

THE IMPACT OF WETLAND VEGETATION DRYING TIME ON ABUNDANCE OF MOSQUITOES AND OTHER INVERTEBRATES

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ABSTRACT. Vegetation management for constructed treatment wetlands often involves knocking down emergent vegetation with heavy equipment and inundating the dead vegetation after a period of drying. Such practices create favorable conditions for larval mosquitoes. We studied the relationship between length of the drying period for an emergent macrophyte, *Typha* sp., and the abundance of aquatic invertebrates in replicated 0.18-m² wading pools. The mosquito, *Culex tarsalis*, was significantly more abundant in pools containing vegetation aged for 2 wk before inundation compared to pools containing vegetation aged 5 wk, freshly cut vegetation, or without vegetation. Potential larval mosquito food resources (particles between 2 and 61 µm in equivalent spherical diameter) in the 2-wk aging treatment did not differ significantly from the other treatments during the 5-wk experiment. The abundance of other larval culicids, noncullicine Diptera, and potential mosquito predators (i.e., Dytiscidae and Aeshnidae) did not differ significantly among the vegetation aging treatments.

KEY WORDS *Culex*, constructed wetlands, vegetation management, Diptera, colonization

INTRODUCTION

Constructed wetlands located near housing developments and densely populated areas can produce large mosquito populations, which cause a nuisance and may pose a serious health hazard as vectors of pathogens causing diseases in humans (Walton et al. 1998, Russell 1999, Walton 2002). In addition to providing water treatment, man-made wetlands may be used to enhance wildlife habitat and therefore maintaining the diversity of invertebrate populations, a major food source for migratory waterfowl, is a goal of wetland managers. Large areas of senescing emergent vegetation can reduce the efficacy of constructed wetlands to purify water (Sartoris et al. 2000) and may lower habitat quality. Vegetation management in wetlands may be needed to maintain a properly functioning wetland as well as to control mosquito populations (Schollsberg and Resh 1997, Thullen et al. 2002).

Vegetation management practices such as mowing and discing may leave large mats of senescent vegetation in wetlands that provide a refuge for mosquitoes after inundation (Schaefer and Miura 1985). Berkelhamer and Bradley (1989) suggested that dense mats of rotting vegetation provide nutritional enhancement to developing larvae. Plant decay also may attract gravid mosquitoes. Infusions of decaying vegetation, such as Bermuda grass (Isoe et al. 1995a), hay (Hazard et al. 1967), and bulrush (Walton and Workman 1998) attract gravid *Culex*. Organic enrichment sustained oviposition and larval production in field microcosms (Rodcharoen et al. 1997). Hazard et al. (1967) demonstrated that bacteria are responsible for the ovipositional attractancy of these infusions.

Vegetation management at the Prado Constructed Wetlands in western Riverside County, California,

usually involves draining marshes, knocking over the vegetation with heavy equipment, drying the vegetation, and then inundating the dried plant material. Although these practices are thought to improve water-quality performance of treatment wetlands by enhancing denitrifying bacteria populations, larval mosquito abundance in 3- and 5-ha marshes at the Prado Constructed Wetlands increased significantly after vegetation management (Keiper et al. 2003). In this study, we examined the relationship between the length of time that vegetation is dried before inundation and the abundance of mosquitoes and other invertebrates in small plastic wading pools.

MATERIALS AND METHODS

Study site: This study was conducted at the Prado Constructed Wetlands during August and September 1999. The Prado Wetlands consist of approximately 50 marshes and ponds encompassing 186 ha. The wetlands were designed to remove nitrate from the Santa Ana River (Mills et al. 1998). Emergent vegetation is dominated by bulrush (*Schoenoplectus californicus* (Meyer) Soják) and cattails (*Typha* sp.).

Vegetation aging experiment: We used a randomized complete block design consisting of 4 treatments within each of 5 blocks of small plastic wading pools (diameter = 107.3 cm, depth = 20.3 cm) situated on berms adjacent to the marshes at 5 sites. Three vegetation treatments were created by using 1-m-long stems of cattails that had been aged in the field for 5 wk, 2 wk, and 0 wk (fresh). Plant material was aged in full sun on the banks of the marshes. To add equivalent plant biomass to each pool, 5 stems (1 m long) of *Typha* sp. were added to the most aged treatment (5 wk), 4 stems were added to the intermediate treatment (2 wk), and 3 stems were added to the least aged treatment (0 wk). The control treatment assessed mosquito abundance in the water used to fill the pools and contained no vegetation.

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Table 1. Proportion of total collection, functional feeding group association, and days until 1st collection of invertebrate taxa through the course of the study.

Taxa	Proportion	Functional feeding group ¹	Days until 1st collection ²			
			Con	0 wk	2-wk	5-wk
Diptera						
Culicidae						
<i>Culex</i> L1/L2 ³	0.418	FF	6	6	6	6
<i>Cx. tarsalis</i>	0.138	FF	13	13	13	6
<i>Cx. quinquefasciatus</i>	0.006	FF	13	13	13	13
<i>Cx. stigmatosoma</i>	0.012	FF	20	13	6	13
<i>Cx. restuans</i>	0.001	FF	—	—	—	13
<i>Cx. pupae</i>	0.021	—	20	13	13	13
<i>Anopheles</i> L1/L2	0.017	FF	13	20	13	26
<i>An. hermsi</i>	0.007	FF	20	33	20	33
<i>An. pupae</i>	0.002	—	36	20	36	26
Chironomidae	0.297	CG	6	6	6	0 ⁴
Ceratopogonidae	0.007	CG	26	33	33	33
Ephydriidae						
<i>Brachydeutera sturtevanti</i>	0.027	CG	6	6	6	6
<i>Ephydra</i> spp.	0.001	CG	33	33	—	33
Ephemeroptera						
Baetidae	0.003	CG	36	33	36	20
Coleoptera						
Hydrophilidae (larvae + adults)	0.007	P/S	26	13	13	13
Dytiscidae (larvae + adults)	0.027	P	20	13	6	13
Odonata						
Aeshnidae	0.005	P	—	36	26	26
Coenagrionidae	0.001	P	—	—	—	36
Hemiptera						
Corixidae	0.005	G	20	—	—	20

¹ FF, filter feeder; CG, collector gatherer; P, predator; S, scavenger; G, generalist.

² Treatments: Con, no-vegetation control; 0 wk, freshly cut vegetation; 2 wk, 2-wk-old vegetation; 5 wk, 5-wk-old vegetation.

³ L1/L2, 1st- and 2nd-stage larvae.

⁴ A small 1st-stage chironomid larva was detected that must have passed through the screen when water from the wetland was added to the pools.

Each block consisted of 4 pools placed approximately 1 m apart, each weighed down by a red patio brick (30.1 × 29.6 × 5.7 cm). Vegetation was added and the pools were filled with water sieved (mesh aperture 0.73 × 0.32 mm) from the wetlands to remove macroinvertebrates. Pools were filled to a depth of approximately 8 cm on August 11 and maintained at that depth by biweekly additions of screened water until the end of the experiment.

Sampling: Three 350-ml dips were taken weekly in each pool. Each sample was concentrated by using a 148- μ m-mesh screen, preserved with 70% ethanol, and counted in the laboratory. Third- and 4th-stage larval mosquitoes were keyed to species by using the keys of Meyer and Durso (1998), and all other specimens were keyed to at least the family level by using the keys of Merritt and Cummins (1996). A water sample (60 ml) was taken for particle counting. Samples were placed on ice and immediately counted upon arrival in the laboratory with an electronic particle counter (Multisizer II, Beckman Coulter, Miami, FL). The counter was equipped with a 100- μ m aperture. Particles be-

tween 2 and 61 μ m (equivalent spherical diameter) were counted.

Statistics: Statistical tests were run for taxa comprising greater than 1% of the total specimens collected. Repeated measures analyses of variance (ANOVA) were run by using $\log_{10}(n + 1)$ -transformed abundance data from the dip samples as well as the particle counts. A Tukey's honestly significant difference multiple comparison test was run if significant differences ($P < 0.05$) were found in the ANOVA. All statistics were computed with the computer software package SigmaStat version 2.03 (Fox et al. 1995).

RESULTS

Mosquitoes

Among mosquito taxa, early instars of *Culex* and *Culex tarsalis* Coquillett were most abundant (Table 1), with maximum numbers collected at 12 days after inundation (August 23). Significant differences for mosquito abundance were observed between

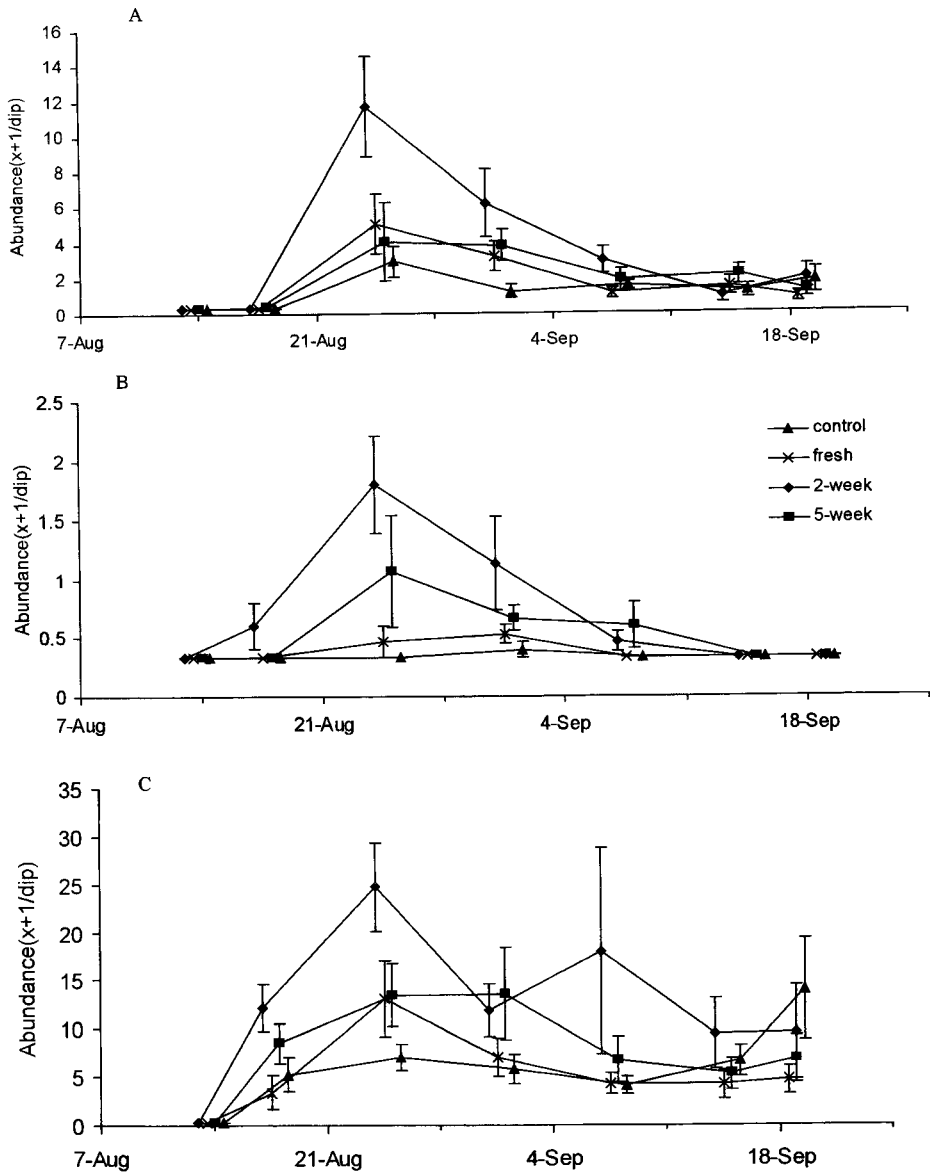


Fig. 1. Abundance (mean \pm SE) of larval *Culex tarsalis* (A, 3rd and 4th instars), larval *Culex stigmatosoma* (B, 3rd and 4th instars), and larval *Culex* spp. (C, all instars) in dipper samples from 4 vegetation aging treatments during late summer 1999. Points are offset horizontally to facilitate illustration.

dates ($F_{5,20} = 8.756$, $P < 0.001$). A steady decline in larval mosquito abundance occurred after August 23 until the end of the study on September 16.

The period of drying macrophytes before inundation significantly affected larval mosquito abundance. Larval *Cx. tarsalis* were significantly more abundant in pools containing 2-wk-old vegetation than in pools containing 5-wk-old ($F_{3,12} = 8.268$, $P = 0.003$, $q = 4.694$, $P = 0.027$), fresh ($q = 5.306$, $P = 0.013$), or no vegetation ($q = 6.589$, $P = 0.003$) (Fig. 1A). The number of mosquito larvae collected from the 5-wk aging treatment did not

differ significantly from the control ($q = 1.895$, $P = 0.557$) or the fresh treatment ($q = 0.612$, $P = 0.972$). Also, larval mosquito abundance in the fresh treatment did not differ significantly from the control ($q = 1.283$, $P = 0.802$).

Culex stigmatosoma Dyar exhibited a trend among treatments (overall treatment effect: $F_{3,12} = 5.963$, $P = 0.010$) similar to that for *Cx. tarsalis*, with abundance being greatest in pools containing 2-wk-old vegetation vs. control ($q = 5.449$, $P = 0.011$) or fresh vegetation ($q = 4.704$, $P = 0.027$) (Fig. 1B). Significant differences for larval mos-

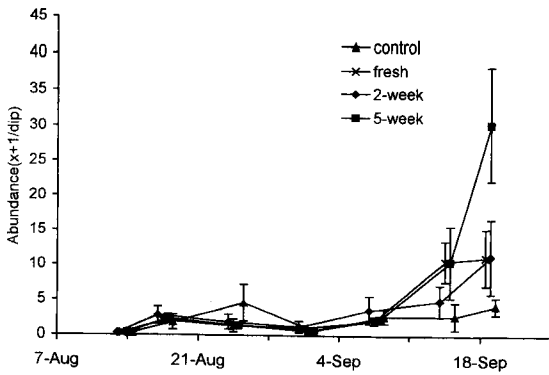


Fig. 2. Average number of larval Chironomidae collected per dip from 4 vegetation aging treatments during late summer 1999. Points are offset horizontally to facilitate illustration.

quito numbers were not found between pools containing 2-wk-old vegetation vs. 5-wk-old vegetation ($q = 2.627$, $P = 0.296$). Although *Culex quinquefasciatus* Say followed a similar trend for abundance, no significant differences were detected among the treatments ($F_{3,12} = 0.758$, $P = 0.539$). Site by date interactions were significant ($F_{6,24} = 10.731$, $P < 0.001$) because significant differences of larval mosquito abundance among treatments were observed only during the early part of the experiment.

When the early instars of *Culex* were combined with all *Culex* species, larval abundance differed significantly among treatments ($F_{3,12} = 4.003$, $P = 0.035$; Fig. 1C). Abundance of combined *Culex* differed significantly only between the 2-wk-aging treatment and fresh vegetation ($q = 4.571$, $P = 0.032$). No significant treatment by date interaction was found ($F_{18,72} = 1.614$, $P = 0.079$).

Anopheles hermsi Barr and Gupta vanij was the only *Anopheles* species collected; therefore, all larval instars were combined for abundance measurements. No significant differences in larval abundance were observed among treatments ($F_{3,12} = 2.210$, $P = 0.140$). Significant differences in abundance were observed among dates ($F_{6,24} = 10.586$, $P < 0.001$). Larval *Anopheles* were most abundant during the last 3 wk of the study and colonized the pools later than the *Culex* spp. (Table 1).

Other invertebrates

Chironomidae were among the 1st taxa to colonize the pools, and were collected on the 1st sampling date (Table 1). Chironomid abundance did not differ significantly among treatments ($F_{3,72} = 1.706$, $P = 0.219$), with highest abundance occurring late in the study (Fig. 2). Midge abundance differed significantly among dates ($F_{6,72} = 21.292$, $P < 0.001$). Chironomidae were the most abundant insects collected throughout the study, representing ~30% of total collected macroinvertebrates.

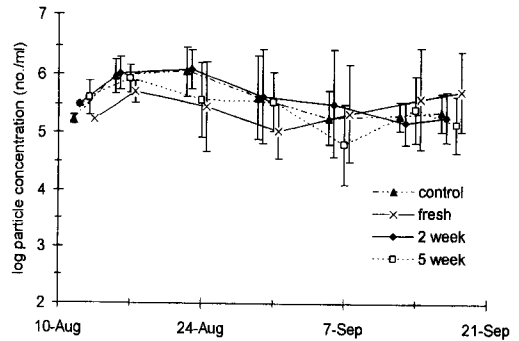


Fig. 3. Number of particles (mean \pm SE) between 2 and 61 μ m (equivalent spherical diameter) per milliliter collected from 4 vegetation aging treatments. Points are offset horizontally to facilitate illustration.

Among cyclorrhaphan flies, the shore fly *Brachydeutera sturtevantii* Wirth was the most common throughout the study and the 1st higher dipteran taxon to colonize the pools (Table 1). No significant differences for larval shore fly abundance were observed among treatments ($F_{3,12} = 2.270$, $P = 0.133$), but differences of abundance were observed among dates ($F_{6,24} = 4.605$, $P = 0.003$). Adult *B. sturtevantii* were present on the surface of the pools on the 1st sampling date and larvae were found on the 2nd date, only 6 days after inundation (Table 1), and numbers rose steadily throughout the study.

Predators readily colonized the pools, although more slowly than did most of the dipteran taxa. Dytiscidae were numerically dominant (Table 1). No significant differences were observed among treatments ($F_{3,12} = 3.085$, $P = 0.068$) for dytiscid beetles. Hydrophilid larvae were the 2nd most abundant predator ($n = 35$, 0.6% larvae) in the pools. Dragonfly nymphs (Odonata: Aeshnidae) were the 3rd most abundant predator colonizing the pools ($n = 26$, 0.5%). Aeshnidae were found to be most abundant in the 2-wk treatment, although most of these nymphs were limited to a single pool and collection date.

Particle abundance

Particle abundance did not differ significantly among treatments ($F_{3,12} = 2.080$, $P = 0.156$; Fig. 3). Mean particle abundance among all treatments averaged between 100,000 and 1,000,000 particles/ml. Significant differences were detected among sites ($F_{4,12} = 9.330$, $P = 0.001$). Particle numbers among sites differed by as much as 0.87 standard deviations (standard error = 0.0738). This may be due to placement of pools near trees and access roads where dust, pollen, and other particles could have fallen into them. Overall, treatment particle concentrations rose slightly on the 2nd sampling date then declined slightly before rising again during the last 3 sampling dates of the study (Fig. 3).

DISCUSSION

The period of aging of harvested emergent macrophytes before inundation significantly influenced the abundance of *Culex*, particularly *Cx. tarsalis*. The time-integrated mean abundance of larval *Cx. tarsalis* in pools containing vegetation aged 2 wk was approximately 2 times greater than that in pools containing either vegetation aged 5 wk or freshly cut vegetation and was 3 times greater than that in the control pools during the 5-wk study. Wetland vegetation management often includes knocking down standing plants and drying vegetation before reflooding (Keiper et al. 2003) because the decaying vegetation is thought to provide an important carbon source for denitrifying bacteria in the wetland (Baharie, personal communication). The results of our study suggest that the potential of wetland ponds to produce large numbers of mosquitoes may be reduced if ponds are flooded either immediately after plants have been knocked down or after knocked down vegetation has been dried for >1 month. The benefit of lowered mosquito production provided by drying times >5 wk was not addressed in the current study; however, the time that wetland ponds are nonoperational typically needs to be minimized and longer drying times may not be practical for treatment wetland operations.

Culex tarsalis was the most abundant mosquito collected throughout this study and is the most common mosquito encountered in dipper samples taken in the Prado Wetlands (Keiper et al. 1999). *Culex stigmatosoma* and *Cx. quinquefasciatus* were less abundant but followed a similar trend, with highest abundance at 2 wk after inundation. Populations of larval *Anopheles hermsi* established after the *Culex* spp., which is a trend previously observed in the Prado Wetlands (Keiper et al. 1999). The number of days until larval *Anopheles* spp. were collected was twice the number of days until the 1st collection of *Culex*.

Other dipteran taxa were not attracted to a particular aging treatment but colonized pools regardless of the age of the dry vegetation in them. Wetland managers may be interested in stimulating Chironomidae production for waterfowl utilization during the waterfowl breeding season (Euliss and Grodhaus 1987). Larval midges were the most abundant insect collected across all treatments for the 5 wk of this study. Chironomid numbers were not affected by duration of vegetation drying and larval chironomid abundance increased toward the end of our study. It may be possible to manage wetlands to ensure chironomid production while keeping mosquito production low if ponds are reflooded immediately after vegetation management, but a longer-term study in wetland ponds would help to elucidate the temporal dynamics of the Chironomidae.

The abundance of particles in the range of 2–61

μm was not significantly affected by the vegetation aging treatments. Particles in this size range usually are important food resources for mosquito larvae (Merritt et al. 1992b); however, particles <2 μm can also be components of larval mosquito diets (Walker et al. 1988, Merritt et al. 1992a). The particle types most important to ovipositional attractancy and as larval food may be attached to the substrate or to the decaying vegetation. Bacteria present in aquatic habitats produce volatile compounds that can attract mosquitoes to oviposition sites as well as other compounds in the water that stimulate oviposition (Hazard et al. 1967, Isoe et al. 1995b) and are likely to be <2 μm in size. Their presence in the wetland habitat and the importance of bacterial processes in water treatment warrants detailed measurement of bacterial responses to wetland management practices.

Organic enrichment (Rodcharoen et al. 1997), flooding duration (Beehler and Mulla 1993), the influence of potential predators (Stav et al. 1999), and volatile chemicals produced by bacteria (Hazard et al. 1967) all have been implicated in the ovipositional attractancy of various aquatic habitats to mosquitoes. The factors present in nature that make one habitat more suitable than another for oviposition and subsequent production of mosquitoes are complex and interrelated. Mosquito production from man-made wetlands can be reduced by incorporating design features into a wetland that limit the proliferation of emergent vegetation (Thullen et al. 2002) or by removing harvested vegetation (Keiper et al. 2003). When wetland management requires the inundation of dead plant material to enhance water-quality performance, the period of aging harvested macrophytes before inundation influences abundance of *Culex* sp. Further studies in operational wetlands are necessary to determine whether the results of our mesocosm study are applicable to the larger, more complex wetland ecosystems.

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