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ECOLOGY OF MOSQUITOES AND LACK OF ARBOVIRUS ACTIVITY AT MORRO BAY, SAN LUIS OBISPO COUNTY, CALIFORNIA[1]

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ABSTRACT. During 1994–95, totals of 17,656 adult females and 111,104 adults reared from field-collected immatures comprising 19 species in 4 genera of mosquitoes were collected from Morro Bay estuary and surrounding environs in San Luis Obispo County, California. *Aedes dorsalis* was the dominant summer mosquito, whereas *Aedes squamiger* and *Ae. washinoi* were abundant during winter and early spring. Host-seeking *Culex tarsalis* were collected infrequently, even though immatures were collected frequently from freshwater surface pools. Overall, 13,561 adults (386 pools) and 91,547 adults reared from field-collected immatures (3,027 pools) were tested for arboviruses by plaque assay in Vero cell culture. Morro Bay virus, a member of the California serogroup, was isolated from 4 pools of *Ae. squamiger* reared from field-collected immatures (minimum field infection rate = 1.07 per 1,000), verifying the maintenance of this virus by vertical transmission. All remaining pools were negative. Three flocks of 10 sentinel chickens and one group of 5 sentinel rabbits were bled biweekly and tested for arbovirus antibodies with negative results. Neither horizontal nor vertical transmission of western equine encephalomyelitis virus was detected.

INTRODUCTION

In the irrigated inland valleys of California, western equine encephalomyelitis (WEE) virus is transmitted horizontally during summer within an enzootic cycle among wild birds and the primary mosquito vector, *Culex tarsalis* Coquillett (Reeves 1990). A secondary cycle involving *Aedes melanimon* Dyar and lagomorphs occurs in the Central Valley during late summer (Hardy 1987), after WEE amplification in the primary *Cx. tarsalis*–bird cycle. *Culex tarsalis* expands is host range at this time to feed more frequently on mammals than during spring (Tempelis et al. 1965, Nelson et al. 1976). Transmission of WEE to humans is tangential and, in recent years, infrequent. Although these summer transmission cycles are relatively well understood, mechanisms that enable the persistence of WEE virus between periods of active transmission and that initiate the entrance of virus into the summer bird–*Cx. tarsalis* cycle remain undocumented, and the role of secondary or alternative transmission cycles among *Aedes* and mammals in enzootic maintenance is poorly understood (Reisen and Monath 1989). Elsewhere in western North America, *Aedes dorsalis* (Meigen) has been found infected with WEE virus, at times in the absence of concurrent infections in *Cx. tarsalis* (Reisen and Monath 1989). Both *Ae. melanimon* and *Ae. dorsalis* oviposit drought-resistant eggs capable of surviving cold winter and dry summer conditions and of remaining infected with California encephalitis virus for extended periods (Turell et al. 1982). However, attempts to demonstrate vertical transmission of WEE by *Ae. melanimon* by testing adults reared from field-collected immatures has been unsuccessful, even during years when WEE virus was isolated repeatedly from host-seeking females (Reisen et al. 1990). Laboratory experiments using parenterally inoculated female *Ae. dorsalis* and *Ae. melanimon* also have not been able to document vertical transmission of infectious virus (Hardy and Reeves 1990).

In August 1991 and 1992, Fulhorst et al. (1994) made 3 isolations of WEE virus from pools of adult *Ae. dorsalis* that were reared from immatures collected from one salt marsh site at Morro Bay estuary, California. These isolations were important, because they were the first indication of vertical transmission of WEE virus by any mosquito species, the first isolations of WEE virus from *Ae. dorsalis* in California, and one of the few indications of WEE virus activity in coastal California. Even low levels of vertical transmission by *Aedes* ovipositing drought-resistant eggs could provide a mechanism for WEE virus persistence during periods when WEE virus cannot be detected within the primary bird–*Cx. tarsalis* cycle and may explain, in part, the long-recognized correlation between WEE epidemics and wet spring conditions (Hess and Hayes 1967).

Several arboviruses in addition to WEE virus have been isolated from the Morro Bay area. Morro Bay (MB), a California encephalitis (CE)-like virus, was isolated repeatedly from adult *Aedes squamiger* (Coquillett) reared from field-collected immatures collected from salt marsh habitat (Eldridge et

[1] A brief unrefereed summary of this research was presented previously (Reisen et al. 1996).

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al. 1991). Morro Bay subsequently has been isolated from *Ae. squamiger* from coastal California as far south as San Diego (Fulhorst et al. 1996b), but less is known of its horizontal transmission cycles and possible public health significance. Other viruses isolated from Morro Bay include an unidentified CE-serotype and Jamestown Canyon viruses isolated from *Ae. dorsalis* and a Northway (NOR) serotype virus isolated from *Culiseta particeps* (Adams) (Fulhorst 1994[4], Fulhorst et al. 1996a). The NOR-serotype virus subsequently was identified as Stanfield (Fulhorst, unpublished data), characterized previously by Campbell et al. (1991).

To extend the findings of Fulhorst et al. (1994), we conducted an in-depth investigation of mosquito and arbovirus ecology at Morro Bay during 1994–95. Our hypothesis was that WEE virus was maintained by vertical transmission within *Ae. dorsalis* populations, amplified by horizontal transmission among rabbits, and then introduced into the *Cx. tarsalis*–bird cycle. To test this hypothesis we monitored: 1) adult mosquito population dynamics and arbovirus infection rates throughout the year, 2) seasonal changes in vertical infection rates among immature mosquitoes, and 3) virus transmission rates to sentinel chickens and rabbits.

MATERIALS AND METHODS

Study area: The physiography of Morro Bay estuary is dominated by a salt marsh created by alluvial deposits from Turri and Chorro Creeks (Fig. 1A). The marsh is dry during neap tides, but may be completely inundated by seawater during flood tides. The salt marsh is vegetated by dense stands of pickleweed (*Salicornia*), which is replaced by freshwater marsh species such as cattails (*Typha*) wherever freshwater intrudes. The marsh is bordered in all directions by foothills of the Coast Range. Upland chaparral and grasses are replaced by riparian vegetation along water courses and by stands of imported eucalyptus. The California Department of Parks and Recreation operates a campground in a dense stand of pine and eucalyptus along the northern boundary of the salt marsh (near sites 1 and 16, Fig. 1A).

Weather at Morro Bay is characterized by cool summers and mild wet winters (Fig. 2A), with frequent coastal fog and overcast conditions. A data logger (Datapod, Omnidata, Logan, Utah) recorded temperature hourly at the surface of a permanent pool near breeding source E (Fig. 1B). Interestingly, solar radiation and bacterial action produced mean midsummer water temperatures that were warmer than mean maximum air temperatures. Rainfall during the winter of 1994–95 was extraordinarily high and caused considerable flooding. Spates from Turri and Chorro creeks inundated the salt marsh with freshwater and deposited up to 15 cm of silt, which covered large stands of *Salicornia* and presumably smothered some *Aedes* eggs.

Mosquito sampling and processing: Adult host-seeking female mosquitoes were collected biweekly from March 1994 through November 1995 by 20–24 CDC-style traps baited with 1–2 kg of dry ice and operated without lights (CO_2 traps) from 1100 through 0830 h on fixed standards placed in salt marsh, ecotonal, riparian, and upland habitats (Fig. 1A). Additional adult mosquitoes were collected by 2 NJ light traps (near sites 4 and 11), by sweeping in *Salicornia* using an AFS sweeper (Meyer et al. 1983) or net, and by a hand-held aspirator while landing on human bait. Human baits were protected from mosquito bites by applying repellent to exposed surfaces such as hands and face. When available, immature mosquitoes were collected by dipping, transported to the Arbovirus Field Station insectary (22–25°C, 14h:10h light : dark photoperiod) in Bakersfield, reared to adults, and maintained for >4 days on 10% sucrose. After April 1995, collections of immatures emphasized habitats supporting populations of *Ae. dorsalis* and *Cx. tarsalis*. Voucher specimens of selected mosquito species were deposited at the Bohart Museum, University of California, Davis and in the collections of B. F. Eldridge, University of California, Davis and R. P. Meyer, Orange County Vector Control District, Santa Ana.

Field-collected and reared adults were anesthetized with triethylamine, sorted by sex and/or female reproductive status (unfed, bloodfed and gravid), counted, pooled into lots of 50 each, and stored at −70°C until tested for virus. Subsamples of reared *Aedes* females were held >7 days on 10% sucrose and dissected, and follicular maturation was scored using the scheme of Christophers (1911). Females with several advanced follicles at ≥stage IV were considered to be autogenous.

Mosquito pools were shipped to the Arbovirus Research Laboratory at Berkeley where they were tested for infectious virus using a plaque assay on Vero cells (Hardy et al. 1993). Virus-positive Vero cell passage 1 or 2 cultures were identified using the enzyme immunoassay (EIA) described by Kramer et al. (1992), except that 3,3′ diaminobenzidine was used as the substrate for final color development. Pools of *Ae. dorsalis* collected during summer as immatures near site 9 where Fulhorst et al. (1994) isolated WEE virus also were tested for WEE virus genomic RNA using a reverse transcriptase–polymerase chain reaction (RT-PCR) assay similar to that described by Howe et al. (1992).

Sentinels: Virus transmission was monitored by bleeding 3 flocks of 10 sentinel chickens and one group of 5 sentinel rabbits biweekly throughout the year. Sentinel locations are shown in Fig. 1A; chickens at site 3 in 1994 were transferred to near site 29 during 1995 in an attempt to detect transmission in inland agricultural habitat. In addition,

[4] Fulhorst, C. F. 1994. The epidemiology and ecology of mosquito-borne viruses in coastal areas of California. PhD. dissertation. University of California, Berkeley.

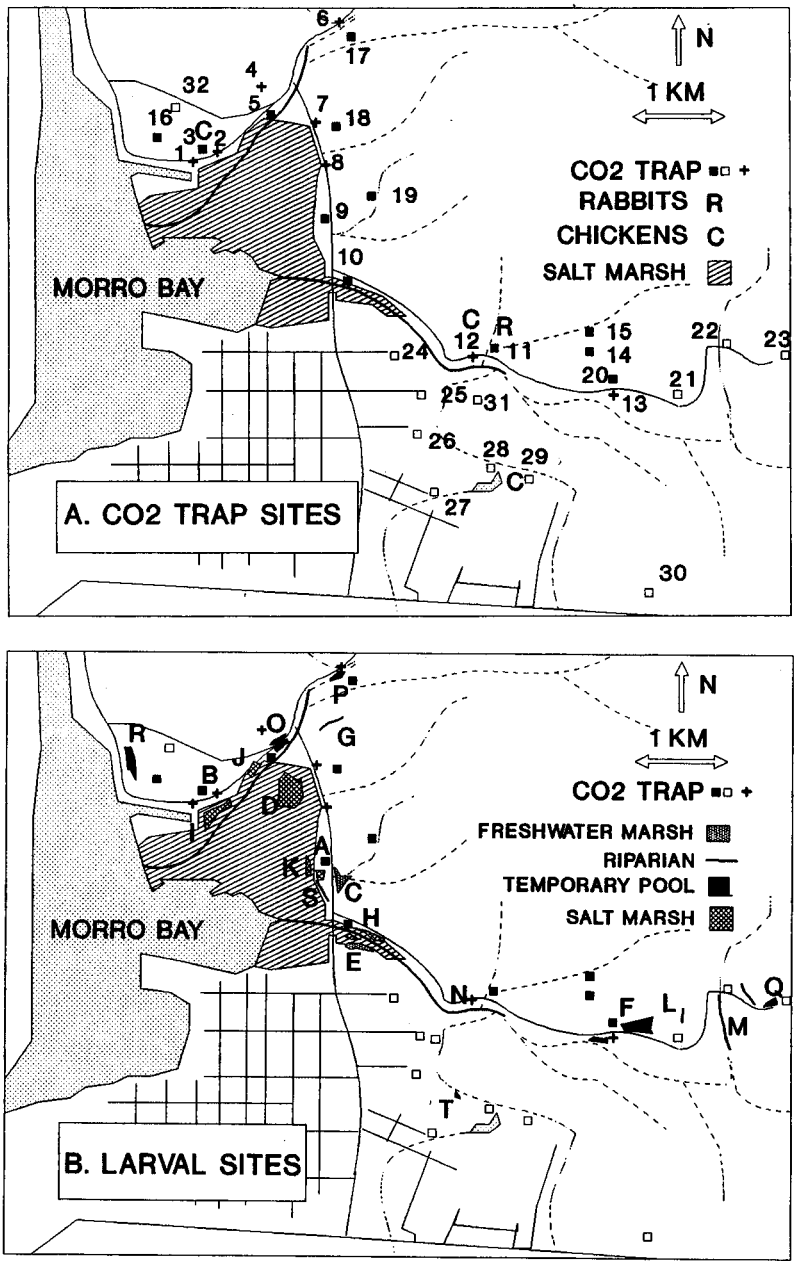

Fig. 1. Map of Morro Bay estuary showing: (A) CO_2 trap sites and sentinel chicken and rabbit locations, and (B) locations of positive immature collections. Traps indicated by + were operated during spring and early summer 1994, those indicated by filled squares were operated during both 1994 and 1995, and those indicated by open squares were operated only during 1995. Larval sites indicated by letters included semipermanent and permanent freshwater marshes (FRESHWATER MARSH), slow-moving streams and associated overflow pools (RIPARIAN), temporary surface rain water pools (TEMPORARY POOLS), and intermittently flooded salt and brackish pools on salt marsh (SALT MARSH).

23 local chickens of mixed ages housed at a farm near site 25 were bled during 1994. Chickens were bled from the comb (Reisen et al. 1994) and rabbits were bled from the ear vein. Sentinel bloods were tested for WEE, St. Louis encephalitis, and California-group virus antibodies by appropriate EIAs. Protocols for the care and use of vertebrate animals in this research were described in Animal Use Protocol R009-0695B "Arbovirology Ecology and Vector Competence Studies" approved by the Animal Care and Use Committee of the University of California, Berkeley.

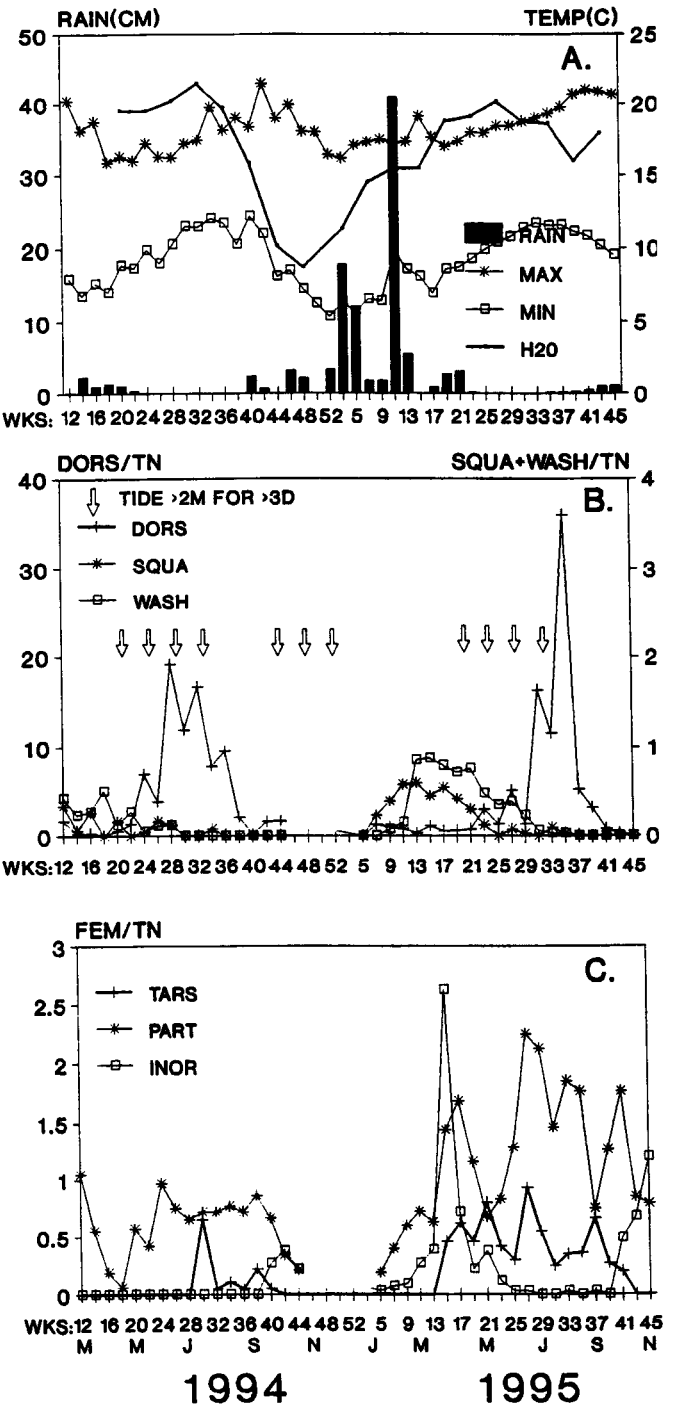

Fig. 2. A) Temporal changes in weather: biweekly total rainfall and mean maximum–minimum ambient temperature recorded at Morro Bay by the California Irrigation Management Information System (CIMIS) and water temperature recorded at site E. B) Temporal changes in mosquito abundance: biweekly geometric mean number of females collected per CO_2 trap night for *Ae. dorsalis* (DORS), *Ae. squamiger* (SQUA), and *Ae. washinoi* (WASH), and C) *Cx. tarsalis* (TARS), *Cs. particeps* (PART), and *Cs. inornata* (INOR). Arrows in panel B indicate periods when flood tides exceeded 2 m height for ≥3 consecutive days.

Table 1. Total adults reared from field-collected immatures and adult females collected at Morro Bay and tested for virus infection, 1994–95.

Species	Immatures				Adults		
	Sites positive	Larvae collected	No. tested	No. pools	Females collected	No. tested	No. pools
Aedes							
dorsalis (Meigen)	57	45,399	44,641	1,042	12,928	10,592	233
sierrensis (Ludlow)	0		0		7	0	
squamiger (Coquillett)	33	16,724	3,733	111	219	179	15
washinoi Lanzaro and Eldridge	14	8,533	8,550	212	363	302	17
Anopheles							
franciscanus McCracken	11	41	32	17	330	16	3
hermsi Barr and Guptavanji	11	111	102	27	16	1	1
occidentalis Dyar and Knab	34	633	425	103	102	2	1
Culex							
apicalis Adams	10	285	279	34	0	0	
boharti Brookman and Reeves	6	87	0		0	0	
erythrothorax Dyar	5	17	16	6	251	21	3
quinquefasciatus Say	15	734	601	60	303	125	8
reevesi Wirth	1	4	0		2	0	
restuans Theobold	20	1,777	1,155	57	0	0	
stigmatosoma Dyar	46	7,379	3,841	137	109	59	5
tarsalis Coquillett	57	16,208	16,151	639	330	252	19
thriambus Dyar	5	178	178	20	3	0	
Culiseta							
incidens (Thomson)	41	8,276	7,269	259	299	207	21
inornata (Williston)	32	3,409	3,265	187	364	290	15
particeps (Adams)	13	1,309	1,309	116	2,030	1,515	45
Totals		111,104	91,547	3,027	17,656	13,561	386

RESULTS

Mosquito abundance and ecology: A total of 111,104 adults reared from field-collected immatures and 17,656 field-collected adult females comprising 4 genera and 19 species of mosquitoes were collected from Morro Bay on 39 occasions during 1994 and 1995 (Table 1). *Aedes dorsalis* was the most abundant species, comprising 41% of the immatures reared to adults and 85% of the adults (mostly host-seeking females collected by CO_2 traps), followed by *Cx. tarsalis* (15 and 2%), *Ae. squamiger* (15 and 1%), *Ae. washinoi* (8 and 2%), *Culex stigmatosoma* (7 and 1%), *Culiseta incidens* (7 and 2%), *Cs. particeps* (1 and 11%), and *Culiseta inornata* (3 and 2%), respectively. The locations and temporal occurrence of positive larval collections are summarized for each of the common species in Table 2 and depicted spatially in Fig. 1B. Sites J and E were classified as salt marsh habitat during 1994, but were silted heavily by winter runoff from Chorro and Turri creeks during March 1995, respectively. Increased elevation apparently prevented the tidal intrusion of saltwater, and these sites were inundated with freshwater runoff and

Table 2. Site utilization, habitat, and seasonality of common mosquitoes collected as immatures at Morro Bay, 1994–95.

Species	Sites[1]	Habitat[2]	Months
Ae. dorsalis	D, E, H, I, K	SM	Jan–Dec
Ae. squamiger	A, D, H, J	SM	Nov–Mar, Jun
Ae. washinoi	B, J, O	TP	Jan–Feb
Cs. incidens	B, M	TP, R	Feb–Nov
Cs. inornata	F, L, M	TP, R	Oct–Apr
Cs. particeps	B, M	TP, R	Feb–Apr, Oct–Dec
Cx. stigmatosoma	E, L, M, S, T	FM, TP, R	Feb–Nov
Cx. tarsalis	C, E, F, L, M	FM, TP, R	Feb–Nov

[1] Sites where greatest numbers of specimens were collected; shown in Fig. 1B.
[2] Habitat types: SM, salt marsh; TP, temporary pool; R, riparian; FM, freshwater marsh.

seepage, respectively, changing both vegetation and mosquito fauna. Unexpectedly, large numbers of immature *Ae. squamiger* (4,441 total), normally a winter–early spring species (Bohart and Washino 1978), were collected during June of both 1994 and 1995 from site J. Also of interest was the repeated recovery of small, but consistent, numbers of *Cx. tarsalis* immatures from salt marsh habitat. Based on months when immatures were collected, the common species were classified as winter (*Ae. squamiger, Ae. washinoi*), spring/fall (*Cs. inornata, Cs. particeps*), or summer (*Ae. dorsalis, Cs. incidens, Cx. stigmatosoma, Cx. tarsalis*) abundant (Table 2). In addition to photoperiod and temperature, seasonality was determined by winter rains inundating eggs (e.g., *Ae. washinoi*) and creating riparian overflow and other temporary surface pool breeding sites.

Adult host-seeking abundance in females per trap night was estimated by 20–24 CO_2 traps operated biweekly and analyzed after $\ln(y + 1)$ transformation by 2-way ANOVAs with months and trap sites as main effects. *Aedes dorsalis* was most abundant from July through August, peaking during week 28 in 1994 and week 35 in 1995 (Fig. 2B). Delayed peak abundance during 1995 was attributed to the early season mortality of eggs covered by silt deposited during late winter. In general, increases in host-seeking female abundance followed approximately 2 wk after flood tides >2 m occurred on >3 successive days. Rapid immature development was attributed to relatively warm water temperatures (Fig. 2A). Significantly highest mean catches were made at traps 5, 14, 15, 18, and 32, indicating that with the exception of an open meadow (site 5), females were collected most frequently host-seeking on hillsides in grass/chaparral (sites 18, 32) or pasture (sites 14, 15) habitats.

Aedes squamiger and *Ae. washinoi* abundance was unimodal, with peaks during winter and early spring (Fig. 2B). *Aedes squamiger* was collected most frequently at traps in chaparral vegetation to the south of the salt marsh (sites 24, 25, 31), whereas *Ae. washinoi* was collected most frequently in vegetated areas along or near water courses (sites 5, 26, 27, 28, 31). The abundance of *Ae. washinoi* at traps positioned far south of principal breeding sites (Table 2) indicated that we may have failed to locate one or more productive breeding sites.

Autogeny affects adult sampling by CO_2 traps by delaying the time after emergence when host-seeking commences and by reducing the number of females surviving to begin host-seeking. Reproduction without imbibing blood meals also may facilitate the vertical transmission of arboviruses. On 9 occasions during May–September 1994 and May–July 1995, 20–60 female *Ae. dorsalis* collected as immatures and then reared and maintained for >7 days in the insectary were dissected to determine ovarian maturation. Overall, 26 (7.3%) of 356 females had ≥4 follicles matured to ≥stage IV and were considered to be autogenous. The percentage of females autogenous ranged from 0 on 4 occasions to 30% (n = 47) on May 16, 1994. A similar low rate of autogeny was reported for *Ae. dorsalis* collected in Nevada (Chapman 1962). In addition, all of the 80 *Ae. squamiger* collected on 3 occasions and the 23 *Ae. washinoi* collected on one occasion were anautogenous.

Culex tarsalis was most abundant during late summer 1994 and from April through September 1995 (Fig. 2C). The greatest numbers of adults were collected at trap sites 18, 19, 24, 25, and 31. Sites 24, 25, and 31 were near breeding site E, which was especially productive during 1995. Although collected infrequently during 1994, *Cs. inornata* was abundant during April and November 1995 (Fig. 2C). Mean catch size was not significantly different among trap sites, even after $\ln(y + 1)$ transformation, with highest counts at site 32. *Culiseta particeps* was consistently abundant throughout summer (Fig. 2C), with greatest trap counts at sites 14, 15, 24, 25, and 31 on hillsides near Turri Creek. Productive breeding sites for *Cs. particeps* were never located, and the few positive sites were limited to shaded riparian habitat (Table 2).

Virus detection: Overall, 91,547 adults collected as immatures and 13,561 females collected mostly by CO_2 traps were tested for arboviruses in 3,027 and 386 pools, respectively (Table 1). From March 1994 through March 1995 adults of most species reared from immatures were tested for virus infection, but after March 1995 virus testing was limited to *Ae. dorsalis* and *Cx. tarsalis,* potential vectors of WEE. All host-seeking females collected were tested, with the exception of the 3 *Anopheles* species. Four of 111 pools of *Ae. squamiger* collected as immatures were positive for MB virus (minimum infection rate [MIR] = 1.07 per 1,000 tested), whereas 179 host-seeking females (15 pools) were negative. All remaining pools of all species were negative for virus infection, including 15,131 *Ae. dorsalis* collected at site A where Fulhorst et al. (1994) previously isolated WEE virus. In addition, 111 pools of *Ae. dorsalis* collected as immatures during July and August from sites K, D, and H were tested for WEE virus genomic RNA by RT-PCR with negative findings. Tests on sera from sentinel chickens and rabbits as well as resident chickens were negative for antibodies to WEE, St. Louis encephalitis, and California-group viruses.

DISCUSSION

Mosquito ecology: The lack of organized mosquito control, diverse and abundant larval and adult habitats, and persistent surface water, especially during spring, produced a diverse mosquito fauna at Morro Bay estuary and surrounding upland areas. Large numbers of immatures were collected from a variety of surface water habitats; however,

adults of all species were difficult to collect in CO_2 traps. Low trapping effectiveness was unexpected, because these same CO_2 traps and dry ice holders collected large numbers of both *Cx. tarsalis* and *Ae. dorsalis* elsewhere in California (e.g., Reisen et al. 1995) and because the 3 abundant *Aedes* species were mostly anautogenous. Attempts to enhance catch size by changing trap position or by extending the trap operation period to include afternoon as well as night met with limited success. Collection of *Ae. dorsalis* was greatest in grass/chaparral habitat on hill slopes near cattle, >1 km from salt marsh breeding sites. Traps operated in ecotonal vegetation near the salt marsh were generally unproductive. To increase the number of adults available for virus testing, we augmented the CO_2 traps with lights, low-release-rate packets of octenol (American Biophysics Corporation, Jamestown, RI), and chemical or battery-operated heat sources without any noticeable increase in catch size (data not shown). We also tested malaise traps, Prince and Fay *Aedes* traps, chicken-baited lard can and modified flap traps, and afternoon and evening human landing catches with minimal success (methods and trap designs described in Service 1993). Landing catches were productive only for *Ae. dorsalis* following large emergences. Collecting *Aedes* adults resting in *Salicornia* using nets or a mechanical sweeper (Meyer et al. 1983) was labor intensive and specimens were mostly newly emerged males and females (i.e., not different from an arbovirus standpoint from specimens collected as immatures and then reared to adults) or gravid females. Additional research is indicated to improve sampling methodology for host-seeking mosquitoes in these cool coastal environments.

The abundant mosquito species were well segregated in time and/or space. *Aedes dorsalis* used the same salt marsh breeding sites as *Ae. squamiger*; however, these species were separated temporally, with *Ae. dorsalis* abundant during summer and *Ae. squamiger* abundant during winter. *Aedes squamiger* and *Ae. washinoi* were abundant concurrently during late winter and early spring; however, breeding sites were separated spatially, with *Ae. squamiger* confined to salt marsh habitat and *Ae. washinoi* confined to freshwater pools along streams. Similarly, the 3 *Culiseta* species were found in different habitats at different times of the year. *Culiseta incidens* larvae were collected in sunlit pools along foothill rivulets and from an ornamental pond, whereas *Cs. particeps* larvae were found mostly in shaded riparian pools. *Culiseta inornata* adults were active for limited time periods during the fall and spring, and immatures were most abundant in freshwater marshes created by runoff from fall and winter rain. *Culex tarsalis* was a generalist, exploiting some salt marsh habitats, flooded hoof prints and small surface pools, and freshwater marsh sites. Other species such as *Culex restuans, Culex apicalis, Cx. thriambus,* and *Anopheles franscicanus* were focally abundant in microhabitats associated with vernal rivulets.

Arbovirus ecology: Tests of 105,108 mosquitoes in 3,413 pools and 30 chicken and 5 rabbit sentinels bled biweekly from March 1994 through November 1995 failed to detect the presence of WEE virus at Morro Bay estuary or surrounding areas. In addition, 111 pools of *Ae. dorsalis* tested by RT-PCR also were negative. Collectively, these data indicate that WEE virus probably has not persisted at Morro Bay, either horizontally in a *Cx. tarsalis*–bird or an *Aedes*–rabbit cycle, or vertically within *Aedes* or other mosquito species populations. Although *Cx. tarsalis* larvae were abundant in a variety of surface-water habitats, adults were collected infrequently and overall abundance averaged <1 female per CO_2 trap night except at site 25, where the geometric mean was 1.2 females/trap night. Similar results were reported by Fulhorst (1994)[4] using both CO_2 traps and evening landing catches. Analyzing data collected in the Central Valley, Olson et al. (1979) and Milby et al. (1978) indicated that *Cx. tarsalis* abundances of >1 female per New Jersey trap night or >10 females per CO_2 trap night per year were necessary to support WEE virus enzootic transmission to sentinel chickens. Consistent with these thresholds, sera from 3 flocks of sentinel chickens and from local chickens housed at Morro Bay were negative for antibodies against WEE.

Previous isolations of WEE virus from Morro Bay were limited to *Ae. dorsalis* collected as immatures at site A during August; tests on *Ae. dorsalis* from other sites or other times of the year were negative (Fulhorst 1994[4], Fulhorst et al. 1994). MIRs recorded by Fulhorst (1994)[4] were 0.42 per 1,000 for site A (his site #8) and 0.21 per 1,000 for immatures from all sites (n = 14,038) that were reared to adults and tested for virus infection using a plaque assay in Vero cells. Our data from Morro Bay including site A indicated that vertical WEE transmission either had not been maintained or has persisted at a very low level (MIR < 0.02 per 1,000, n = 44,641 *Ae. dorsalis* immatures). It should be mentioned, however, that the 95% confidence limits on Fulhorst's MIR ranged from 0 to 0.45 per 1,000 and therefore were not statistically different from our rate of 0. Although site A was flooded and large numbers of *Ae. dorsalis* were collected and tested during spring and fall, site A remained dry during the summers of both 1994 and 1995. A total of 6,750 *Ae. dorsalis* were collected from sites within 1 km of site A during August and tested for virus with negative findings. During August and September 1995 when site A was dry, soil/*Salicornia* samples were collected on 3 occasions from site A, returned to the laboratory, and flooded with water from site K, but this failed to stimulate *Aedes* eclosion. Viable eggs apparently were present at site A at this time, because larvae were collected from our marked soil sampling sites after natural flooding the following October.

During the summer of 1994, we attempted to demonstrate vertical transmission experimentally by testing the progeny from *Ae. dorsalis* females that were collected from Morro Bay and then inoculated with the DAV 3340 strain of WEE virus isolated by Fulhorst et al. (1994) from *Ae. dorsalis* collected at Morro Bay. Unexpectedly, tests on 759 adults (40 pools) reared from combined 1st and 2nd ovipositions were negative for infectious virus by plaque assay in Vero cells (unpublished data). These and previous experiments (Hardy and Reeves 1990) indicate that *Ae. dorsalis* is not an efficient vertical vector of WEE virus. Collectively, field and experimental data question the long-term vertical maintenance of WEE virus in *Ae. dorsalis* populations at Morro Bay. Additional research is planned to further clarify these negative findings.

Evidence for horizontal transmission of WEE virus among mammals and *Aedes* also was minimal. Similar to our negative findings with host-seeking females, Fulhorst (1994)[4] did not recover WEE virus from 15,803 *Ae. dorsalis* or 8,738 other species collected as host-seeking adults. In a serosurvey of potential mammalian blood meal hosts of *Ae. dorsalis*, Fulhorst (1994)[4] found that the sera of 2 of 155 cattle and 1 of 42 *Peromyscus maniculatus* neutralized WEE virus, but sera from 91 dogs, 9 deer, 6 *Lepus californicus*, 21 *Sylvilagus bachmani*, 29 *Citellus beecheyi*, and 39 *Neotoma fuscipes* were negative. In the current study, sera from sentinel rabbits positioned between salt marsh breeding sites and productive CO_2 trap sites 14 and 15 remained negative throughout.

Morro Bay virus appears to be maintained in nature by vertical transmission and has been recovered from most *Ae. squamiger* populations sampled in central and southern California (Eldridge et al. 1991, Fulhorst 1994[4], Fulhorst et al. 1996b). Experimentally, *Ae. squamiger* from Morro Bay were shown to be effective horizontal and vertical vectors of MB (Kramer et al. 1992). However, similar to previous studies (Fulhorst 1994[4]), MB was not isolated from host-seeking *Ae. squamiger* females and antibodies were not detected in sentinel rabbits. Fulhorst (1994)[4] isolated MB from host-seeking *Ae. washinoi* and a California encephalitis serotype virus from host-seeking *Ae. dorsalis* collected at Morro Bay, and antibodies to MB were detected in 3.5% of 372 human and 1% of 95 horse sera tested from San Luis Obispo County; none of 27 lagomorph sera were positive. These results differed markedly from research on California encephalitis (CE) virus in the Central Valley, where CE is maintained by vertical transmission within *Ae. melanimon* populations, amplified by horizontal transmission among *Ae. melanimon* and lagomorphs, and tangentially transmitted to humans (Hammon and Reeves 1952, Gressikova et al. 1964, Reisen et al. 1990). As pointed out by Fulhorst (March 1996, personal communication), low horizontal transmission and amplification rates at Morro Bay also may be indicated by the apparent decline over time in the minimum vertical infection rate per 1,000 immatures reared and tested as adults: 5.74 in January 1989 (Eldridge et al. 1991), 1.52 during the winters of 1991–92 (Fulhorst 1994[4]) and 1.07 during the winters of 1994–95 (current study). Further research will be required to understand the horizontal transmission cycles and possible public health importance of MB in coastal California.

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