A REVIEW OF METHODS FOR RECOVERING BITING MIDGE LARVAE (DIPTERA: CERATOPOGONIDAE) FROM SUBSTRATE SAMPLES

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ABSTRACT

Ten methods for sampling larvae of biting midges are reviewed: sieving; salt-flotation; sieving and salt-flotation combined; sugar-flotation; Berlese funnel-extraction; light-extraction; sand-extraction; agar-extraction; decanting; and carbon dioxide-flotation. In general, agar-extraction and salt-flotation are the most effective methods for collecting larvae. Sodium chloride used in flotation results in faster extraction of the larvae but causes higher mortality of first and second instars; magnesium sulfate results in slower collection of larvae but fewer deaths of younger instars. Sieving, sieving plus salt-flotation, and Berlese funnels can be used successfully with certain substrates, such as rotting leaves, aquatic plants, mosses, and tree-hole material. Sugar-flotation provides results similar to salt-flotation with the advantage of less larval mortality. Sand-extraction, light-extraction, and decanting have produced less satisfactory results. Carbon dioxide-flotation is a promising technique which should be explored further.

Key Words: Ceratopogonidae, Diptera, larvae, immature insects, collecting techniques.

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The biting midges, family Ceratopogonidae, are a widespread and taxonomically diverse group of flies. They are virtually worldwide in distribution (Wirth et al. 1974), with 36 genera and over 500 species described from North America north of Mexico (Wilkening et al. 1985). The Ceratopogonidae are of interest due to their medical importance as disease vectors (Kettle 1962, Linley et al. 1983) and their ecological importance in aquatic communities (Cushman et al. 1986).

The majority of the research conducted on this family has been directed toward the adult stage. There is a critical need, however, for studies of the taxonomy and biology of the immature stages. Larvae of the majority of ceratopogonid species remain unknown. It has long been recognized that the systematics of this family will be fully understood only when characters of all life stages are examined (Saunders 1956, Ewen and Saunders 1958, Kettle and Elson 1975, Glukhova 1977).

Ceratopogonid larvae inhabit a diversity of aquatic and semiaquatic habitats (Mullen and Hribar 1988). Recovering larvae from these habitats has been a challenge to biologists for many years. Several techniques for collecting larvae of the ceratopogonid genus Culicoides have been discussed in short reviews by Kline et al. (1975) and Jones (1978). The following ten methods for sampling larvae of biting midges have been developed: sieving; salt-flotation; sieving and salt-flotation


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combined; sugar-flotation; Berlese funnel-extraction; light-extraction; sand-extraction; agar-extraction; decanting; and carbon dioxide-flotation.

Sieving

Sieving can be a very productive technique for recovering biting midge larvae. This method is most successful when a series of graduated mesh sizes is used. The substrate is placed in the topmost sieve and washed through with water. The sieves retain extraneous matter while the larvae are collected in the bottommost sieve, if the mesh is fine enough, or in a pan. Larvae of the following ceratopogonid taxa have been reported collected by sieves: several species of Culicoides (Jamnback and Wirth 1963); C. furensoides from Sphagnum moss (Cochrane 1973); C. sanguisuga (Kwan and Morrison 1974); C. furens, C. hollensis, and C. melius (Kline et al. 1975); unidentified Ceratopogonidae from Eurasian watermilfoil, Myriophyllum spicatum (Pardue and Webb 1985); Alluaudomyia, Culicoides, and Dasyhelea spp. from rotting leaves, decaying wood, and tree-hole material (L. J. H., unpublished data).

Salt-flotation

The salt-flotation method is a relatively easy and productive means for recovering ceratopogonid larvae. The substrate sample is immersed in a saturated aqueous salt solution. Because of the difference between the specific gravities of the larvae and the solution, the larvae float to the top where they can be collected with a pipette or a curved needle. Magnesium sulfate and sodium chloride have both been used for collecting midge larvae.

Ladell (1936) used magnesium sulfate to collect terrestrial arthropods from soil samples, the first reported use of salt solutions to collect insects from substrate samples. His technique included bubbling air through the salt solution, to help dislodge the insects from the soil. Several investigators have used magnesium sulfate-flotation to recover ceratopogonid larvae: various larvae (Williams 1960); several Culicoides spp. (Jamnback and Wirth 1963, Chaker 1982); and C. variipennis (Mullens and Rutz 1983, Mullens and Rodriguez 1984, 1988). Davies and Linley (1966) used a unique modification of this method to collect larvae of Leptoconops spp. By their method, the substrate was gently sprinkled into the salt solution, and the larvae floated up to the surface where they were recovered from the meniscus.

Rees and Winget (1979) and Rees et al. (1971) used saturated solutions of sodium chloride to collect larvae of the Leptoconops kerteszii complex. Boreham's (1981) salt-flotation method utilized an inverted funnel. After the substrate was placed in a container and mixed with the saturated salt solution, a funnel was inverted over the substrate and then filled with more salt solution. Water of lower salinity was added to the container to cover the tip of the funnel to a depth of three centimeters. The larvae swam up into the funnel and out into the lower salt concentration and were collected outside the funnel. Boreham (1981) further noted that this method worked better when the apparatus was kept in the dark.

Mullens and Rodriguez (1984) tested the relative efficiencies of magnesium sulfate and sodium chloride for collecting C. variipennis larvae. They found that using sodium chloride resulted in faster collection of third and fourth instars, but that many of the first and second instars do not survive the process. Solutions of magnesium sulfate were less harsh, permitting live recovery of more of the smaller larvae; however, a longer time was required to collect larvae. Although both salts
have been used successfully, sodium chloride may be preferable for economic reasons. Table salt (NaCl) is inexpensive and easily purchased in most places. Larvae of *Alluaudomyia*, *Bezzia*, *Culicoides*, *Palpomyia*, and *Stilobezzia* spp. have been collected from sandy and muddy substrates using salt flotation (L. J. H., unpublished data).

There are some disadvantages to using salt-flotation. The technique is time-consuming. The solution must be stirred several times during the collection process to assure that the salt solution penetrates the sample thoroughly. As long as one-half hour may elapse before the first larvae are collected. The larvae do not simply float to the top of the solution, as many previously-mentioned authors imply. They swim actively, and if one fails to capture them on the first try, they will often swim down into the substrate for some minutes before reappearing at the surface. The larvae must be collected quickly since they do not survive very long in the saturated salt solution. In spite of these drawbacks, salt-flotation is a valuable collecting technique, and live larvae may be available within an hour or two after returning from the field.

**Sieving and salt-flotation combined**

The combined use of sieves and the salt-flotation method is often more effective than using either of the two methods alone. The substrate sample is washed through a series of sieves as previously described. The material recovered in the lowermost sieve is then placed in a container with a saturated salt solution, where the larvae are subsequently collected on the surface of the water. The sieves remove much of the coarse debris which can interfere with the detection and final recovery of the swimming larvae.

Kettle and Lawson (1952) were the first to combine the two techniques; they collected larvae of several species each of *Ceratopogon*, *Culicoides*, and *Stilobezzia*. Other researchers have used this combination of methods to collect various *Culicoides* spp. (Glukhova 1967, Blanton and Wirth 1979), *C. circumspectus* (Becker 1958), *C. franclemontii* and *C. pechumani* (Cochrane 1974), and *Leptoconops irritans* (Clastrier 1971). The combined method has also been used to collect *Alluaudomyia*, *Culicoides*, *Dasyhelea*, and *Forcipomyia* larvae from tree-hole material, decaying wood, and the rhizosphere of sedges and grasses (L. J. H., unpublished data). This combined method significantly reduces the time spent examining various substrates for larvae.

**Sugar-flotation**

Using sugar to extract larvae from substrate samples works on the same principle as does salt-flotation; the difference in specific gravity between the larvae and the solution causes the larvae to float on top of the liquid. Blanton and Wirth (1979) mention this method for collecting *Culicoides* larvae. One drawback to the sugar extraction method is the high cost of sugar relative to that of salt. When sugar is used, the larvae remain alive considerably longer than they do in salt solutions, allowing one to scrutinize the sample for first and second instars after the larger larvae have been removed.

**Berlese funnel-extraction**

Berlese funnels are commonly used to collect invertebrates from samples of duff and soil. They can also be used for collection of ceratopogonid larvae with
excellent results, particularly if one is interested in obtaining living larvae from a
substrate which does not lend itself well to other collecting methods (e.g., aquatic
plants, rott ing leaves, some tree-hole material). Berlese funnels have been used to
collect several species of *Culicoides* (Jammback 1965, Blanton and Wirth 1979) and
*Forcipomyia* (Saunders 1959). Berlese funnels have been used to extract larvae of
*Alluaudomyia*, *Atrichopogon*, *Bezzia*, *Culicoides*, *Dasyhelea*, and *Forcipomyia* from
samples of aquatic mosses (*Fontinalis* spp.), liverworts, and decaying leaves (L. J.
H., unpublished data).

**Light-extraction**

Light-extraction works because larvae are attracted to light and thus can be
concentrated and collected. In one variation of this method, the substrate is placed
in a vessel with a layer of gauze covering an opening on the bottom. The vessel is
placed in a pan of water, and the entire apparatus is exposed to light. The biting
midge larvae swim toward the light source and can be collected from the water in
the pan. Smith and Lowe (1948) collected *Leptoconops* larvae in this manner,
whereas Dzhafarov (1964) used this method to collect larvae of *Culicoides*, *Lepto­
conops*, and *Forcipomyia* subgenus *Lasiohelea*. Another variation of this method
requires only that the substrate be immersed in water and then exposed to a
strong light on one side. This method is useful for collecting smaller instars from
sediment samples, where they are usually visible only with difficulty (L. J. H.,
unpublished data).

There has been some disagreement in the literature concerning the response of
biting midge larvae to light stimuli. A negative response to light has been noted
for *C. furens* and *C. phlebotomus* (Painter 1927), *C. dovei* (Hull et al. 1934), *C.
accarensis* (Carter et al. 1920), and for several other *Culicoides* spp. (Kettle and
Lawson 1952). Megahed (1956), however, reported that larvae of *C. nubeculosus*
responded positively to light. Becker (1958) correlated the response of *C. circum-
scriptus* to light with their feeding behavior, observing that recently fed larvae
retreated from light while unfed larvae moved toward light. However, Becker
(1958) was unable to demonstrate similar behavior by *C. maritimus*. It appears that
the successful use of light-extraction may be influenced by the species being
collected and the time since the larvae have last fed. Light-extraction is rarely
used anymore, as more efficient and simpler methods of collection have been
devised. Glukhova (1967) found this method to be so unsatisfactory that it was
abandoned for other, more efficient techniques.

**Sand-extraction**

Sand-extraction has proven to be a useful technique for collecting some species
of ceratopogonid larvae, particularly *C. furens* (Bidlingmeyer 1957, Kline et al.
1975). In this method, the substrate was placed in a container and covered with
clean sand. The sand was then covered with water and allowed to stand for 24
hours. The larvae moved upwards through the substrate to just below the surface
of the sand. The sand was then removed from the container and passed through
sieves to recover larvae.

**Agar-extraction**

Agar-extraction was developed by Kline et al. (1981) for collection of *C.
mississippiensis* larvae from soil samples taken in salt marshes. A 2% water-agar
mixture was poured onto the substrate sample and allowed to harden. The larvae entered the agar via the agar-soil interface and burrowed upwards to the surface. It was believed that the anaerobic conditions created by the agar drove the larvae upward. They were prevented from reentering the agar by the high surface tension. The larvae were easily removed from the agar surface by washing them into a jar with a spray of water from an ordinary laboratory wash bottle. Kline et al. (1981) recommended that 300 ml of agar be used for a sample of 0.8·1 volume. Within 48 hours, they collected 72% of the larvae present in their samples.

This method has been used successfully to collect Alluaudomyia, Bezzia, Culicoides, Palpomyia, Sphaeromias, and Stilobezzia larvae from sandy or muddy substrates (L. J. H., unpublished data). In general, this technique works well. The sides of the container into which the substrate is placed must be kept clean and dry or the agar will not adhere to form a seal along its edge. Care should be taken to avoid excessive water in the sample or the agar will be too soft for the technique to work properly.

Decanting

Decanting larvae from standing water, or elutriation, has been used to collect C. melleus larvae (Jamnback and Wall 1958), several other Culicoides spp. (Jamnback 1965), and Leptoconops spp. (Smith and Lowe 1948). Jamnback (1965) decanted larvae of tree-hole breeding species of Culicoides from the water found in cavities of trees. Jamnback and Wall (1958) and Smith and Lowe (1948) extracted larvae from soil samples by flooding the sample with water and then decanting the top layer of the water containing the larvae from the mixture. Simple decanting of larvae has proven to be a tedious and often unproductive process, and for this reason has not been widely used.

Carbon dioxide-flotation

Peters and Soponis (1983) devised a method for using carbon dioxide to extract small chironomid larvae from sand samples. They used club soda as a source of gas in their original method, although they later modified the technique to use pressurized gas from a tank. They claimed that, with three or four rinses, between 80% and 95% of the invertebrates in a sample could be extracted. Hribar (unpublished data) used this technique once with a sandy-clay substrate which had been found to contain larvae of Bezzia nobilis on previous occasions. When club soda was added to the substrate, larvae of this species quickly floated to the surface where they were easily collected. This technique could prove to be a valuable collecting method for various taxa in a number of different substrates.

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