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SEASONAL DISTRIBUTION AND BEHAVIOR OF CALIFORNIA ANOPHELINE MOSQUITOES

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For many years biological and ecological studies of malaria mosquitoes by entomologists were of a field research nature, by necessity, as a part of and tied to control. The initial period of problem solving was led by works of Freeborn (1921, 1926), Bradley and King (1941), Aitken (1945), Markos (1950) and many others. This was followed by a great proliferation of information in various basic phases of mosquito bionomics, including physiology, genetics, photoperiodicity, and communication, much of which has been financed by grants and other special funds. This report brings together detailed field and laboratory observations gathered at the University of California at Davis as a part of general ecological investigations of the four species of California anophelines: *Anopheles punctipennis* Say, *A. franciscanus* McCracken, *A. freeborni* Aitken, and *A. occidentalis* Dyar and Knab.

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STUDY AREA AND METHODS.–The main area under investigation was a transect of northern California from the Sierra Nevada Mountains at Emigrant Gap to the Pacific Ocean at Point Reyes Station (Bailey and Baerg 1966). Supplemental surveys were taken from the Oregon border south to Pismo Beach, and from the northeastern portion of the State and the desert area to the southeast.

It was difficult to locate comparable semi-permanent breeding sites throughout the transect largely because of pollution by cattle, variable and unpredictable flushing by spring rains and irrigation practices. In the Sacramento Valley proper it was rare to find any natural, undisturbed breeding areas. No larvae of *A. punctipennis* were found on the floor of the Valley. Nearly all stream beds had been filled, diverted, or modified for irrigation or flood control purposes. As a result, *A. franciscanus*, also once common in the Valley, was found primarily in the peripheral foothills. As seasonal pools dried, particularly on the west side which lacks the snowmelt water seepage of the eastern slope, it was necessary to shift our collecting sites to those still producing larvae.

Immatures obtained in collections were laboratory-reared to adults to enable positive identifications. Adult mosquitoes were aspirated from resting stations and their physiological condition determined visually. Individuals that were classified as "with blood" were wholly or partially engorged or had a portion of the blood meal digested with eggs apparent. Christopher's stage of follicle development was determined only in the case of *occidentalis*. Many hundreds of adult and larval samples were taken year-round in almost every conceivable place of resting or breeding. Two species, *franciscanus* and *occidentalis*, were studied in detail for doctoral theses (Baerg 1967; Christensen 1968).

Laboratory colonies of *A. freeborni* and *franciscanus* were maintained for several years, and *occidentalis* was colonized for several generations. The southern strain of *occidentalis*, commonly referred to as "*freeborni*" by various writers, was maintained in laboratory culture for over one year. Attempts to establish a breeding colony of *punctipennis* consistently failed, although the species was collected regularly throughout the program. It is noted, however, that laboratory colonization of eastern strains of *punctipennis* have been successful (Boyd and Mulrennan 1934; Dr. Martin D. Young, personal communication).

DISTRIBUTION.—Each of the four species presents a distinct dispersal picture which is best described ecologically. Various writers (Herms 1921; Aitken 1945; Freeborn 1949; Markos 1950; Freeborn and Bohart 1951; Loomis et al. 1956) have given many details on the distribution and exact type of environment in which each species is found. This information need not be repeated here.

Our extensive series of collections of the aquatic stages of these anophelines are given in Table 1. The tabular data summarize a total of 412 collections at 219 stations from 1964 to 1967. New findings of interest might be mentioned. Larval populations were sampled extensively along the northeastern border of the state in 1966, which is the western edge of the Great American Basin. *A. franciscanus* was found in Lassen and Plumas counties. At the other extreme, this species was taken near the Salton Sea at -200 feet elevation. In 1967 the breeding of *occidentalis* in Del Norte County at Crescent City and at Pismo Beach, San Luis Obispo County, was verified. In the pine belt, above 4,000 feet, anophelines are very rarely collected. In open sagebrush-juniper areas, however, *franciscanus* was found in receding streams up to 5,200 feet.

Table 1.—Summary of *Anopheles* Larval Collections, North-Central California, 1964-67.

Area	Species	Number stations species occurred	Relative frequency of species collection
East of Sacramento River (72 Stations)	*franciscanus*	21	52
	freeborni	53	98
	occidentalis	0	0
	punctipennis	34	83
West of Sacramento River (38 Stations)	*franciscanus*	27	51
	freeborni	13	15
	occidentalis	0	0
	punctipennis	12	55
Coastal watershed (109 Stations)	*franciscanus*	51	88
	freeborni	13	31
	occidentalis	41	104
	punctipennis	53	123
TOTALS (219 Stations)	*franciscanus*	99	191
	freeborni	79	144
	occidentalis	41	104
	punctipennis	99	261

We have recorded previously the finding of an "overlap area" near Napa where *occidentalis* and *freeborni* were present. Near and southwest of Healdsburg in grassy side pools along the Russian River during 1965-67 we found these species. *A. freeborni* was collected at Monte Rio five airline miles from the ocean and *occidentalis* was obtained five miles south of Healdsburg, which is the greatest distance inland for this anopheline. During World War II, in April, 1944, the senior author collected and reared *freeborni* taken from a grassy fresh water seepage pool in the former U. S. Marine Base dairy barn at Mare Island Navy Yard. This site was not along the ocean proper, but was less than 100 yards from San Pablo Bay.

No new or unusual records of *punctipennis* were gathered. As this species is very specific in its habitat, it is nearly always possible to anticipate the finding of the larvae by recognizing the proper ecological conditions. These niches, however, are entirely absent in such areas as desert regions, alkaline marshes, polluted farm ponds, and snow fields. Even when what appears to be an ideal environment is found, at moderate to high elevations in the Sierra Nevada Mountains none have been collected by us. Carpenter (1969) obtained *freeborni* at 6,000 feet in Sierra County. Aitken's record of *franciscanus* in Alpine County (7,300 feet) still remains unique.

SEASONAL INCIDENCE.—The relative abundance of anophelines varies from year to year and locality to locality. Several important factors, including the total rainfall and its distribution, the spring and summer maximum and mean temperatures, and the type and acreage of irrigated crops and economic conditions directly or indirectly influence seasonal fluctuations. Our observations in two contrasting ecological areas in northern California, the coastal belt and the Sierra foothills, clearly illustrate a predictable pattern of appearance and abundance of the four anophelines.

These stations were not sampled regularly each season, therefore, direct comparison of the population densities of the anophelines in the same year is not available.

On the coast at Pt. Reyes Station, 22 larval collections were made between January 7 and December 30 of 1966. The data gathered from an established farm pond, a ditch and a small swamp were as follows:

	Seasonal Range of Larvae	Total No. Collected
A. franciscanus	Jun. 21–Oct. 29	185
A. occidentalis	Mar. 22–Nov. 12	1,530
A. punctipennis	Mar. 22–Nov. 12	364

Table 2.—Summary of abundance and seasonal feeding activity of *Anophelines* in resting stations at Pt. Reyes Station, Calif.

MONTH	*franciscanus*		*occidentalis*		*punctipennis*	
	no. females collected	percent blood-engorged or gravid	no. females collected	percent blood-engorged or gravid	no. females collected	percent blood-engorged or gravid
1966						
April	0		1	0	10	10
May	0		2	100	3	100
June	1	100	124	37	16	81
July	2	50	96	91	15	40
August	3	100	126	69	1	0
September	1	100	82	51	7	14
October	2	0	52	26	16	6
November	1	0	23	0	17	0
December	0		45	9	36	0
1967						
January	0		14	29	9	22
February	0		1	100	5	0
March	0		0		0	
TOTALS	10	0-100	566	0-100	135	0-100

From the same locations, in abandoned buildings and a culvert, 23 adult collections were taken over a two-year period, 1966-67 (Table 2). During the summer in this cool coastal climate *franciscanus* persists only marginally, *punctipennis* is common and widespread, *freeborni* does not occur and, as is obvious by the above data, *occidentalis* is the most abundant species. As *punctipennis* does not congregate in protected stations until the colder, rainy season begins, this species actually may be more numerous before this time.

During 1965, 19 visits were made to the foothill study area, Wolf Creek, from March 30 to December 16. A tabular summary of larvae from a sunlit, established farm pond (1,314 feet elevation) and its shaded seepage ditch is given below.

	Seasonal Range of Larvae	Total No. Collected
A. franciscanus	Jul. 1—Nov. 19	518
A. freeborni	Mar. 30—Nov. 19	299
A. punctipennis	Mar. 30—Dec. 2	1,393

Samples of adult anophelines taken from farm buildings in this area during 1965, and less frequently 1966-67, are shown in Table 3. These data illustrate two generalizations. First, in a brushy, rocky hill area the innumerable resting sites result in dispersal of the adults. Thus, manmade structures under these conditions give much lower adult counts in relation to the larval density. Secondly, in this area, which is a preferred breeding area for *punctipennis* (note larval density), the adults did not congregate in buildings but were scattered in shady thickets adjacent to the pond.

The above findings contrast with results of our collections from a barn near a long established local breeding area west of Vacaville. The latter station served as an ideal resting site of *punctipennis*. No breeding of *freeborni* occurred in the area.

It is interesting to compare the figures obtained by Herms (1919) in California's first mosquito survey in 1916-17. In the Sierra counties he found that *freeborni* comprised 16%, *punctipennis* 67%, and *franciscanus* 17%. In the Sacramento Valley counties his collections totaled 82% *freeborni*, 7% *punctipennis*, and 11% *franciscanus*. Our data on anophelines sampled from the Nevada County study area in 1965-66 produced strikingly similar figures; 14% *freeborni*, 63% *punctipennis*, and 23% *franciscanus*. In the Capay Valley on the west side of the Sacramento Valley proper (Table 4) our 1964-65 adult collections, in sufficiently large numbers to give good reliability, gave 92% *freeborni*, 6% *franciscanus*, and 2% *punctipennis*. Conditions in the farming areas have changed much more drastically in the past 50 years than those in the foothills. Nevertheless, the comparison demonstrates that the reproductive potential of these species has changed little in undisturbed areas.

Other series of collections show a different relationship. In the course of extensive flight range studies of *Culex tarsalis* (Bailey et al. 1965), CO_2 traps were employed in the Sacramento Valley, primarily in rice fields. These traps, in 37 experiments, caught 475,408 *tarsalis*, 18,502 *freeborni* (including one male), three *franciscanus* and no *punctipennis*. Anophelines are not attracted readily to this type of trap, as are *Culex*, but the numbers captured are an excellent index of the relative number in flight in a major agricultural area.

HOST PREFERENCE AND BITING ACTIVITY.—In most cases, the proximity, availability, and relative abundance of several acceptable warm-blooded hosts appear to determine the so-called host-preference. Mosquito blood-feeding patterns in California have been investigated by Reeves, Tempelis and Washino (1964, 1967). They found that man is not a common host. Rabbits, horses and cows rank at the top of the list of mammals, with other domestic mammals as well

Table 3.—Adult Anopheline collections and seasonal feeding activity in resting stations at Wolf Creek Ranch, Nevada County, California. 1965-67.

	franciscanus		*freeborni*		*punctipennis*	
	number females	percent blood-engorged	number females	percent blood-engorged	number females	percent blood-engorged
1965						
May 4	0		0		0	
18	0		0		0	
June 3	0		0		0	
15	0		0		0	
July 1	1	0	2	100	4	100
15	5	33	0		1	0
27	11	33	0		0	
Aug. 10	13	100[a]	1	100	1	100
24	22	63	5	66	1	0
Sept. 9	12	0	0		4	100
21	14	29	1	0	0	
Oct. 5	12	0	3	67	0	
19	5	100	25	13	4	0
Nov. 4	4	0	24	55	5	0
19	2	0	lost		13	0
Dec. 2	4	0	55	0	13	0
1966						
Jan. 13	0	46	46	0	7	0
Feb. 10	0	23	23	9	2	0
Apr. 28	0	6	6	100	5	80
May 26	1	5	5	100[a]	0	
Oct. 26	10	58	58	19	10	0
Nov. 16	6	58	58	7	4	0
30	3	73	73	1	10	0
Dec. 14	0	65	65	0	7	0
1967						
Jan. 16	0	41	41	15	5	0
Nov. 8	0	8	8	100	5	100
TOTALS(males)	125(70)	0-100	499(10)	0-100	101(6)	0-100

[a]Includes gravid specimens.

as rodents making up the total tested, chiefly by the precipitin method.

In the field we have been bitten by all species with the exception of *occidentalis:* this anopheline readily feeds upon man under laboratory conditions. *A. freeborni* and *punctipennis* will bite during daylight hours, and the former is the only one to invade residences and bite indoors. *A. punctipennis* will seek blood in open sheds, barns and at the mouths of caves or old mine shafts. Along the coastal region, where three species of anophelines occur commonly, many residents were interviewed and none complained of mosquitoes (the salt marsh *Aedes* excepted).

Certain observations can be made from blood-feeding data in our samples (Tables 2-4). November and December normally are the months of minimal feeding, and no *punctipennis* with blood were seen during this period. During the winter in the coastal area, blood-engorgement was most consistent in *occidentalis. A. occidentalis* also showed egg development every month except April and November. Unfortunately the very sparse population of *franciscanus* found on the coast makes it difficult to draw a comparison with populations of the species in the other study areas. On the west side of the Sacramento Valley, blood-engorged *franciscanus* specimens were collected each month. Resting stations with a high percentage of immigrant adult *freeborni* (Capay Valley) exhibited a much lower ratio of blood-fed specimens than those harboring a strictly local population (Wolf Creek). Because of its aggressive and migratory habits, *freeborni* is the only anopheline in California considered as a pest mosquito.

The fall transition in *freeborni* feeding behavior is very dramatic. For example, in warm weather on September 28, 1965 near east Nicolaus, Sutter County, in the heart of a

Table 4.–Adult Anopheline collections and seasonal feeding activity in resting stations at Capay Valley, Yolo County, California[a], 1964-65.

	franciscanus		*freeborni*		*punctipennis*	
date	percent number females	percent blood-engorged or gravid	number females	percent blood-engorged or gravid	number females	percent blood-engorged or gravid
1964						
May 28	1	100	0		1	0
June 8	0		0		6	0
23	4	75	0		1	0
July 16	3	66	0		1	0
29	24	62	1	0	0	
Aug. 11	31	75	0		4	0
25	12	81	46	35	0	
Sept. 1	1		147	9	0	
11	9	20	485	1	0	
15	12	33	520	2	0	
22	14	40	615	1	0	
29	24	38	837	4	1	0
Oct. 6	28	65	404	15	3	66
13	11	57	269	9	2	0
20	12	55	186	6	1	0
27	55	46	306	8	3	0
Nov. 3	34	28	413	6	16	0
10	28	12	310	9	26	0
17	18	0	213	2	15	0
24	10	0	2 155	1	6	0
Dec. 1	19	0	181	4	18	0
8	13	22	133	3	9	0
15	20	20	154	8	13	0
29	11	0	234	8	14	0
1965						
Jan. 5	9	22	68	6	5	0
12	7	0	91	4	5	0
19	10	0	114	15	3	0
26	8	14	72	6	2	0
Feb. 4	5	0	59	9	6	0
11	4	25	60	18	4	25
18	4	50	63	38	2	50
25	2	50	40	80	1	100
Mar. 4	2	0	8	50	2	100
11	3	66	7	43	0	
18	2	50	0		0	
TOTALS	450	0-100	6,191	0-80	170	0-100

[a]Five to 19 stations were collected each date, depending on accessibility. Data tabulated from stations collected ten or more times during study period.

rice growing area, 3,000 *freeborni* were collected from under a small bridge. These mosquitoes did not attempt to feed upon us in the field, and only 90 (3 percent) took a blood-meal in the laboratory. In the fall of each year large numbers of *freeborni* congregate under an abandoned bridge over the Sutter Bypass, in the center of the Sacramento Valley on the southwest side of the Marysville Buttes. During late September, October and early November we have estimated over 100,000 adults resting at one time under this structure, and of the many thousands collected only a very small number attempted to take blood. It is interesting to note that no other anophelines were seen here.

FLIGHT HABITS.–During the breeding season anophelines appear to disperse very short distances from the emergence

sites. If hosts are in close proximity, the flight range need be but a few hundred yards or less. As the winter season approaches, preceded by a physiological interruption in egg production, the flight range of females is extended. At this time sheltered niches offering greater protection than the summer resting are sought. *A. punctipennis* and *occidentalis* congregate in shelters adjacent to the breeding locales, while *franciscanus* disperses into shelters often a mile from known summer larvae sites. The migratory species, *freeborni*, is the only anopheline that can be found in significant numbers remote from breeding sites. Flights into these winter hibernating areas, such as Capay Valley (Table 4) and the foothills bordering the Sacramento Valley (Table 5), can be observed in the same stations from year to year.

A. freeborni was studied in detail by us for four years (Bailey and Baerg 1967). We conducted 18 release-recapture experiments with marked mosquitoes totaling 54,800 wild-caught specimens with a 0.032% recovery. Following the releases, 89,685 unmarked and 328 marked specimens were collected at preselected stations. Some of the most important findings were as follows:

Table 5.–Distribution of Anophelines in relation to altitude in North-Central California 1963-64.

A. State Highway 20, West. Colusa-Lake Co. 10/1 - 3/19.

Elevation (feet)	Number adult females in resting stations.		
	franciscanus	*freeborni*	*punctipennis*
335	37[a]	1,187[a]	0
380	18	369[a]	0
640	22	108	0
790	9	57	0
817	3	24	0
1,016	34	335	1
1,055	1	5	0
1,135	20	29	0
1,725	307	35	0
TOTALS	451	2,149	1

B. State Highway 20, East. Yuba-Nevada Co. 9/11 - 1/30.

268	2[a]	327[a]	11
297	0	623[a]	8
395	3[a]	466[a]	13
683	0	220	7
1,300	0	16	32
1,451	0	56	12
2,040	0	1	3
2,640	0	2	0
TOTALS	5	1,711	86

[a]Includes male specimens (limited numbers observed and collected only in early fall).

"Flights as far as 17.5 miles do occur on the part of some individuals; however, we feel the great majority of mosquitoes seek, and do find adequate overwintering quarters within five miles of their breeding site in most farming areas."

The mean distance from the release point for 18 recaptured marked mosquitoes was 3.05 miles. Large numbers of marked mosquitoes (310) did not leave the release site or its immediate vicinity.

The majority of marked specimens were recaptured in the rice growing area during October; at the release sites only during cold weather in December, and again in much smaller numbers after the middle of January during the spring movement.

Movement of the "prehibernation" population into the foothills at the margins of the valley definitely takes place up to at least ten miles, and abundantly up to 400 feet elevation (see Table 5).

During the winter there is a continual "relocation" of the semidormant females, with the exception of periods when the temperature is below 45°F.

The female of this mosquito (*freeborni*) commonly lives five months in the winter and occupies many different resting sites, often many miles from its point of origin.

OVERWINTERING.–In recent years a great deal of laboratory experimental work has been conducted on photoperiodicity in relation to the physiological cycles and dormancy in insects. Beck (1968), and more recently Washino (1970),

Table 6.–Life span of caged, hibernating female *Anopheles* in natural resting stations November-March, 1966-67. (Percent surviving, with sugar and water).

Holding period (weeks)	*franciscanus* 80 females	*freeborni* 80 females	*occidentalis*[a] 25 females	*punctipennis* 80 females
1	95	69		89
2	74	47	100	85
3	41	34		81
4	22	19	96	72
5	6	12		60
6	4	10	56	45
7	4	7		31
8	1	6		20
9	1	5	24	12
10	1	2		9
11	0	2	12	7
12		2		6
13		2	12	6
14		2		6
15		2	0	6
16		2		5
17		2		1
18		2		0
19		1		
20		0		

[a]Laboratory-reared and tested in Point Reyes Station, October, 1967. Minimum temperature approximately 33°F. Other three species tested in Davis at a temperature range of 38-62°F.

have reviewed the highlights of the subject, the latter particularly in relation to mosquitoes.

Several types of conditions appear to affect (or determine) the nature and composition of overwintering populations of the California anopheline mosquitoes. The first is characterized by *punctipennis*, in which the adults usually originate from very localized and somewhat isolated breeding females, therefore, are rather uniformly aged individuals resulting from the normal, late summer generations. In the seasonal study of the farm pond in the Sierra foothills, two periods of abundance were observed for *punctipennis* larvae, with maximum numbers collected on July 15 and October 19. The latter produced adults that entered directly into hibernation.

In a study by Washino and Bailey (1970) it was found that *punctipennis* normally overwintered as inseminated females which did not take blood-meals, and developed fat bodies during September through December. Individual variation may produce occasional non-conformity as evidenced by one gravid female collected October 5, 1964, that laid 290 eggs in the laboratory in the first week, and another collected November 22, 1964, that produced 80 eggs after 11 days.

Weather conditions such as heavy late spring rainfall may be detrimental to the larval population by flushing out stream pools. Under these conditions diapausing females must arise from a population made up largely of individuals hatching from the early fall brood.

A mixed population of *occidentalis* occurred in the fall of 1967. In the other years, if the seasonal larval peak is well defined and is reached in mid-summer a high percentage of the fall adult population does not enter diapause but continues to reproduce. With the exception of 1967, blood-engorged or gravid *occidentalis* females were found from May through February.

A. freeborni are distributed throughout a wide range in latitude from Boundry County, Idaho (45.5°N. lat.) to El Paso, Texas (32°N. lat.). In the southeastern segment, cold winters undoubtedly have a great influence on the cessation of its egg development. However, this species is markedly influenced by the shortened daylight hours of late summer (Depner and Harwood 1966). Each year the majority of *freeborni* females comprising the overwintering population exhibit gonotrophic dissociation in California (Washino 1970). The artificial breeding area of thousands of acres of rice fields in the Sacramento Valley is generally infested with *freeborni*. Bailey and Gieke (1968) and others have shown that a peak of larval density is reached in late August through early September. The fields are drained abruptly, usually beginning early in September and continuing into October. On September 12, 1967, at Grimes (Colusa County) 93% of the larvae collected from two rice fields and a seepage ditch were first and second instars, suggesting that large numbers of mosquitoes might be produced during short daylight hours under special circumstances, i.e., in extensive and undisturbed ponded areas and late maturing rice fields (see also Sherman and Kramer 1970). During drainage of rice fields the larvae are flushed out into ditches and sloughs or are stranded in the muddy fields before completing development. However, many do survive, and the resulting adults are of nearly the same age.

The sampling of *freeborni* in the Capay Valley, Yolo County, showed blood-engorged females comprise anywhere from less than 1 to 15% of the population during the period of fall movement. In contrast, an isolated breeding population in the Sierra foothill pond produced 15 to 55% blood-engorged females from October 5 to November 4 in adjacent resting stations. There were three *freeborni* larval abundance peaks in this pond during the breeding season; June 3, July 27, and September 21. These examples illustrate the tendency in the species for some individuals to take blood during the fall (the extent depending on local conditions) and to physiologically divert the acquired energy away from egg development.

Only a very small percentage of the many thousands of wild *freeborni* adults collected in the winter months laid eggs within a few days after being brought into the laboratory. Many individuals developed eggs 4-10 days after taking a blood-meal, but withheld them until induced to oviposit by removal of their wings. We find a specific example in our notes of a gravid female taken about ten miles west of Davis on January 1, 1965 that laid immediately when brought into the laboratory. These experiences, obtained as a by-product of handling large numbers of wild adult *freeborni* (150,000), are cited to support the conclusions of Washino (1970) who stated "the overwhelming majority of females in September that enter diapause were nulliparous. . . No further ovary development was observed until diapause was terminated . . . there was no evidence that any single factor was responsible for termination of diapause."

We have found gravid females and early instar larvae of *franciscanus* in December at Mecca, in Riverside County (33.5°N. lat.). Other investigators also have reported this anopheline breeding in some part of California throughout the year, indicating that overwintering specimens are in a quiescent state rather than a true diapause. Washino and Bailey (1970) stated that blood-engorged *franciscanus*, in a few instances only, do not develop eggs in the fall and early winter in the Davis area. In the northern portion of the state breeding as well as feeding in this species normally ceases in cold weather.

In the Sierra foothills and along the central coastline, *franciscanus* larvae are the last of the anophelines to appear in the spring. In 1965 in Nevada County the first larvae were found July 1 and in 1966 at Pt. Reyes Station, on June 21. The larval populations increased to a maximum density in late August and persisted at a high level well into September; September 21 at the first above mentioned site and August 30 at the coastal site. Since there usually are not distinct broods, as in *punctipennis*, adults of various ages and physiological condition probably enter the winter quiescent phase with the onset of cold weather. There is a limited surviving adult population to initiate breeding in the spring. The cool water is less favorable to larval development of *franciscanus* than for other California anophelines.

The foregoing data illustrate some of the difficulties involved in attempting to categorize the types of dormancy exhibited by these species. While the unique record of a "last" larva in the fall or a "first" in the spring is not significant in itself, it is an indication that some individuals of a species population have an inherent capacity to be active and to survive extreme conditions within its range, although at times in precariously small numbers.

FACTORS INFLUENCING ADULT AND LARVAL POPULATIONS.–Temperature, the extremes and the average maximum and minimum, can limit the distribution as well as

seasonal abundance of virtually all mosquitoes. One anopheline, *punctipennis*, is widespread in North America, but seeks larval breeding niches providing a cool environment. Many areas in which it occurs normally have a low winter minimum. However, the shelters used for hibernation, whether natural or manmade, would have a somewhat higher temperature than more exposed areas. We have collected females of all four species at readings in the low 30's; in such instances, the mosquitoes were reluctant to fly and often were flattened close to the particular resting surfaces. They all seek locations protected from the wind, thus minimizing desiccation. In the mountainous counties of Plumas, Shasta, Siskiyou, and Trinity, *franciscanus*, *freeborni* and *punctipennis* were collected. These areas have low winter temperatures. Since *occidentalis* is found only along the coast, it is not known whether the adults could withstand ambient temperatures close to freezing.

Limited experimental work has been conducted by us on this environmental factor. At a constant 43°F adult *franciscanus* and *punctipennis* survived five weeks--as indicated by normal activity when they were returned to room temperature. *Freeborni* lived nearly twice as long. Table 6 presents the data obtained on the life span of wild-collected hibernating females (except *occidentalis*, which were laboratory-reared) exposed to normal winter temperatures at low elevations in north-central California. Sugar and water were available to the caged specimens, as in nature the adults are free to move about on warm days and obtain these items necessary for survival. The age composition of the specimens obtained in the field could not be determined. *A. franciscanus* appeared to be the least tolerant of the prolonged (fluctuating) winter temperatures of the Sacramento Valley. This is compatible with its absence in Idaho, Washington, and Oregon (except the southwestern segment) whereas *punctipennis* and *freeborni* occur widely in these states (Stage, Gjullin, and Yates, 1952).

Tolerance of the adults of these species to high temperature also has been viewed in the laboratory (Bailey and Baerg, 1966). In these tests, specimens were not preconditioned (acclimated) before exposure. The purpose of the experiments was to demonstrate that innate differences in susceptibility do exist between species. Under controlled relative humidity conditions, exposure to 110°F for nine minutes produced 100 percent mortality of all laboratory-reared specimens except *franciscanus*. Female *franciscanus* survived up to 14 minutes, while males succumbed in one-half this time. High temperatures (90°F and above) in the field have a depressing effect on the adult population of *freeborni* (Bailey and Gieke, 1968). Also, *punctipennis* becomes scarce in hot summer weather; King, Bradley, Smith and McDuffie (1960) reported similar observations in Louisiana. The coastal species, *occidentalis*, is prevented from invading the valleys opening to the coast by summer isotherms. In cool summers, penetration into the interior is greater but still very limited. Optimum conditions for this species are found at Pt. Reyes Station, having very cool summers. The maximum reading at which *occidentalis* has been collected in a resting site is 88°F. In the hot desert region, *franciscanus* is the only anopheline able to survive. We have collected adults in a culvert at Mecca at greater than 95°F. This species was commonly found in resting sites at the periphery of the Sacramento Valley at 95-97°F. On one occasion only *punctipennis* was collected at 94° and *freeborni* at 101°. These isolated cases are significant primarily in that they indicate the approximate tolerated maxima of individuals before they may attempt to relocate themselves under natural conditions.

In field breeding sites larvae of the four species have been taken at water temperatures in the 91-99°F range. *A. punctipennis* larvae have been found at a low of 44° and the others at approximately 55°. Laboratory experiments demonstrated that at 43°F all *franciscanus* first instar larvae die within four days, whereas *freeborni* and *punctipennis* survive twice as long. This would suggest that in a cold spell in early spring after egg-laying has begun, *franciscanus* could be eliminated more quickly. Nonacclimated laboratory-reared larvae also were tested for tolerance at 108°F. Data gathered indicated *franciscanus* has the greatest and *occidentalis* the least tolerance to this high temperature. In each species, a decreased tolerance was exhibited by the succeeding instars. Similar findings with *freeborni* and other anophelines have been published by Barr (1953).

Strains of *franciscanus* from the far ranges of its latitudinal distribution apparently have a correspondingly greater ability to withstand the respective temperature extremes. This capacity is evidenced by the high density of larvae of this mosquito in algal mats in receding pools in hot creek beds in summer. Seasonal differences in larval tolerance were illustrated in *occidentalis* by a laboratory experiment. Early season first instar larvae suffered 99 percent mortality when exposed five minutes in water with a temperature of 108°F. In sharp contrast, late season larvae exhibited only 17 percent mortality. Fourth instar larvae of *occidentalis* reared at 60° and 80° exhibited a marked increase in ability to withstand the temperature range at which they were reared. Similar tests were not conducted with other species. We have found in other tests, utilizing a thermal gradient tank with water ranging from 38° to 105°F, that larvae of *freeborni* and *punctipennis*, and *occidentalis*, when introduced at the 70-75° area distributed themselves randomly. Individuals moving into the lower range were killed or became moribund as did those reaching the 93-105° area.

Experiments on the effect of water temperature on the growth of *freeborni* larvae (Bailey and Gieke 1968) showed that this mosquito could tolerate fluctuating (natural) winter exposure in the 40-72° range (mean 52.6°), but only a few individuals matured, and after 81-84 days. In the laboratory, at a controlled temperature of 55°, which is near the threshold for completion of development of each stage for all four species, larval growth of *freeborni* was completed in 31-62 days with a 66 percent mortality. The maximum temperature at which *freeborni* immatures can develop successfully is between 90° and 95°, with a mortality of about 90%. These latter studies were made to provide a general tool for determining population development in rice fields. Variations in the mortality of early and late blood larvae to high temperature and other details remain to be investigated.

It is obvious that the late instar larvae can move about quickly and, in a few inches, can find lower surface water temperatures (in shade among plant growth, or debris) in the summer thus avoiding lethal extremes. This perhaps compensates for their decreased tolerances in comparison with the early instars. However, in the late winter an entire population is chilled and individuals are not able to seek a more favorable environment.

In concluding this phase of the subject, namely, the limitations of anophelines to or by temperature, one has to "read in" the factors of inherent resistance and adaptability. Additionally, it must be emphasized that laboratory experiments expose the organism to artificial conditions not found in nature.

Many other factors, both physical and biological, have their influence on mosquito larvae. Some of these are readily measurable and others are quite elusive and difficult to record in the aquatic environment. Since anophelines are surface feeders, water depth apparently is of little direct importance. Larvae can be collected at the margins of reservoirs, lakes, and deep forest pools, regardless of the total water depth. Water movement, however, is quite critical. Undisturbed water is necessary for feeding, pupation and emergence of the adult. The wind-swept shoreline of a pond or lake will have few larvae in contrast to the lee side. Occasionally, larvae in small numbers can be found at the edge of a canal or stream bank among algal growth or patches of grass, but away from this limited protection they are swept away by the water flowing past. Algal mats anchored in a slowly moving stream can support larvae on the surface even if the water flows beneath the algae.

In the distributional study of *freeborni* larvae in rice fields Markos (1950) stated that "variations in the density of the rice plants within normal field limits had no apparent influence on the number of larvae that developed". Research personnel at the Tennessee Valley Authority water impoundment also studied *A. quadrimaculatus* larval density in detail in relation to the type and density of vegetation. Erect, emergent vegetation supported, or rather offered protection, to the heaviest concentrations of larvae. Our observations on *freeborni* and *punctipennis* in particular indicate this condition: reeds, erect grasses, *Typha* sp. and similar plants, (as well as watercress in the case of *punctipennis)* harbor the largest populations. Floating vegetation, particularly a heavy growth of *Lemma*, can exclude mosquito larvae.

As anopheline larvae increase in size they have a tendency to scatter and distribute themselves. When a larva comes in contact with other larvae, it readily moves away from them. Crowding of the larvae rarely was observed in the field. There appears to be some competition, however, for the most favorable environmental conditions in relation to protection, shade, food, and temperature. Our records seldom show more than 10-15 fourth instar larvae taken at one time in the standard type of dipper. Much larger numbers of early instar larvae, especially *franciscanus* (in algal mats), often are collected.

In the laboratory, crowding is a factor that must be considered in maintaining strong colonies and a dependable supply of larvae. In the standard white enameled pans normally employed for larval rearing, about 12 x 7 inches, the concentrations of larvae had to be serially reduced during growth. *A. freeborni* and *punctipennis* are most adversely affected by crowding; 100 fourth instar larvae per pan was about the maximum number that could be reared without nearly constant attention. The tendency of the larvae to cluster in the corners resulted in injury by the chewing of their bristles. The large larvae could not maintain themselves at the surface film without a complement of supporting bristles, and sank to the bottom where they died after a few hours. These larvae also were rendered more susceptible to secondary bacterial infection (particularly *Pseudomonas*). Feeding every few hours reduced "cannabalism" and aided in separating the larvae for longer intervals. Grass clippings, algae, or excelsior in some cases, were used to provide additional feeding surfaces. Up to 300 larvae per pan of *franciscanus* could be reared successfully. While the additional factors of temperature and light were involved, our laboratory investigations have shown the more rapid growth rate and agressive character of *franciscanus* enable this species to clearly assert its dominance when reared in pans with the other California anophelines.

Our collections in northern California have been made in waters varying in pH from 6.4 to 10.3. Not all four California anophelines have been collected in water within this range of hydrogen ion concentration, although preliminary laboratory experiments indicated they can tolerate these extremes. Essentially, the pH of fresh water suitable for anophelines in this area is not of sufficient acidity or alkalinity to prohibit larval development.

Of the three anophelines inhabiting the coastal area, *occidentalis* was collected in the most highly brackish water, i.e., up to 55.7% sea water. Two other species, *punctipennis* and *franciscanus* were found in concentrations of to 20%. The highest salinity in which we have collected *occidentalis* has been 1.95% dissolved salts, indicating it readily tolerates brackish conditions found at the mouths of our small coastal streams.

Culicidologists have made frequent mention of anopheline preference for "clear, fresh, sunlit water". Among the California species, all but *punctipennis* are found in the greatests abundance in a sunny location. We have commented previously upon this phase of larval preferences and its possible over-emphasis (Bailey and Baerg, 1966). All four species have been reared in both complete darkness (with the exception of a few minutes each day to service the pans) and continuous light. It should be noted that under these artificial conditions no predators or toxic by-products from a biological complex were allowed to exist or accumulate. This leads to a brief mention of such influences.

In our studies we have not explored nutritional and antibiotic factors, or predators influencing larval populations. Christensen (1968) examined *occidentalis* larvae for protozoan parasites and believed them to be of greater importance than generally realized. Considerable effort to determine the antibiotic factors of blue-green algae in rice fields which appear to inhibit anopheline larval development has been made with little success by Gerhardt (1956) and Washino (1964). Markos (1950) earlier had reviewed this and related topics. In the past few years *Gambusia* "seeding" of rice fields in large quantities appears to offer the best promise of successful manipulation of the larval predators.

CONCLUSIONS.—If the "growth" of the state continues, together with the exploitation of the natural environment, we foresee a continual reduction in the acceptable breeding sites of *occidentalis* along the narrow coastal strip and, on the other hand, an increase in the local populations of *punctipennis* in the foothill areas. Recreational and second home developments in the Sierra and coastal mountain areas from 1,000 to 3,000 feet elevation have impounded water and, depending on particular plant growth and water management, additionally may favor *freeborni*. With present irrigation practices, the aggressive, very adaptable *freeborni* is given almost unlimited opportunity to multiply in rice fields,

ponding from irrigation of many crops, and in weed-choked, shallow canals. Control of this principal pest species (and potential malaria vector) will need to be continued indefinitely. The fourth species, *franciscanus*, undoubtedly will persist innocuously in desert and outlying hill areas, in sunlit shallow puddles and stream pockets, remaining through late summer and fall if waters are not too heavily polluted. It is well known that modern urbanization and malaria do not go forward together. Thus, over a progressively smaller area in the state we should continue to observe the condition known as "anophelism without malaria".

References Cited

Aitken, Thomas H. G. 1945. Studies on the anopheline complex of western America. Univ. of California Publ. in Entomol. 7:273-364, 39 figs. Univ. Calif. Press, Berkeley.

Baerg, David Carl. 1967. Bionomics of the mosquito *Anopheles franciscanus* McCracken in California. Ph.D. Thesis, Univ. Calif. Library, June 132 pp.

Bailey, Stanley F. and David C. Baerg. 1966. Ecological studies of California anophelines – a preliminary report. Proc. Calif. Mosq. Control Assoc. 34:54-59.

Bailey, Stanley F. and David C. Baerg. 1967. The flight habits of *Anopheles freeborni* Aitken. Proc. Calif. Mosq. Control Assoc. 35:55-69.

Bailey, Stanley F., D. A. Eliason and B. L. Hoffmann. 1965. Flight and dispersal of the mosquito *Culex tarsalis* Coquillett in the Sacramento Valley. Hilgardia 37:73-113.

Bailey, Stanley F. and Paul A. Gieke. 1968. A study of the effect of water temperatures on rice field mosquito development. Proc. Calif. Mosq. Control Assoc. 36:53-61.

Barr, A. R. 1952. The thermal death times of the aquatic stages of some American dark-winged anophelines. Amer. J. Hyg. 55: 170-181.

Beck, Stanley D. 1968. Insect photoperiodism. New York Academic Press VIII. 288 pp.

Boyd, Mark F. and J. A. Mulrennan. 1934. The establishment of a cage colony of *Anopheles punctipennis*. Ann. Entomol. Soc. Amer. 27:311-312.

Bradley, G. H. and W. V. King. 1941. Bionomics and ecology of Nearctic anophelines. In Human Malaria, Amer. Assoc. Adv. Sci. Publ. 15:79-87.

Carpenter, Stanley J. 1969. Observations on the distribution and ecology of mountain *Aedes* mosquitoes in California. XII. Other species found in the mountains. Calif. Vector Views 16:28.

Christensen, Howard Anthony. 1968. Biology of *Anopheles occidentalis* Dyar and Knab. Ph.D. Thesis, Univ. Calif. Library, June, 135 pp.

Depner, K. R. and R. F. Harwood. 1966. Photoperiodic responses of two latitudinally diverse groups of *Anopheles freeborni* (Diptera: Culcidae). Ann. Entomol. Soc. Amer. 59:7-11.

Freeborn, Stanley B. 1921. The seasonal history of *Anopheles occidentalis* Dyar and Knab in California. J. Econ. Entomol. 14: 415-421.

Freeborn, Stanley B. 1926. The mosquitoes of California. Univ. Calif. Tech. Bull., Coll. Agr. 3:333-460.

Freeborn, Stanley B. 1949. *Anopheles* of the Nearctic region. (In Boyd). Malariology. Vol. I and II. W. B. Saunders Co., Philadelphia, pp. 382-384.

Freeborn, Stanley B. and R. M. Bohart. 1951. The mosquitoes of California. Bull. Calif. Insect Survey, Univ. Calif. 1:25-78.

Gerhardt, R. W. 1956. Present knowledge concerning the relationship of blue-green algae and mosquitoes in California rice fields. Proc. Calif. Mosq. Control Assoc. 24:53-55.

Herms, William B. 1919. Occurrence of malaria and anopheline mosquitoes in northern California. U.S.P.H.S., Weekly Publ. Health Rpts. 34:1579-1587.

Herms, William B. 1921. Distributional and ecological notes on anopheline mosquitoes in California. J. Econ. Entomol. 14: 410-414.

King, W. V., G. H. Bradley, Carroll N. Smith and W. C. McDuffie. 1960. A handbook of mosquitoes of the southeastern United States. U.S.D.A. Agr. Handbook 173:188.

Loomis, E. C., R. M. Bohart and J. N. Belkin. 1956. Additions to the taxonomy and distribution of California mosquitoes. Calif. Vector Views 3:38-45.

Markos, Basil George. 1950. The ecology of California *Anopheles*. Proc. Calif. Mosq. Control. Assoc. 18:69-73.

Sherman, Eugene J. and Marvin C. Kramer. 1970. A report on the distribution and density of *Anopheles freeborni* larvae and pupae in the Central Valley from September through November. Calif. Vector Views 17:4-6.

Stage, H. H., C. M. Gjullin and W. W. Yates. 1952. Mosquitoes of the northwestern states. U.S.D.A. Agr. Handbook No. 46:95.

Tempelis, C. H. and W. C. Reeves. 1964. Feeding habits of one anopheline and three culicine mosquitoes by the precipitin test. J. Med. Entomol. 1:148-151.

Tempelis, C. H. and R. K. Washino. 1967. Host-feeding patterns of *Culex tarsalis* in the Sacramento Valley, California with notes on other species. J. Med. Entomol. 4:315-318.

Washino, Robert K. 1964. Rice field mosquito ecology project-progress report, 1963. Proc. Calif. Mosq. Control Assoc. 32: 37-39.

Washino, Robert K. 1970. Physiological condition of overwintering female *Anopheles freeborni* in California (Diptera: Culicidae). Ann. Entomol. Soc. Amer. 63:210-216.

Washino, Robert K. and S. F. Bailey. 1970. Overwintering of *Anopheles punctipennis* (Diptera: Culicidae) in California. J. Med. Entomol. 7:95-98.

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