

recommended concentrations with *A. albimanus* and *A. pseudopunctipennis*. These tests are to be repeated every six months and a comparison made of the data collected at each testing to see if the susceptibility of the species that we are working with changes during the time that we are making the study. At present we have only the first readings; other readings will be made at 6-month intervals.

In the tests made to date we found that *A. albimanus* and *A. pseudopunctipennis* were very susceptible to both insecticides to about the same degree; however, *A. albimanus* did show a slight bit more susceptibility to the test materials.

CONCLUSIONS. Tests were made in Mexico to determine the lasting qualities of several formulations of dieldrin and also of DDT when applied as a residual

insecticide in malaria eradication work. It was found from tests made in three areas in Mexico that the insecticides when applied to mud surfaces gave over 300 days of effectiveness using 25 mg. of dieldrin per sq. ft. in two of the three areas and slightly less in the third area. Higher application rates, 50 mg. to 100 mg. of dieldrin per sq. ft., gave a longer lasting period of effectiveness but not in the same ratio as the quantity of insecticide used. Tests are still under way in the area where the red mud gave a shorter period of effectiveness.

Tests made to date using the World Health Organization's kit for testing the susceptibility of mosquitoes to insecticides showed that the *Anopheles* in the areas where we are working are very susceptible to dieldrin and DDT.

A SAMPLING PROCEDURE FOR *CULICOIDES MELLEUS* (COQ.) (DIPTERA: HELEIDAE) WITH OBSERVATIONS ON THE LIFE HISTORIES OF TWO COASTAL *CULICOIDES*¹

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Culicoides melleus (Coq.). The unusual breeding habitat of *Culicoides melleus* (Coq.), sandy beaches along the coastline, was apparently first recorded by Goulding *et al.* (1953) in Florida. On Long Island, N. Y., small numbers of *Culicoides melleus* larvae were collected from samples of intertidal sand examined under a wide-field dissecting microscope. The larval sampling methods described by Dove *et al.* (1932), Carpenter (1951), Kettle and Law-

son (1952) and Wirth (1952) were tried with only limited success.

LARVAL SAMPLING. In the spring of 1957, a more useful method of sampling was developed after observing the behavior of *C. melleus* larvae in the laboratory. Larvae placed in a 50 ml. graduate containing about 10 ml. of sand and 30 ml. of water stayed beneath the surface of the sand. When the graduate was inverted and returned to an upright position the sand settled in a few seconds and the larvae could be seen swimming in the water. A few swam upward for a short time before sinking slowly to the bottom but most swam downward. In less than two minutes almost all of the larvae disappeared again beneath the sand. These

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observations indicated that the larvae were readily dislodged but that they re-enter the sand a short time after being dislodged. After testing several variations in sampling technique, a "standardized" sampling procedure which took these observations into account was developed. This procedure is as follows:

1. One-third pint samples of sand are collected from suspected breeding areas. A jelly glass of one-third pint capacity is a convenient collecting and measuring container.

2. In the laboratory, the sand is placed in a pint jar and an equal amount of tap water is added.

3. The water is decanted into another pint jar and then poured quickly back into the jar containing sand so that it mixes with it. This process is repeated six times.

4. After the sixth time the sand is allowed to settle for 2-3 seconds, then the water is decanted into a jar without sand.

5. A portion of this water, the amount depending on the turbidity, is then poured into a cylindrical jar and held over a light bulb shielded with white bond paper.

6. *C. melleus* larvae, if present, can be seen clearly from above swimming in the water, which is gently swirled every few seconds. The larvae are removed with a medicine dropper. All of the water in the sample is examined in this way.

7. The entire procedure is then repeated with the same sample and the total number of larvae obtained from two subsamplings is recorded.

8. If more than 50 larvae are present in a one-third pint sample it is recorded as 50+; otherwise all of the larvae are counted.

This technique proved to be satisfactory in estimating *C. melleus* larval populations, but is not directly applicable for use in sampling populations of species breeding in muddy habitats where the water becomes too turbid to see the larvae.*

Some of the larvae present in a given sample of sand remained in the sand after mixing with water and therefore were not counted. The proportion of those present

in a given sample which are actually counted was estimated in the following way. Four one-third pint samples of sand were thoroughly dried. They were then flooded and examined to make sure no larvae were present. Next, 100 *C. melleus* larvae were distributed uniformly in each of the samples. The sampling procedure outlined above was followed. The numbers of larvae collected are shown in Table 1.

The data presented in Table 1 indicate that about two-thirds of the larvae in a given sample are collected by the use of the *C. melleus* sampling procedure described above.

DISTRIBUTION OF LARVAE. The number of *C. melleus* larvae collected from intertidal sand in different localities varied greatly, apparently depending on a combination of factors. Within a given area, i.e., along a particular beach, if present, they occurred in a band between high and low tides. Usually they were not present near the high or low tide marks, except for an occasional larva. The width of the band where the larvae were most numerous varied from beach to beach, apparently depending on the slope and tidal range. Along most of the Long Island beaches it was less than 20 feet wide. The more gradual the slope and the higher the tide, the wider the band where larvae occurred. Another important factor seems to be the exposure of the beach to waves. Larvae were usually found in protected bays or inlets, but were scarce or altogether absent along beaches which are exposed to prolonged and heavy wave action.

VERTICAL DISTRIBUTION OF LARVAE. Laboratory observations indicate that the larvae live close to the surface. When large numbers of larvae were placed in

*Bidlingmayer (1957) recently described a somewhat similar technique for sampling larval populations of *C. furens* (Poey). Since the larvae of this species are found in muddy situations, sand is poured over the mud sample. It is then flooded and allowed to stand over night. A high proportion of the larvae migrate into the sand where they can be removed more readily than from mud.

TABLE 1.—The effectiveness of the *C. melleus* sampling procedure.

Replicate	Numbers of Larvae Collected			Number of Larvae Present
	1st Sub-Sample	2nd Sub-Sample	Combined Total	
a	48	20	68	100
b	44	18	62	100
c	51	21	72	100
d	40	19	59	100

jars of sand covered with a little bay water some could be seen, with a binocular dissecting microscope, gliding just beneath the surface of the sand, at a depth of 1 to 3 grains of sand. If they were not disturbed, some would partially emerge from the sand and remain with their bodies perpendicular to the surface for several minutes. Occasionally one could be seen apparently ingesting minute particles. The sweeping action of the epipharynx as they swallowed could be observed readily. When the jar was tapped the larvae quickly disappeared into the sand but after a few seconds they would suddenly reappear.

Four vertical distribution samples were taken to see if the larvae occurred mostly near the surface under natural conditions. Two of the samples were taken in April and two in July. A total of 121 *C. melleus* larvae were collected in these samples. Of these, 105 were found in the top one-half inch, 16 in the next one-half inch and none in the next 3 inches. There appeared to be no difference in the vertical distribution of larvae collected in April and July. All four samples were taken at about low tide during the daylight hours.

LARVAL ABUNDANCE AND GROWTH. *C. melleus* larvae were abundant during the entire sampling period from March through August. Larvae 1.8 mm. in length or longer were found every month from March through August. The average length of larvae (calculated on a monthly basis increased gradually from 2.5 mm. to 3.3 mm. from March to June. In July and August it decreased to 2.2 mm. and 2.7 mm. respectively, due to the

appearance of small larvae. The smallest larvae collected were 1.5 mm. long. These were consistently present in July and August collections but were not found earlier in the season.

PUPAE. In 1957, the first *C. melleus* pupation noted took place in the laboratory. The larva had been collected on April 8 and pupated on May 3. The first pupa found in the field was collected on May 11. Table 2 shows the seasonal changes in relative abundance of *C. melleus* larvae and pupae collected in 1957.

TABLE 2.—The relative abundance of *C. melleus* larvae and pupae collected on Long Island in 1957

Period	Number of Larvae	No. of Pupae per 100 Larvae
March	105	0
April	256	0
May 1-15	759	0.9
May 16-31	232	1.3
June 1-15	667	2.6
June 16-30	1347	6.8
July 1-15	258	2.7
July 16-31	1025	0.7
August	1009	0.3
Total number collected	5658	135

The number of pupae collected increased gradually in May and early June, and reached a sharp peak in late June. Relatively few pupae were found after the first half of July. The sharp peak in numbers of pupae collected in June and the relatively small number of pupae collected before and after this period suggest that *C. melleus* has only one generation per year on Long Island.

The duration of the pupal stage of two *C. melleus* females, reared from larvae, was 5 and 14 days respectively. Adults emerged from 12 pupae collected in the field an average of 6.3 days after they were collected.

ADULTS. *C. melleus* adults are widely distributed on Long Island. They were collected at Asharoken, Hampton Bays, North Sea, Noyac, and Orient Point. The seasonal distribution of annoying adult coastal *Culicoides* on Long Island is discussed in a companion paper (Jamnback, Wall and Collins, 1958).

Culicoides canithorax HOFFMAN. *C. canithorax* is also widely distributed on Long Island. Adults have been collected from Jones Beach, Asharoken, Hampton Bays, North Sea, and Orient Point. Larvae and pupae were collected from salt marsh sod samples dried in Berlese funnels. As with *C. melleus*, larvae and pupae were reared in individual corked vials containing a few drops of bay water and bits of marine algae. The earliest pupation noted occurred in the laboratory. A larva collected on March 26, 1957 pupated on April 23. A male emerged 5 days later. Seven larvae were reared to adults, 6 larvae to pupae, and 4 pupae to adults. The pupal period of the seven adults reared from larvae ranged from 4 to 8 days, with an average of 5.7 days. In addition to these, 18 females and 10 males were recovered from Berlese funnel collecting jars. These presumably hatched from pupae in the sod samples. Larvae, pupae, or adults of this species were recovered from sod samples taken in salt marshes bordering or adjoining Long Island

Sound, Peconic Bay, Moriches Bay, and Shinnecock Bay on Long Island. They were widespread in the salt marshes and were recovered from sod samples which had *Spartina alterniflora*, *Spartina patens*, *Distichlis spicata*, *Scirpus* spp., and *Salicornia* spp. as the dominant plant cover. They appeared to be particularly abundant in wet marsh that was covered by almost every high tide, where the *Spartina alterniflora* grew to a height of two feet or more, and the sod had a thin covering of mud.

It is interesting to note that the breeding habitats of these two salt-water species are mutually exclusive, i.e., *C. melleus* was never found breeding in the salt marsh proper and *C. canithorax* was never found breeding in intertidal sand.

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