

Environmental Factors Affecting the Western Treehole Mosquito (*Aedes sierrensis*)

Abstract

Water-filled treeholes were investigated to document environmental factors affecting the western treehole mosquito, *Aedes sierrensis*. Waters in northern California treeholes were characterized by relatively high pH, high conductivity, high potassium, and high boron concentrations. Some of the chemical factors (nitrate, chloride and iron) were correlated with emergence of *A. sierrensis*. The physical factors of treehole surface area, number of stems and the amount of organic matter in the treehole also were correlated with the emergence of *A. sierrensis* from treeholes. Interspecific biological interactions, such as predation or competition among aquatic macroinvertebrates, were not found to be important for *A. sierrensis*. Intraspecific competition for limited food resources was important for *A. sierrensis*. Resource limitation might also affect other organisms inhabiting other phytotelmata.

Introduction

Phytotelmata are small aquatic habitats associated with living or dead plants. Water-holding flower bracts, modified leaves, leaf axils or stem internodes are found in at least 29 plant families distributed throughout the world (Fish 1983). Conspicuous phytotelmata include pitcher plants and bromeliad axils. Water-filled treeholes occur over a vast geographic range, largely corresponding to that of hardwood trees. Crotches or rotted limbs or trunks of trees often form cavities which collect rainwater. Treeholes support unusual assemblages of aquatic biota. The treehole water may be very turbid and the treeholes dark. Photosynthetic plankton are rare in these microlentic habitats. Low dissolved oxygen concentrations may further limit the diversity of biota. In California, treehole water may freeze in the winter, and some treeholes dry up by late May (summer rains are rare or absent), so the productive season for aquatic organisms may be brief.

Oligochaetes and acarines occur in treehole waters, but immature insects usually dominate the macroinvertebrate biomass. Throughout the world, mosquitoes are among the most frequent and numerous inhabitants of treeholes (Bradshaw and Holzapfel 1991). Five species of mosquito larvae have been collected from northern California treeholes (D.L.W. unpublished data). According to the classification of Rohnert (1950), two of these species (*Culiseta incidens* and *Culex stigmatosoma*) are dendrolimnetophiles (generalists attracted to a wide variety of aquatic habitats); one (*Aedes increpitus*) is a dendrolimnetoxene (an accidental inhabitant which is not a regular member of the treehole com-

munity); and two (*Orthopodomyia signifera* and *Aedes sierrensis*) are dendrolimnetobionts (organisms largely restricted to treeholes).

Some parasites (e.g., Egerter and Anderson 1985, Washburn *et al.* 1989) and pathogens (e.g., Pinnock *et al.* 1973, Samson and Soares 1984) have been studied more than predators in treeholes of western North America. The role of interspecific competition in western treeholes is not known.

Abiotic factors in western treeholes have not been thoroughly studied. Water chemistry, amount of organic matter in the treehole, physical dimensions and factors relative to the location of a treehole might influence mosquito production directly or by affecting the biotic factors. The importance of interactions among biotic and abiotic factors in affecting larval mosquito populations in phytotelmata is poorly known (Walker *et al.* 1991).

Aedes sierrensis is the most common insect in California treeholes. This species is important because it is a vector of deer bodyworm (*Setaria yehi*) and canine heartworm (*Dirofilaria immitis*) (Weinmann *et al.* 1973, Weinmann and Garcia, 1974). The present study was conducted to investigate physical, chemical, and biological factors associated with some California treeholes and to determine which factors affect the emergence of *A. sierrensis*.

Materials and Methods

Twenty-four trees in nine different areas of Lake and Mendocino counties were included in this study. Species examined included California black oak (*Quercus kelloggii*), interior live oak (*Quercus*

wislizenii), blue oak (*Quercus douglasii*), valley oak (*Quercus lobata*) and Pacific madrone (*Arbutus menziesii*).

According to Munz's (1959) classification of California plant communities, areas K, JM and G are northern oak woodlands and areas HR, EB, WB, MC, RH and HS are foothill woodlands. Areas K and G were the most densely forested sites. Areas HR and MC had the lowest density of trees.

Latitude, longitude and elevation were determined from topographic maps. The direction and angle of slope of the ground were determined with a compass and clinometer. Each study tree was identified (Munz 1959) and its height determined with a clinometer.

All leaves, organic detritus, etc. were removed from each treehole, drained, weighed and recorded as treehole "organic matter." "Treehole volume" was determined by adding known quantities of water to an empty treehole. Water surface area was calculated from multiple width and length measurements. Maximum treehole depth was also measured. The compass direction of the treehole opening (which could influence the amount of leaves, rainwater or sunlight entering a treehole) was recorded.

On 21 February 1985 a water sample was obtained from each treehole and water quality factors were determined according to standard methods (Greenberg 1985). When *A. sierrensis* pupae began to appear, emergence traps (Woodward *et al.* 1988) were placed over 13 treeholes. Twice each week, emerged adults were collected from each trap and identified (Bohart and Washino 1978, Woodward *et al.* 1988). Emergence of *A. sierrensis* typically ceases by August (Garcia *et al.* 1989) so emergence was monitored until 02 September.

The total number of *A. sierrensis* emerging was regressed on various physical, chemical and biological factors (Northwest Analytical 1986). Percentages were transformed to arcsine square roots prior to regression analyses. Differences between tree species (except the two *Q. lobata*) and between slope directions were tested by one-way analysis of variance and Newman-Keuls multiple range tests.

Results and Discussion

The water quality data appear in Table 1. There was relatively little variability in some chemical fac-

tors (e.g., magnesium and nitrate). However other factors showed considerable variability among treeholes. For instance, the maximum potassium concentration (2,610 mg/l) was more than 104 times greater than the minimum concentration (25 mg/l).

Boron concentrations have not been reported in most treehole studies, but the average boron concentration in natural fresh waters is ca. 0.01 parts per million (ppm) (Wetzel 1975). The mean boron concentration in treehole waters of the present study was 0.36 ppm.

Vrtiska and Pappas (1984) found that Nebraska treeholes had water hardness values (mean = 9.3 ppm) significantly lower than waters from other mosquito larval habitats. Water hardness in the present study was relatively high (mean = 113 ppm).

Treehole waters often have high potassium concentrations (Peterson and Chapman 1969, Washburn and Anderson 1986). Vrtiska and Pappas (1984) reported that potassium concentrations (298 ppm) in treehole waters were significantly higher than in other mosquito larval habitats. In the present study the mean potassium concentration was very high (520 ppm).

The mean pH was 7.8 and the mean conductivity was 1,286 $\mu\text{mhos/cm}$ (Table 1). Kitching (1983) reported a mean pH of 6.4 and a mean conductivity of 339 mS/cm in some European treeholes, and a mean pH of 5.9 and conductivity of 227 mS/cm in Australian treeholes. Vrtiska and Pappas (1984) reported a mean pH of 6.9 and mean conductivity of 456 μmhos in waters of Nebraska treeholes.

Lunt and Peters (1976) found *Aedes hendersoni* in treehole water with a pH of 9.3, but *Anopheles barberi* larvae were not found in treeholes with a pH greater than 8.7. In a study of Louisiana treeholes (Peterson and Chapman 1969), some larvae (e.g., *Toxorhynchites rutilis septentrionalis*) were collected in water having a pH of 9.9, but the treehole mosquito *Aedes triseriatus* was absent from all treeholes having a pH greater than 8.8. In the present study, *A. sierrensis* larvae were present (in low numbers) in the treehole with the highest pH (8.5 in EB-1).

Peterson and Chapman (1969) found that *A. triseriatus* was absent in treehole waters having electrical conductivities greater than 4,100 $\mu\text{mhos/cm}$. Washburn and Anderson (1986) collected *A. sierrensis* larvae from treehole waters and

TABLE 1. Analyses of water samples collected from treeholes in northern California.

Tree	pH (units)	Chemical												
		oxygen demand (mg/l)	Potassium (mg/l)	Electrical conductivity (μ mhos/cm)	Total dissolved solids (mg/l)	Iron (mg/l)	Manganese (mg/l)	Copper (mg/l)	Magnesium (mg/l)	Calcium (mg/l)	Hardness (mg/l)	Nitrate (mg/l)	Chloride (mg/l)	Boron (mg/l)
K-2	7.5	710	240	664	986	0.3	1.70	0.01	11	44	155	0.4	7	0.23
K-4	8.3	390	200	658	756	0.2	0.50	0.01	8	21	93	0.5	5	0.72
K-8	8.2	1340	462	2041	1786	0.5	0.20	0.01	13	20	103	0.6	13	0.37
K-9	8.6	380	350	977	954	0.2	0.80	0.01	10	17	84	0.4	6	0.06
JM-1	6.6	450	33	452	424	0.3	1.20	0.01	6	19	72	0.4	9	0.24
JM-2	7.4	260	27	183	224	0.2	0.20	0.01	3	15	50	0.4	4	0.29
HR-1	7.4	2590	481	1492	2520	0.7	0.50	0.01	7	31	106	0.8	11	0.79
HR-2	8.6	1280	848	1675	2072	0.6	0.50	0.17	9	31	114	0.8	17	0.37
FB-1	8.6	9830	2610	4780	11670	7.0	3.10	0.03	49	64	362	1.0	23	1.63
EB-2	8.7	1900	1460	2771	3544	4.0	1.40	0.03	21	24	146	1.0	17	0.67
WB-1	6.2	630	57	311	832	0.2	0.30	0.03	4	23	74	0.7	7	0.28
MC-1	9.5	1240	657	1203	1656	4.0	0.30	0.04	7	22	84	0.7	8	0.30
RH-1	8.8	4480	1416	2486	830	2.0	1.80	0.20	27	37	204	0.8	29	0.19
HS-1	9.4	3120	1445	5933	6950	4.0	0.40	0.02	20	34	167	1.0	17	0.39
G-1	7.0	310	140	454	420	0.4	0.09	0.01	8	19	80	3.0	2	0.02
G-2	8.0	280	166	413	518	0.3	0.09	0.01	7	23	86	7.0	6	0.25
G-3	8.8	240	317	832	776	0.2	0.04	0.01	15	19	109	0.7	5	0.05
G-4	7.4	610	310	245	720	5.0	0.80	0.02	5	16	61	0.6	6	0.18
G-5	8.1	990	489	913	1430	2.4	1.80	0.01	8	31	110	0.7	19	0.26
G-6	6.7	440	36	187	390	0.9	0.10	0.01	5	23	78	0.7	2	0.13
G-7	6.9	180	25	174	255	0.2	0.30	0.01	6	19	72	7.0	4	0.03
G-8	6.5	1200	129	349	1360	3.0	0.90	0.02	9	40	137	0.8	9	0.31
G-9	6.5	530	58	375	268	1.8	0.70	0.01	4	12	46	0.7	10	0.61
Mean	7.8	1451	520	1286	1797	1.7	0.77	0.03	11	26	113	1.3	10	0.36

electrical conductivities of 70 to 8,370 $\mu\text{mhos/cm}$. This is similar to the conductivity range (174 to 5,933 $\mu\text{mhos/cm}$) of waters harboring *A. sierrensis* in the present study.

Peterson and Chapman (1969) collected *A. triseriatus* larvae in waters having a magnesium concentration as high as 8.6 milligram equivalents per liter and calcium concentration as high as 27.1 milligram equivalents per liter. Washburn and Anderson (1986) found *A. sierrensis* larvae occurred in treeholes with magnesium concentrations as high as 125 ppm and calcium concentrations as high as 258 ppm. In the present study *A. sierrensis* larvae occurred in waters having a magnesium concentration as high as 49 ppm and a calcium concentration as high as 64 ppm. These concentrations of magnesium and calcium are higher than those occurring in most lentic habitats (Wetzel 1975).

Additional factors associated with treeholes appear in Table 2. The mean treehole surface area was 554 cm^2 and the mean depth was 22.2 cm. Kitching (1983) reported a mean surface area of 422 cm^2 and a mean depth of 15.8 cm in Australian treeholes, and a mean surface area of 423 cm^2 and a mean depth of 25.2 cm for some treeholes in England.

The size (volume) of the treehole (Table 2) was not significantly correlated ($P > 0.05$) with the species of tree. *Quercus douglasii* treeholes were significantly ($P < 0.05$) higher above the ground than treeholes in other species. There were no differences ($P > 0.05$) among tree species in treehole depth, treehole surface area or the amount of organic matter in a treehole. There was no correlation ($P > 0.05$) between the height of the tree and the height, depth, surface area, or volume of the treehole.

Some of the ions were positively correlated with each other, so they may have a common source (Table 3). This finding is consistent with reports that stemflow (rain water which runs down the side of a tree) is important in bringing some inorganic ions into treeholes (Fish 1983, Wallace *et al.* 1991).

Emergence traps over selected treeholes (Table 4) collected nine species including a mosquito (*A. sierrensis*), a marsh beetle (*Cyphon* sp.), a moth fly (*Telmatoxypus* sp.), a hover fly (*Blera humeralis*), two midges (*Polypedilum pedatum* and *Limnophyes hamiltoni*) and two biting midges

(*Culicoides neofagineus* and *C. cavaticus*). The emergence of adult male *A. sierrensis* was most highly correlated ($P < 0.01$) with the amount of organic matter in the treehole (Figure 1). The organic matter may include leaf litter, mistletoe and dead organisms which have fallen in the hole, dead moss and lichens which occur near some treeholes, twigs, catkins, acorns, pollen, seeds, etc. Decayed organic matter is an ovipositional attractant for *A. sierrensis* (Ahmadi and McClelland 1983), so the treeholes with greater amounts of organic matter may have had more eggs.

Larvae of some treehole mosquitoes (e.g., *Anopheles plumbeus*, *O. signifera*) feed by filtering suspended particles while hanging from the water surface (Zavortink 1985, Bradshaw and Holzapfel 1991). In addition to filtering, *A. sierrensis* larvae commonly dive to the bottom of the treehole to feed by browsing or gnawing on organic matter or associated microbes. *Aedes sierrensis* might be more successful than other treehole insects (Table 4) partly because it is relatively euryphagous.

There can be large numbers ($> 10,000$) of early instar *A. sierrensis* larvae and very little organic matter in some treeholes. This can result in intraspecific competition for the limited amount of resource available. An inadequate food supply for the larvae may result in smaller adults or in fewer mosquitoes emerging from treeholes. Figure 1 shows that *A. sierrensis* emergence is consistent with the reports that populations of eastern treehole mosquitoes are limited by the amount of available food (Fish and Carpenter 1982, Carpenter 1983). Low per capita resources can result in retarded larval growth, reduced pupal weight, reduced survivorship, reduced pupation success and reduced emergence of some species (Hawley 1985, Bradshaw and Holzapfel 1991).

The emergence of females was negatively correlated with the concentration of chloride ions (Figure 2). The maximum chloride concentration found in a treehole (23 ppm) apparently adversely affected *A. sierrensis* development. Vrtiska and Pappas (1984) indicated tree holes containing *A. triseriatus* were characterized by low chloride (mean = 6.2 ppm) but Peterson and Chapman (1969) collected living *A. triseriatus* larvae in treeholes having chloride concentrations as high as 12.5 milligram equivalents per liter. *Aedes sierrensis* (or some important microbe) may be relatively sensitive to chloride ions.

TABLE 2. Physical and other factors relative to water-filled treeholes in northern California.

Tree	Latitude (North)	Longitude (West)	Elevation (meters)	Slope direction (degrees)	Slope angle (percent)	Tree (species)	Tree stems (no.)	Tree height (meters)	Treehole direction (degrees)	Treehole height (cm)	Treehole depth (cm)	Surface area (sq cm)	Treehole volume (cc)	Organic matter (grams)
K-2	38°56'	122°47'	563	228	19	<i>Quercus kelloggii</i>	2	17.1	315	9	29.0	210	4150	815
K-4	38°56'	122°47'	566	228	24	<i>Quercus kelloggii</i>	2	13.7	225	0	9.0	198	970	200
K-8	38°56'	122°47'	563	228	16	<i>Quercus kelloggii</i>	4	14.0	235	10	22.0	595	24550	865
K-9	38°56'	122°47'	564	233	19	<i>Quercus kelloggii</i>	3	16.2	54	16	28.0	437	12800	685
JM-1	38°55'	122°46'	651	23	9	<i>Quercus kelloggii</i>	4	20.7	90	80	10.0	630	—	1105
JM-2	38°55'	122°46'	652	113	9	<i>Quercus kelloggii</i>	2	18.6	0	50	9.0	90	1000	105
HR-1	38°59'	122°49'	418	198	3	<i>Quercus douglasii</i>	4	5.9	355	102	2.0	36	730	62
HR-2	38°59'	122°49'	418	198	2	<i>Quercus douglasii</i>	1	7.3	10	510	170.0	516	—	—
EB-1	38°59'	122°40'	436	233	23	<i>Quercus douglasii</i>	5	9.4	270	8	3.0	24	2900	10
EB-2	38°59'	122°40'	436	298	27	<i>Quercus douglasii</i>	3	9.8	300	43	19.5	81	5600	50
WB-1	38°59'	122°41'	411	273	11	<i>Quercus wislizenii</i>	2	14.0	10	50	9.0	96	—	10
MC-1	39°12'	122°55'	475	223	6	<i>Quercus lobata</i>	5	19.8	190	130	42.0	4800	192831	—
RH-1	39°07'	122°54'	430	73	15	<i>Quercus douglasii</i>	3	15.8	125	400	33.0	2097	85163	125
HS-1	38°56'	122°56'	561	118	5	<i>Quercus douglasii</i>	1	17.1	165	200	22.0	—	15740	—
G-1	39°14'	123°06'	330	86	4	<i>Arbutus menziesii</i>	5	12.5	105	9	12.5	442	10200	1570
G-2	39°14'	123°06'	332	95	7	<i>Arbutus menziesii</i>	3	13.1	240	5	7.0	570	3200	1340
G-3	39°14'	123°06'	332	95	7	<i>Arbutus menziesii</i>	2	13.4	285	5	12.0	160	650	100
G-4	39°14'	123°06'	333	27	27	<i>Quercus wislizenii</i>	2	14.3	260	5	7.0	144	960	170
G-5	39°14'	123°06'	332	12	6	<i>Quercus wislizenii</i>	2	16.5	130	9	6.0	126	700	260
G-6	39°14'	123°06'	333	50	17	<i>Quercus wislizenii</i>	5	15.5	135	24	11.0	294	4600	950
G-7	39°14'	123°06'	333	53	16	<i>Quercus wislizenii</i>	5	16.2	—	75	28.0	475	12555	1540
G-8	39°14'	123°06'	332	51	21	<i>Quercus wislizenii</i>	2	4.9	87	30	4.0	36	390	100
G-9	39°14'	123°06'	332	55	22	<i>Quercus wislizenii</i>	3	15.7	163	20	4.0	144	—	—
Mean			441	141	13		3	14.0	163	77	22.2	554	19983	529

TABLE 3. Matrix of Spearman's rank correlation coefficients (rho) of physical and chemical measurements of 13 water-filled treeholes.

	Volume	Surface area	Depth	pH	Conductivity	T.D.S.	C.O.D.	Nitrate	Iron	Manganese	Copper	Magnesium	Calcium	Hardness	Chloride	Boron	Potassium
Organic mat.	.438	.897**	.353	-.526	-.438	-.587*	-.460	-.288	-.361	-.251	-.360	-.371	-.309	-.466	-.530	-.620*	-.501
Volume		.543	.725**	.273	.165	-.027	.099	.082	-.011	.129	.132	.152	-.155	-.110	-.102	-.033	.010
Surface area			.620*	-.345	-.262	-.507	-.507	-.326	-.485	-.299	-.338	-.135	-.424	-.419	-.532	-.592*	-.370
Depth				.356	.231	-.022	-.121	-.099	-.418	-.052	-.088	.323	-.144	.071	-.192	-.203	.022
pH					.672*	.540	.441	.088	-.032	.576*	.335	.477	.363	.471	.456	.669*	.565*
Conductivity						.907**	.703**	.420	.313	.514	.409	.790**	.526	.725**	.813**	.571*	.923**
T.D.S.							.890**	.453	.571*	.508	.591*	.707**	.655*	.786**	.934**	.654*	.885**
C.O.D.								.412	.709**	.525	.610*	.433	.694**	.615*	.852**	.731**	.687**
Nitrate									.536	-.065	.549	.393	.265	.357	.269	.104	.398
Iron										.418	.827**	.221	.383	.352	.599*	.286	.495
Manganese											.420	.224	.468	.434	.650*	.456	.659*
Copper												.522	.409	.519	.582*	.478	.500
Magnesium													.497	.828**	.593*	.301	.641*
Calcium														.845**	.637*	.537	.433
Hardness															.728**	.401	.632*
Chloride																.665*	.852**
Boron																	.489

* P < 0.05

** P < 0.01

TABLE 4. Mean numbers of insects collected per trap per night in emergence traps from 28 March to 02 September 1985.

Tree	<i>Cyphot</i> sp.	<i>Telmatoscopus</i> sp.	<i>Blera</i> <i>humeralis</i>	<i>Limnophyes</i> <i>hamiltoni</i>	<i>Polypedilum</i> <i>pedatum</i>	<i>Calicoides</i> <i>cavaticus</i>	<i>Calicoides</i> <i>neofagineus</i>	<i>Aedes sierrensis</i>		
								♀ ♀	♂ ♂	total
K-2	0.08	0.06	0.00	0.01	0.00	0.00	0.10	0.06	0.04	0.10
K-4	0.00	0.10	0.04	0.00	0.00	0.13	0.06	0.61	0.62	1.23
K-8	0.35	0.09	0.00	0.00	0.00	0.16	0.15	0.98	2.38	3.36
J-2	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.39	0.25	0.64
H-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.88	2.00	2.88
EB-1	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.03	0.02	0.05
EB-2	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.06	0.03	0.09
G-1	0.00	0.03	0.01	0.02	1.88	0.11	0.00	3.11	5.37	8.48
G-3	0.00	0.11	0.01	0.03	0.00	0.10	0.00	0.04	0.04	0.08
G-4	0.00	0.00	0.01	0.01	0.00	0.02	0.00	0.03	0.00	0.03
G-5	0.00	0.00	0.06	0.04	0.00	0.18	0.02	0.49	0.16	0.65
G-6	0.00	0.00	0.00	0.01	0.00	0.16	0.01	3.95	4.99	8.94
G-8	0.00	0.07	0.00	0.00	0.00	0.57	0.01	0.55	0.73	1.28
Mean	0.03	0.04	0.01	0.01	0.14	0.12	0.03	0.86	1.28	2.14

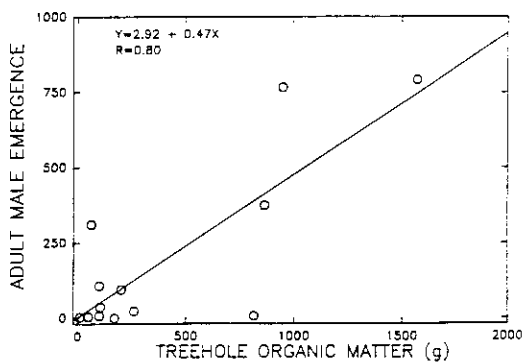


Figure 1. Relationship between the amount of organic matter in California treeholes and emergence of adult male *Aedes sierrensis*.

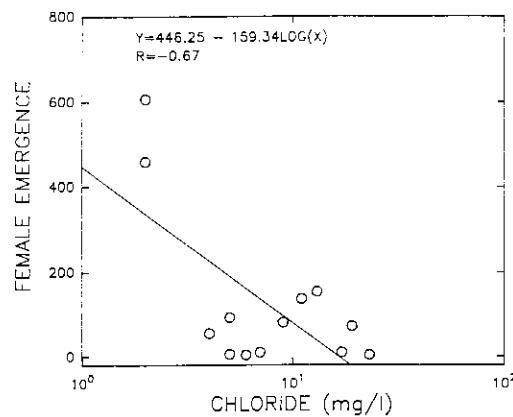


Figure 2. Relationship between the chloride concentration in treehole waters and the emergence of adult female *Aedes sierrensis* from northern California treeholes.

It has been suggested that some mosquitoes in phytotelmata experience delays in larval development times and a sex ratio distorted in favor of males. When larval resources are scarce there could be reduced production of adult females, the heavier and more expensive sex, and the reduction might serve as a feedback mechanism to regulate population size (Frank *et al.* 1985). In the present study females constituted only 40.1% of the emerged adults, but there was no significant correlation between sex ratio and amount of organic matter in the treehole. Female *A. sierrensis* emerge later than males (Garcia *et al.* 1989), so deeper treeholes might be expected to be better

suitable for the females. However there was no correlation between treehole depth and percentage of females emerging from treeholes.

Of the factors in Tables 1-4, the percentage of females was positively correlated only with the concentration of iron in the treehole water (Figure 3). Iron may not be immediately toxic to *A. sierrensis* larvae, but can have complicated effects on other factors including nitrogen, chloride, phosphorous, pH, redox potential, primary production, and bacterial metabolism (Wetzel 1975).

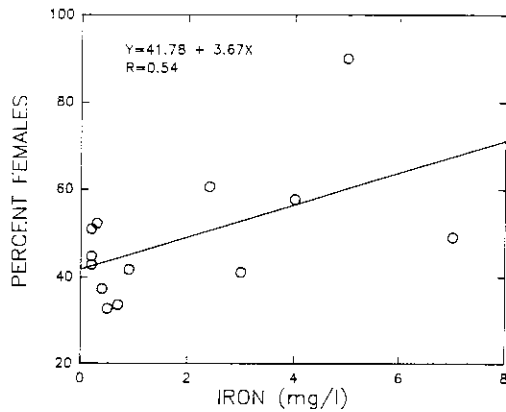


Figure 3. Relationship between the iron concentration in treehole waters and the percentage of adult female *Aedes sierrensis*.

The total adult *A. sierrensis* emergence also was compared with chemical, physical and biological factors in Tables 1-4. Cadavers of other organisms might serve as food for *A. sierrensis* larvae. Of the insects in these treeholes, only the *Culicoides* are considered to be possible predators (Merritt and Cummins, 1984). Other treehole insects could be considered as possible competitors of *A. sierrensis*. However, there were no significant correlations ($P > 0.05$) between the emergence of *A. sierrensis* and the emergence of any of the other species in Table 4, so interspecific biological interactions were not found to be important for *A. sierrensis*. Also the number of insect species per treehole was not correlated with *A. sierrensis* emergence.

Larval predators (e.g., *Toxorhynchites* mosquitoes or cyclopoid copepods) can reduce some populations of treehole mosquitoes (Bradshaw and Holzapfel 1984, Riviere *et al.* 1987). However these predators are rare or absent in California treeholes.

The lower elevation treeholes were in hotter, drier areas and may have dried out sooner than the higher elevation treeholes. However the *A. sierrensis* in the lower treeholes may have started emerging sooner than the higher elevation mosquitoes, and there was no ($P < 0.05$) significant correlation between number of adults emerging and elevation. There was no correlation between slope angle and *A. sierrensis* emergence. The number of mosquitoes emerging was highest ($P < 0.05$) on south-facing ($121-240^\circ$) slopes, which are commonly hot and dry in California. Bradshaw and Holzapfel (1991) reported higher survivorship of a treehole mosquito (*A. plumbeus*) on south-facing slopes in England.

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Non-deciduous trees (e.g., interior live oak and madrone) may produce more of the chemicals that deter herbivores when compared to deciduous oaks because evergreen leaves are vulnerable all year. High concentrations of tannins and lignins reportedly make *Q. wislizenii* less palatable and less nutritious to insects and mammals (Pavlik *et al.* 1991). Tannins produced by some species of trees may have effects on *A. sierrensis* larvae or their natural enemies (Mercer and Anderson 1994). However in the present study there were no significant differences between tree species in the number of adult mosquitoes emerging or their sex ratios.

Treeholes high in trees may receive nutrients from flying organisms and from wind-borne material (anemophilous nutrition). Lower treeholes may also receive acorns and other materials falling into the water or washed by rainwater into the treehole (dendrophilous nutrition according to Frank 1983). The height of the treehole is important for some mosquito species (Sinsko and Grimstad 1977). There was no significant correlation between treehole height and *A. sierrensis* emergence, but treeholes on the highest limbs were not sampled in this study.

Copeland and Craig (1990) found *A. triseriatus* in more shallow treeholes and fewer deep treeholes than expected, and the reverse was true for *Orthopodomyia alba*. In the present study the numbers of emerged *A. sierrensis* were not correlated ($P > 0.05$) with treehole depth.

Total emergence was not correlated with latitude, longitude, or height of tree, but was correlated with the number of major stems (Figure 4).

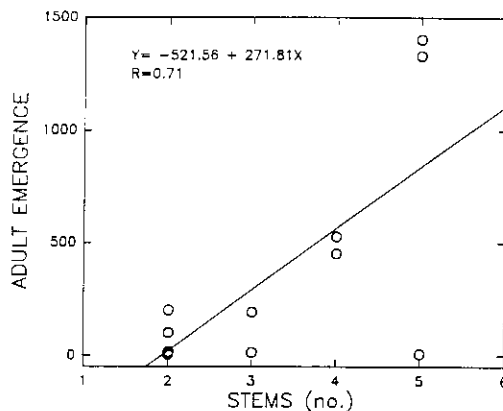


Figure 4. Relationship between the number of major trunk stems on a hardwood tree and the total emergence of adult *Aedes sierrensis* from northern California treeholes.

Some of the trees in this study apparently had been cut to near ground level years ago. The old stump may have become the bottom of a treehole when several trunks sprouted around its circumference. The multiple trunks might funnel stemflow, falling leaves, etc. into these basal treeholes.

Lunt and Peters (1976) found *O. alba* only in treeholes with narrow openings (1.5-1.7 cm). They indicated the surface area of the treehole apparently was not important for *A. hendersoni* or *A. triseriatus*.

The total emergence of *A. sierrensis* was positively correlated with three possibly associated factors: surface area, organic matter, and nitrate (Figure 5). The surface area was positively correlated ($P < 0.05$) with the amount of organic matter in the treehole (Table 3). A treehole with a larger opening would probably have more leaves and other materials falling into it so it would therefore have a greater accumulation of organic matter than treeholes with smaller surface areas. Water with greater organic loading could have greater nitrate concentrations. Nitrate was significantly ($P < 0.05$) correlated with the amount of organic matter in the treehole (Table 3). However, some nitrate also enters treeholes via stemflow. Nitrate can stimulate microbial respiratory metabolism and growth, and detritus decomposition on leaf and bark surfaces and in the water column, thereby providing more mosquito food (Walker *et al.* 1991). Figure 5 is consistent with the finding of Carpenter (1982) that mosquito growth was enhanced in microcosms enriched with nitrate.

The organic matter in many treeholes is largely leaves, which can be used for rearing *A. sierrensis* (Pyle *et al.* 1989). Parts of leaves may be directly ingested by mosquito larvae, or may indirectly provide nourishment through decomposers. Biological transformation of organic matter may be important in treeholes. Fungi are decomposers which can be ingested by treehole mosquito larvae. Carpenter (1983) has shown that leaves in water without mosquito larvae develop massive hyphal growths, but leaves in the presence of eastern treehole mosquito larvae are nearly free of fungi. This is reportedly due to browsing by the larvae. Some protozoans, bacteria and other microorganisms on the leaves may also be ingested by treehole mosquito larvae.

It should be noted that nonaquatic organisms may alter the leaves and other organic matter

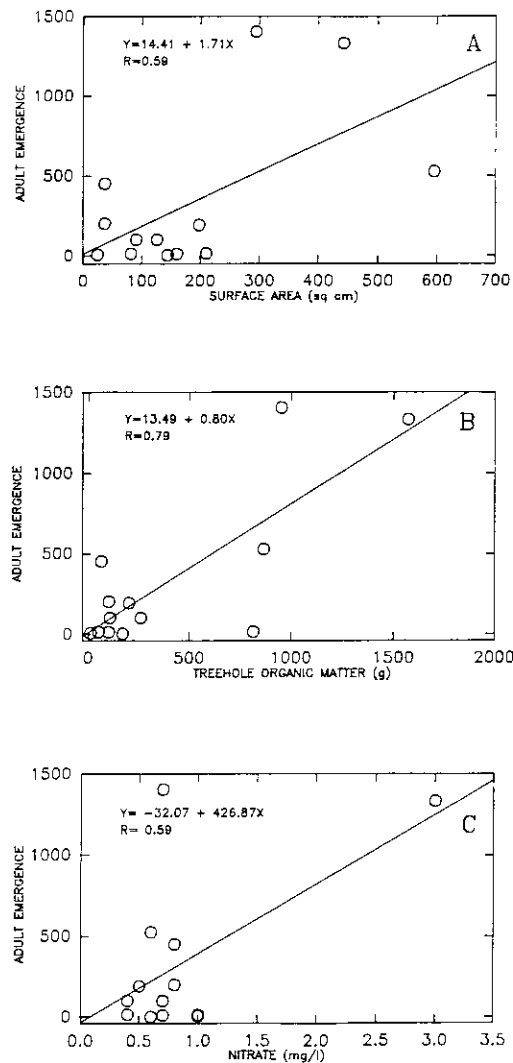


Figure 5. Relationship between treehole surface area (A), treehole organic matter (B), and concentration of nitrate in treehole water (C), and the emergence of adult *Aedes sierrensis*.

when California treeholes are dry in the late summer and early autumn. The leaves and organic matter then provide habitat for numerous invertebrate and vertebrate species (Pavlik *et al.* 1991). The partial processing of the leaves as well as the exuviae, dead organisms, feces, etc. in the treeholes when they are flooded by winter rains may affect the mosquito productivity of a treehole.

The association of emergence with the amount of organic matter in treeholes is consistent with the

idea that treehole mosquito populations are often limited by the larval food supply (Bradshaw and Holzapfel 1991). Eggs, pupae and adults do not feed on the treehole organic matter so the larval stage is the limiting one.

Walker and Merritt (1988) found no difference in the number of *A. triseriatus* emerged from treeholes without leaves or treeholes with leaves. However, emergence of *A. sierrensis* is affected by the amount of leaf litter. Figure 5 is consistent with the idea that *A. sierrensis* is food limited (Gilpin and Langford 1978). Even those adults which do emerge from food-limited phytotelmata may exhibit relatively small size, low survivorship, or low fecundity (Hawley 1985, Bradshaw and Holzapfel 1992).

There are other possible environmental factors which could impact *A. sierrensis*. Some parasites and pathogens have been examined extensively (e.g., Sanders 1972; Sanders and Poinar 1976; Soares 1982; Hawley 1985; Washburn and Anderson 1986; Saunders *et al.* 1988; Washburn *et al.* 1988a, 1988b, 1991; Norton *et al.* 1992) and were not further evaluated in the present study. Interspecific competition may be important in treeholes having higher densities of organisms than in the present study. Also factors which could affect eggs before they contact the water (e.g., predation by ants) or adults after they leave a treehole

(Washburn 1991) were not examined in this study. It is possible that there are water-filled cavities in the center of a tree or very high on limbs which were not found for this study. For eggs hatching after the fall, intermittent drying or freezing can affect the larval development. Heavy rains can wash out eggs, larvae, nutrients or waste products (Walker *et al.* 1991, Washburn and Anderson 1993). Furthermore, some of the factors (e.g., hydrochemical conditions) may change considerably during the course of a season. The factors which limit mosquito production in a treehole may change between high rainfall years and drought years. For these reasons, the results of this study should not be overgeneralized. The roles of possible indirect or interacting biotic and abiotic factors (e.g., number of stems and treehole surface area may affect stemflow which may alter concentrations of nitrate or iron or tannins which may influence bacteria or fungi which may affect food availability for macroinvertebrates) warrant further research. Additional studies are also needed to determine the roles of chemical, biological and physical factors in phytotelmata in other regions.

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