A STANDARDISED FLOTATION METHOD FOR SEPARATING *Leptoconops* (Diptera:Ceratopogonidae) AND OTHER LARVAE FROM SAND SAMPLES

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The following method was developed for separating *Leptoconops bequaerti* Kieffer larvae and pupae from sand samples. The method gives results sufficiently consistent for use in quantitative larval population studies. It is also suitable for recovery of other sand dwelling larvae and pupae.

A cylinder, about 22 inches long and 4 inches in diameter is required, open at one end. Such a cylinder can be constructed from 4 one-quart motor oil tins (tops and bottoms removed) soldered together, but plastic, metal, or asbestos piping would serve equally well. The cylinder was used in an upright position and ¾ filled with magnesium sulphate solution (specific gravity 1.15—1.20). The level of flotation fluid in the cylinder should in fact be regulated according to the amount of sand to be processed, but it is essential that sufficient volume be left to accommodate fluid displaced on addition of the sample.

The sand sample can be added either by sprinkling gradually into the cylinder, or by gentle washing from a tablespoon moved slowly to and fro just beneath the fluid surface. Whatever method is used, addition of large clumps of sand should be avoided. An entire sample can usually be added in about 5 minutes, but the process should not be rushed.

When the sand is finished, the liquid level may be topped up, if necessary, with fresh solution, till almost level with the top of the cylinder. It should then be left for 5–10 minutes to allow organic material to reach the surface.

To recover immature stages, the fluid around the meniscus is drawn off into a petri dish with a coarse pipette. Special attention should be given to this task as most of the larvae and pupae are found around the tin sides. In addition, a larger quantity of surface liquid is taken by making 3 or 4 careful scoops with a tablespoon. Pipetting and scooping are repeated 3 times for each sample so that 3 petri dishes are examined, ensuring that all larvae and pupae are collected. Third and fourth instar larvae and pupae are readily seen in the petri dish without optical assistance and can be removed with a small hook. For younger stages a systematic search of the fluid surface is necessary, with the aid of a stereoscopic microscope.

For population studies and work on larval development, the method was standardised by mixing known numbers of each stage with about ½ quart of white sand, and processing this as usual. Results of the replicated tests are shown in Table 1.

As would be expected, greater efficiency was observed with the larger immature stages. The method performed well with second, third and fourth instar larvae and pupae, and, as reflected in the standard errors, consistency was obtained between replicate tests, though the value obtained with second instar individuals should be treated with caution since only three tests were completed. The same is true for the results with first instar specimens, where it is seen that extraction efficiency was markedly reduced. A more satisfactory series of tests could be carried out with readily available eggs (Linley, 1965), but hardly surprisingly, scores were low and as indicated by the higher standard error value, less consistency was obtained between replicates. Third and fourth instar larvae were not tested separately because of difficulty in distinguishing between the two in live specimens (hence reliable test groups could not be prepared), and in any case it seemed unlikely that the efficiency of the method would differ significantly between the two.

In using this method, it would be advisable to carry out efficiency tests using the particular material involved in the proposed investigation. Percentage recovery of the different stages might well depend to a considerable extent on the type and texture of the sand used. Table 1 is therefore set out primarily for illustrative purposes. Once suitable tests have been made, the correction factors can be applied to the observed numbers in each stage to give estimates of the actual numbers present in the original sample.

Reference

LINLEY, J. R. 1965. Techniques for obtaining viable eggs of *Leptoconops bequaerti* Kieffer, *Culicoides furens* Poey, and *Culicoides barbosai* Wirth and Blanton, (Diptera:Ceratopogonidae). Mosquito News 25(4):452–456.

TABLE 1.—Efficiency of extraction method in recovering immature stages of *L. bequaerti* from sand samples. Correction factor=no. mixed in/no. recovered.

Stage	No. of replicates	No. mixed in	No. recovered	Mean % recovered. and st. error	Correction factor
Eggs	12	1121	201	17.9±2.8	x 5.59
1st. instar larvae	3	170	50	29.4±0.6	x 3.40
2nd. instar larvae	3	160	115	71.9±2.0	x 1.39
3rd. and 4th. instar larvae	6	510	485	95.0±1.0	x 1.05
Pupae	1	41	35	85.4	x 1.17