SALT MARSH *CULICOIDES* (DIPTERA: CERATOPOGONIDAE): COMPARISON OF LARVAL SAMPLING METHODS[1]

D. L. KLINE, J. C. DUKES AND R. C. AXTELL[2]

Department of Entomology, North Carolina State University, Raleigh, N.C. 27607

ABSTRACT. Five methods for determining the abundance of larvae of *Culicoides furens* (Poey) and *C. hollensis* (Melander and Brues) in salt marsh soil and of *C. melleus* (Coquillett) in intertidal sand were compared. The sieve-flotation method yielded some larvae of all 3 species and was the best method for *C. hollesis*. The sand-flotation method yielded the greatest number of larvae of *C. furens*. Direct flotation, Tulgren funnels and rearing chambers were generally less satisfactory methods.

Recovery of *Culicoides* larvae from the soil in salt marshes is tedious and time-consuming no matter what method is utilized. Several basic methods and variations of each have been used by researchers in the past. Among these methods are: (1) sieve-flotation (Kettle and Lawson 1952, Wirth 1952, Kettle et al. 1956, Jamnback 1965), (2) sand-flotation (Bidlingmayer 1957, Williams 1960), (3) direct-flotation (Ferguson, personal communication, Linley and Kettle 1964, Linley and Adams 1972); (4) Berlese funnels (Jamnback and Wirth 1963; Jamnback 1965); and (5) rearing (Hair et al. 1966, Battle and Turner 1970).

During the summer of 1973 we compared these methods to determine which yielded the greatest number of larvae per standard soil sample, and their advantages and disadvantages in the recovery of the North Carolina salt marsh species *C. furens* (Poey) and *C. hollensis* (Melander and Brues), as well as the intertidal sand species, *C. melleus* (Coquillett).

METHODS AND MATERIALS

Soil samples were collected in Carteret County, North Carolina from (1) a *Spartina* salt marsh located along the Newport River and having a substrate of fine silt, and (2) along the margin of a tidal creek (Hoop Hole Creek) draining into Bogue Sound adjacent to the boundary of Atlantic Beach and having a substrate composed almost entirely of coarse beach sand.

Soil samples for each species were collected from specific areas, which were shown by previous studies to be productive for that species (Kline and Axtell, in manuscript). Samples for *C. hollensis* were taken along a major drainage ditch at the Newport River area. The site was regularly flooded (twice daily) by each high tide and the dominant vegetation was *Spartina alterniflora* Loisel. (smooth cordgrass) averaging 1.2–1.8 meters high. Samples for *C. furens* were taken from an interior portion of this same marsh where the dominant vegetation was the short form of *S. alterniflora* (less than 0.3 m high). Samples for *C. melleus* were taken from non-vegetated intertidal sandy areas at Hoop Hole Creek.

Samples were taken by means of a post hole digger yielding an 0.8 liter soil core (10.2 cm. diam. x 7.6 cm. deep). At each site, 5 replicates were taken side by side. Each replicate was transported to the laboratory in a closed plastic container (16 cm. diam. x 11 cm.). A replicate (chosen randomly) was used for each of the following 5 methods of larval extraction.

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[2] Graduate Research Assistant, Research Associate and Professor, respectively.

(1) SIEVE-FLOTATION. The soil was washed through 2 screens (10- and 40-mesh). Only the residue on the 40-mesh screen was retained and placed into a liter graduated cylinder, which was filled with saturated magnesium sulfate solution. The mixture was thoroughly agitated and larvae collected with an eye dropper or fine forceps as they floated to the surface. When all visible larvae were removed, the mixture was reagitated. Inspection for larvae continued until no larvae were observed for 3 consecutive times.

(2) SAND-FLOTATION. The soil was kept in its field containers, covered with approximately 5 cm of commercially available pre-washed sand, saturated with tap water, and allowed to stand at room temperature (ca. 21° C (70° F)) for either 48, 72 or 96 hours. The sand layer was then removed and placed into a 6-liter bucket. A liter of saturated magnesium sulfate solution was added to the sand, and the mixture thoroughly agitated. Immediately following agitation the liquid was decanted into a white-enameled porcelain pan for examination. Larvae were seen moving on the surface and removed by either an eyedropper or fine forceps. After removal of all visible larvae, the solution was returned to the bucket and the mixture reagitated. This process was repeated until 3 consecutive negative observations were recorded.

(3) DIRECT FLOTATION. The soil was placed into a white-enameled porcelain pan and covered with approximately 2 liters of saturated magnesium sulfate solution. The vegetation and soil were broken apart and thoroughly mixed with the salt solution. *Culicoides* larvae floated to the surface and were visible only by their characteristic sinuous movements. Larvae were removed from the mixture by an eyedropper or fine forceps. Inspection for larvae continued for at least 15 minutes even if no larvae were observed in the sample.

(4) TULGREN FUNNEL. The soil was placed directly into a Tulgren funnel and spread evenly into a layer approximately 2.5 cm thick. The sample was covered with a metal lamp shade and heated by a 60-watt electric light bulb for a period of 48 hours. The larvae were driven from the sample down through the funnel and into water in a 0.5 liter plastic container. After remaining under the heat source for the designated time period, the container was removed and the larvae counted.

(5) REARING CHAMBER. The soil was placed into a rearing chamber constructed from a cylindrical plastic container 16.4 cm diameter and 11.3 cm deep. A 7.6 cm diameter hole was cut in the center of a tight-fitting lid and an inverted 10.2 cm diameter funnel was glued over the hole. The outside of the chamber was painted black to prevent the entrance of all light except through the small opening in the funnel. A hole was cut in the lid of a clear plastic vial and the vial inserted over the stem of the inverted funnel. Emerging adult *Culicoides* were attracted to the light and were collected in the plastic vial. The chambers were held at room temperature inside the laboratory for at least 2 months.

RESULTS AND DISCUSSION

Analyses of variance showed that there were significant differences among the methods in the number of larvae of *C. furens* and *C. hollensis* recovered. Too few larvae of *C. melleus* were recovered to warrant analysis. For each species a different method yielded the greatest number of larvae (Table 1). For *C. furens* the sand flotation method produced nearly 2.5 times more larvae than did the next best methods (rearing chambers and direct flotation). *C. hollensis* was most successfully recovered by the sieve-flotation method, which yielded 5 times more larvae than the second best method (direct flotation). Although few *C. melleus* were recovered, sieve-flotation and direct flotation appeared to be better than the other methods.

The sieve-flotation method was the least time-consuming and produced immediate

Table 1. Comparison of 5 methods for determining the abundance of *Culicoides* larvae in soil samples.

Method	Mean no. larvae recovered per sample[a]		
	furens	*hollensis*	*melleus*[b]
Sieve-flotation	7.0[b, c]	25.9[a]	3.6
Sand-flotation	26.5[a]	1.6[b, c]	0.8
Direct flotation	10.4[b]	5.3[b]	2.8
Tulgren funnel	0.1[c]	1.1[b, c]	0.04
Rearing Chamber	10.4[b]	0.6[c]	0.2
Total No. Samples Per Method	25	39	26

[a] Within each species, means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

[b] Numbers of samples with larvae and no. of larvae were too few to warrant analysis of variance.

results. The average sample required from 5–10 min. to wash through the sieves and recovery of larvae with saturated magnesium sulfate solution required an additional 10–30 min. Thus, results could be obtained within an hour after collecting a soil sample. This method yielded the greatest number of *C. hollensis* and the other species were also recovered. This method may have been less successful for *C. furens* due to the large amount of vegetative matter in the samples from areas in which this species breeds, i.e. during washing some larvae were probably retained with the vegetation on the 10-mesh screen.

The sand-flotation method required 48–96 hrs for the larvae to migrate from the soil up into the sand. Flotation of the larvae out of the sand was rapid and, due to the use of prewashed sand, the larvae were readily seen in the clean salt solution. The time required for larval removal was 10–30 min. The sand-flotation method was best for *C. furens* but very poor for the other species.

Direct flotation yielded moderate numbers of larvae of all 3 species and, like the sieve-flotation method, gave rapid results. However, it was very difficult to see the floating larvae after the saturated salt solution was mixed with the mud. Up to 3 hours were needed to remove all the larvae when they were abundant in a sample.

The Tulgren funnel method was the least successful method overall. Occasional larvae were recovered but not enough to make the method useful. Perhaps different Tulgren funnels could be devised that would adequately recover these species of *Culicoides*.

The rearing chambers had advantages in that they required very little labor and they yielded adult *Culicoides*, making identifications easier. However, this method requires excessive laboratory space, if a large number of samples are processed, and about 2 months is required for emergence of all the adults.

CONCLUSIONS

The method chosen by the individual researcher will depend upon his objectives. If large numbers of samples need to be processed and data on all 3 species are desired in a short time, the sieve-flotation method would be best. If maximum yield of larvae is desired, then the sand-flotation method should be used for *C. furens* and the sieve-flotation method for *C. hollensis*. If the only objective is to determine what species are present, rearing chambers may be advantageous since adults are more easily identified to species than are larvae.

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