito production data were analyzed to determine differences in species contribution to the total number of emerging mosquitoes. Data also were analyzed to determine whether differences occurred in emergence patterns related to depth of water (shore, shallow, and deep), trap location (inflow, middle, and outflow) and bulrush density.

Species-specific differences in the total number of males and females emerging were analyzed using a nonparametric repeated measures analysis of variance based on ranks. The effects of water depth and transect location on *Culex* species were analyzed using a repeated measures analysis of variance on $\log_e(x + 1)$-transformed data. A posteriori comparisons of total mosquito production and depth of water or trap location data were made using an all pairwise multiple comparison procedure (Student–Newman–Keuls).

The relationship between bulrush culm density and the number of emerged mosquitoes was analyzed using a least squares linear regression. Emergence and culm density data were $\log_e(x + 1)$-transformed before analysis.

RESULTS

Culex erythrothorax was the largest contributor to the emerged populations with an average of 59

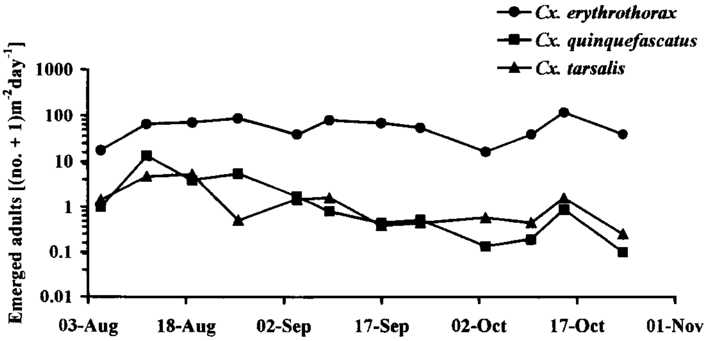

Fig. 2. Total emerged adults from a 0.1-ha (14 × 69-m) experimental wetland (research cell) at the Multipurpose Wetlands Research Facility in San Jacinto, CA, from August 1 to October 24, 1996.

emerged adults/day/m² comprising 94% of the emerged mosquitoes over the course of the study ($\chi_2^2 = 20.3$, $P < 0.001$; Student–Newman–Keuls, $P < 0.05$, Fig. 2). *Culex quinquefasciatus* and *Cx. tarsalis* contributed comparatively less to emerged populations (mean emergence of 2.3 and 1.6 adults/day/m², Fig. 2). The total number of emerged *Cx. quinquefasciatus* vs. *Cx. tarsalis* did not vary significantly across the study.

Comparison of emergence patterns of *Culex* species (Fig. 3) associated with water depth indicated that none of these species had a preference for shore, shallow, or deep water. Both at the species level and when analyzed by sex no significant differences were observed in numbers of emerged adults ($F_{2,6} < 1.204$, $P > 0.363$ in all cases).

Male and female *Cx. erythrothorax* had similar emergence patterns along an inflow–outflow gradient that differed significantly among the 3 transects ($F_{2,6} = 6.074$, $P = 0.036$ and $F_{2,6} = 6.486$, $P = 0.032$, respectively). The middle transect had significantly higher numbers of emerged adult *Cx. erythrothorax* than did the inflow or outflow transects (both male and female, Student–Newman–Keuls method, $P < 0.05$, Fig. 4). Neither male nor female *Cx. tarsalis* or *Cx. quinquefasciatus* showed a significant difference in emergence among transects ($F_{2,6} < 4.113$, $P > 0.075$ in all cases, Fig. 4).

Total emergence of *Cx. erythrothorax* summed across dates was correlated with initial vegetation density in the quadrats ($R^2 = 0.72$, $F_{1,7} = 17.577$, $P = 0.004$, Fig. 5). Initial vegetation densities ranged from 84 to 864 culms/m². Both *Cx. quinquefasciatus* and *Cx. tarsalis* emergence had no correlation with vegetation density (data not shown).

DISCUSSION

Differences in spatial emergence patterns occurred among and within the *Culex* species in this study. *Culex erythrothorax* was the largest contributor (>94% of individuals) to emerging populations during late summer and early autumn. *Culex erythrothorax* was present in 10-fold greater abundance than either *Cx. quinquefasciatus* or *Cx. tarsalis* in early August. This difference increased to approximately 150:1 by late October. At stations along the middle transect in the marsh, 3 peaks (>400 individuals/m²/week) in *Cx. erythrothorax* emergence were observed at approximately monthly intervals. Both other *Culex* species exhibited maximum emergence during August and emerged at low levels (<10 individuals/m²/week) thereafter.

The number of emerged *Culex* spp. did not differ significantly along a gradient of water depth. The lack of preference for water depth by *Cx. erythrothorax* is particularly interesting because larvae of this species are thought to inhabit deep water regions of marshes (Nielsen 1996). Larval developmental sites include thickly vegetated habitats such as alkaline springs, seeps, ditches, river margins, and large permanent swamps (Nielsen and Rees 1961, Chapman 1962, Bohart and Washino 1978, Walters and Smith 1980). The fact that the tule mosquito also emerged in large numbers from shallow water suggests that another factor(s) accounts for the underrepresentation of *Cx. erythrothorax* in dip samples observed in previous studies (Walton and Workman 1998, Walton et al. 1998). Workman (1998) found that several larval behaviors differed significantly among the 4 *Culex* species occurring in the wetland. Species-specific differences in sensitivity to disturbance, aggregation behavior, and association with vertical surfaces could explain the inherent difficulties sampling the immature stages of *Cx. erythrothorax.*

In contrast to emergence patterns associated with water depth, *Cx. erythrothorax* exhibited differences in emergence patterns along an inflow–outflow gradient within the marsh. Significantly greater numbers of *Cx. erythrothorax* emerged from the middle transect than from the inflow or outflow transect. This phenomenon might reflect the influence of several factors including a trade-off between food levels vs. levels of toxic compounds (i.e., ammonia, nitrates, chlorine, trihalomethanes,

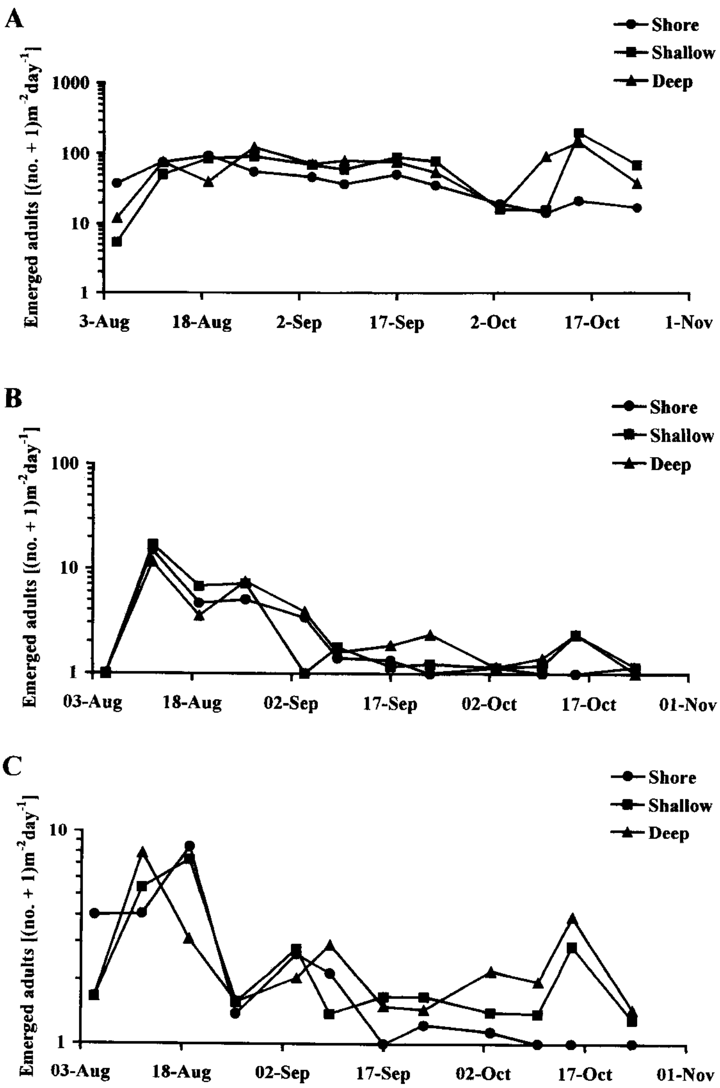

Fig. 3. Number of emerged adults from 3 water depths in a 0.1-ha (14 × 69-m) experimental wetland (research cell) at the Multipurpose Wetlands Research Facility in San Jacinto, CA, from August 1 to October 24, 1996. *Culex erythrothorax* (A), *Culex quinquefasciatus* (B), and *Culex tarsalis* (C).

and chloramines) in the small wetland and the effects of vegetation density.

Previous studies (USBR, NBS, and EMWD 1994) of water quality in the wetlands used in our study found that the concentratio n of small bacteria (total coliform and fecal colif orm bacteria) decreased by >90% as secondary sewage effluent moved through the marsh. Even though TSS, TOC, and turbidity increased along the inflow–outflow gradient (USBR, NBS, and EMWD 1994), the increase in suspended particulate matter was caused by a less favorable food resource (large, filamentous bacteria: e.g., *Beggiotoa* and *Sphaerotilus*) for *Culex* larvae. The significant decline in small suspended bacteria along the inflow–outflow gradient suggests a diminution of the suspended organic matter that is an important resource for developing mosquito larvae (Clements 1992).

Levels of potentially toxic compounds also presumably declined with increasing distance from the inflow. The effluent typically contained a high concentration of ammonium nitrogen (>10 mg/liter: USBR, NBS, and EMWD 1994) and also might contain toxic by-products of chlorination. In a previous study, occasional chlorine contamination of inflow water was observed when levels of residual chlorine in the effluent exceeded the capacity of a dechlorination unit that treated the water feeding the wetlands (USBR, NBS, and EMWD 1994). During the rare instances of chlorine contamination,

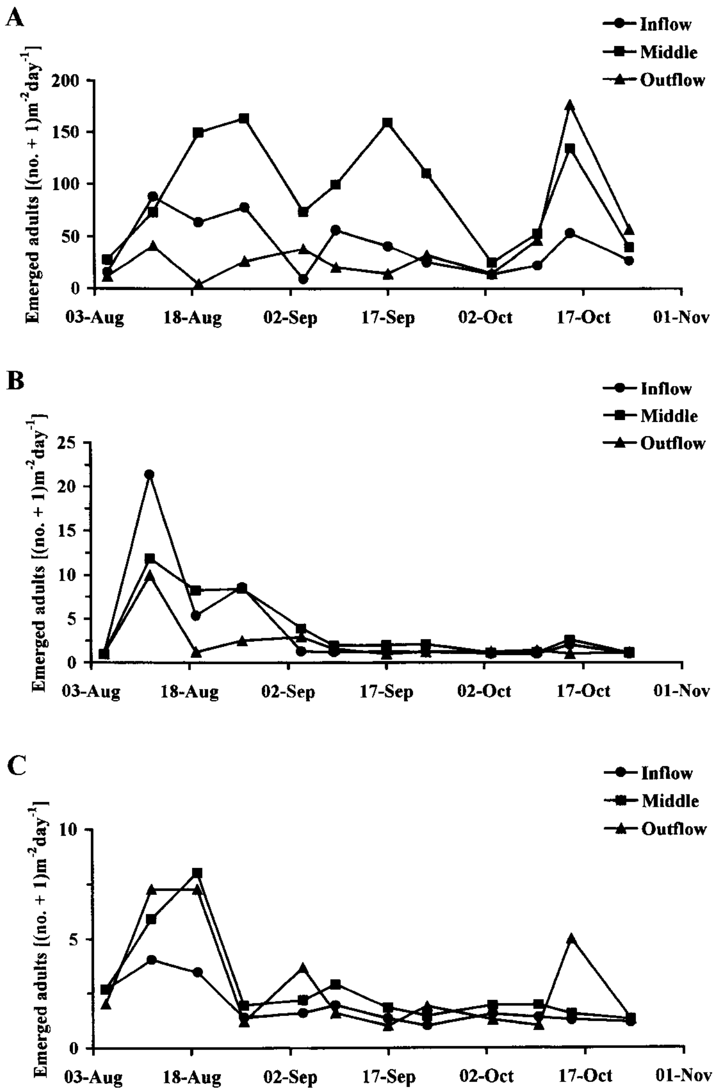

Fig. 4. Number of emerged adults from 3 transects in a 0.1-ha (14 × 69-m) experimental wetland (research cell) at the Multipurpose Wetlands Research Facility in San Jacinto, CA, from August 1 to October 24, 1996. *Culex erythrothorax* (A), *Culex quinquefasciatus* (B), and *Culex tarsalis* (C).

chlorine was not detectable at distances >10 m from the inflow (S. Denison, personal communication). Although larvivorous fish did not occur in the research cell during the present study, previous studies have shown that larvivorous fish were not found in the inflow areas of the research cells (Walton et al. 1996b) and that survival of caged fish was reduced in areas near the inflow of a large (10-ha) wetland receiving similar secondary sewage effluent (Walton et al. 1997). Therefore, the large emerging populations of *Cx. erythrothorax* in the middle of the marsh possibly reflect an ecological partitioning by larvae, and consequent emerging adults, concentrating in areas with more available nutritional resources (i.e., transect nearer the effluent inflow) and where concentrations of potentially toxic chemicals have been reduced. This trend also was influenced by vegetation density.

The most striking trends in emergence patterns of *Cx. erythrothorax* relate to the association of this species with vegetation. Total numbers of emerged *Cx. erythrothorax* adults had a strong positive correlation with bulrush culm density. As vegetation density increased toward a maximum of >800 culms/m^2, *Cx. erythrothorax* adult emergence was >59 adults/m^2/day. Average vegetation densities in the outflow transect were approximately one-half those in the inflow and middle transects. If vegetation densities in the transects are equalized, mosquito emergence (the number of adults emerging

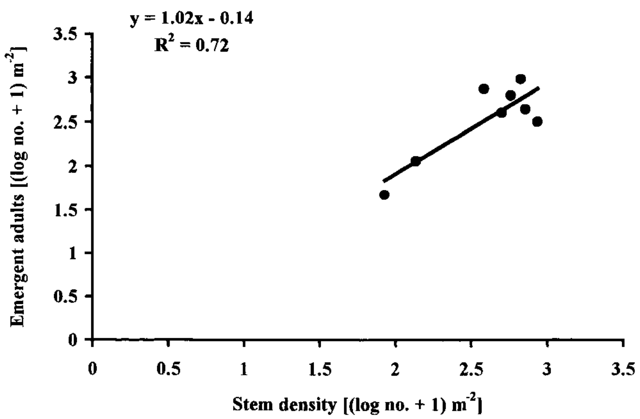

Fig. 5. Least squares linear regression of *Schoenoplectus californicus* density versus total number of emerged adult mosquitoes for *Culex erythrothorax* in a 0.1-ha (14 × 69-m) experimental wetland (research cell) at the Multipurpose Wetlands Research Facility in San Jacinto, CA, from August 1 to October 24, 1996.

per unit area) from the middle and outflow transects is roughly equivalent.

Culex quinquefasciatus and *Cx. tarsalis* showed little association with vegetation and did not exhibit significant emergence patterns along an inflow–outflow gradient. Emergence of *Cx. quinquefasciatus,* a species known to inhabit organically enriched water (Bohart and Washino 1978), was very low throughout the marsh as compared to *Cx. erythrothorax. Culex tarsalis* also was found throughout the marsh and emerging adults were not strongly associated with bulrush density. Unlike *Cx. erythrothorax,* which was virtually absent from protected areas of open water in the wetland, *Cx. tarsalis* was collected both in vegetation and in small areas of open water within the vegetation (Workman 1998). Although emergence traps are not typically used for monitoring mosquito populations (Service 1993), emergence trapping provides a viable means of monitoring adult production and assessing the efficacy of enacted control measures against *Cx. erythrothorax.*

Concern often occurs over abiotic factors such as moderation of internal temperature and lower light levels in the microenvironment under emergence traps (Southwood and Siddorn 1965, Lammers 1977). The placement of the emergence traps in heavy vegetation during this study likely mitigated these problems because the interiors of such vegetation stands are protected from direct exposure to light.

In contrast to larval population monitoring by dip samples (Walton and Workman 1998), sampling of adult emerged populations clearly indicated that *Cx. erythrothorax* was a major contributor to mosquito populations that use this type of marsh as a larval developmental site. Although *Cx. erythrothorax* is not considered to be a pathogen-transmitting mosquito of significant concern for human public health (Meyer et al. 1988, Reisen et al. 1992), it is a pestiferous biter that will seek a blood meal during the day if its resting place is disturbed. In a research cell wetland with approximately 910 m^2 of vegetated surface area and a constantly renewed source of organically polluted water, a potential exists for considerable mosquito production. Because large constructed treatment wetlands receiving secondary-treated effluent have the potential to produce >30,000 host-seeking females per trap night (Walton et al. 1998), control measures might be needed often. *Culex erythrothorax* has a close association with thick stands of vegetation and, when vegetation densities are greater than 300–400 culms/m^2, it becomes difficult to census larvae using conventional dipping methodology and to penetrate the dense vegetation with conventional larvicide formulations (Walton et al. 1998). Designs and operations of constructed treatment wetlands receiving large amounts of reduced nitrogen in the effluent will need to incorporate features and approaches that reduce the density of emergent vegetation.

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