

TECHNIQUES FOR HANDLING MOSQUITO EGG RAFTS AND RAFT SAMPLES (DIPTERA: CULICIDAE)¹

R. G. Weber, Tracy A. Horner²

ABSTRACT: We review methods for handling mosquito egg rafts and describe new tools and techniques that permit rapid handling of the large numbers of individual *Culex* spp. egg rafts collected during field studies of oviposition behavior and vector surveillance. We also describe a method of taking egg samples from individual rafts that maintains the species mix and proportional numbers of eggs deposited nightly on an ovisite. The tools and techniques presented greatly decrease the laboratory space required for hatching large numbers of eggs collected for species determination.

Studies of mosquito oviposition behavior and vector surveillance programs commonly utilize data derived from counts of eggs, or egg rafts, collected from ovitraps (Briand 1964, Fay and Eliason 1966, Frank and Lynn 1982, Holck, *et al.* 1988, Jakob and Bevier 1969, Maw and Bracken 1971, Reiter 1986, Smith and Jones 1972, Surgeoner and Helson 1978). Such studies frequently involve thousands of eggs or rafts (e.g., Lowe, *et al.* 1973 [9,956 rafts], Madder, *et al.* 1980 [13,606 rafts], Maw and Bracken 1971 [9,077 rafts], Smith and Jones 1972 [2,332 rafts]). A variety of devices have been used to collect eggs for study: a plastic scoop (Arredondo-Bernal and Reyes-Villaneuva 1989), metal spoon (Beament and Corbet 1981), small sieve (Chadee and Small 1988), small vial (Guptavanij and Barr 1985), spatula made of fine brass screen (Ilitis and Zweig 1962), the corner of a culture plate lid (Reiter 1986), a spoon-type tissue section lifter (Weber and Weber 1985), and a wire loop (Woke 1937). In many cases the collected eggs or rafts must be held in individual containers for hatching so species may be determined using larval characters. Containers used for hatching have been mentioned infrequently: 200 ml plastic cups (Madder, *et al.* 1980), culture dishes (Weber and Weber 1985), and 24-well tissue culture plates (Reiter 1986).

Part of our oviposition research requires that we collect and identify many individual rafts each summer. Only two mosquito species oviposit in our study sites: *Culex pipiens* L. and *C. restuans* Theobald, species distinguishable with certainty using characters of the first instar head capsule (see keys by Dodge 1966 and Reiter 1986). Larval identification

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² Delaware Agricultural Experiment Station, Department of Entomology and Applied Ecology, College of Agricultural Sciences, University of Delaware, Newark, DE 19717-1303.

is easiest when larvae are dead. The time required for eggs of these species to hatch and the larvae to die (55-65 h) means that considerable material is in the laboratory at any particular time. To reduce space requirements we use 96-well tissue culture plates for collecting and holding eggs until they hatch and larvae can be identified. The wells are filled ca. two-thirds with aged tap water. The small diameter of individual wells in these plates (6.7mm) required us to develop a tool ("raft spoon") for rapid, accurate collection of individual rafts in the field that could be used by relatively inexperienced personnel to collect rafts directly from field ovisites into the wells (Fig. 1). When required, removal of rafts from the wells is equally easy.

The raft spoon is made from brass rod, 4 mm (5/32") in diameter and 12 cm (4-3/4") in length. Brass rod is available from hobby shops; brazing rod of similar diameter should serve as well. Steps in making the spoon are shown in Fig. 2. The taper is 4 cm (1-9/16") long and is formed by filing from each side until the thin end is ca. 0.5 mm (1/32") thick. Both sides are then polished with emery paper. At this stage the thin end is bent to an approximate 9.5 mm (3/8") radius and two punch marks are made where holes will be bored. Holes are required so rafts center on the tip as they are lifted from the surface, instead of sliding off the side. Bending hardens the brass, so the tip must be annealed before holes can be drilled. This is done by heating it to a dull red, then allowing it to cool. The 1 mm (0.04") holes are made with a #60 drill bit, using the punch marks as starting guides, and the square end is rounded with a file. After the holes are drilled, upper and lower surfaces should be repolished to remove burrs. Wrapping the handles with bright-colored plastic tape helps avoid loss of spoons on the ground and, by increasing their diameter, makes them easier to hold.

In some field studies of *Culex* spp. oviposition, we need to identify the species that have laid rafts on test ovisites each night, but without removing complete rafts from an ovisite. Removing a daily sample of rafts for species identification from those laid the previous night would greatly alter larval abundance and would not indicate the exact number of rafts deposited by each species. Removal of complete rafts could also alter species ratio of the remaining rafts and thus might affect attractiveness of the site to gravid females of one species or the other (e.g., Andreadis 1977, Hudson and McLintock 1967, Nakamura 1978). To avoid this problem we remove a sample of 15-25 eggs from each raft for hatching and identification and return the sampled raft to the ovisite. This practice allows us to maintain larval populations in test containers that are proportional, in larval numbers, to nightly oviposition. It has the added benefits of maintaining original species ratios and any attractiveness due to presence of eggs or immature stages.



Figure 1. Using the raft spoon to place a raft into a well of a 96-well tissue culture plate.

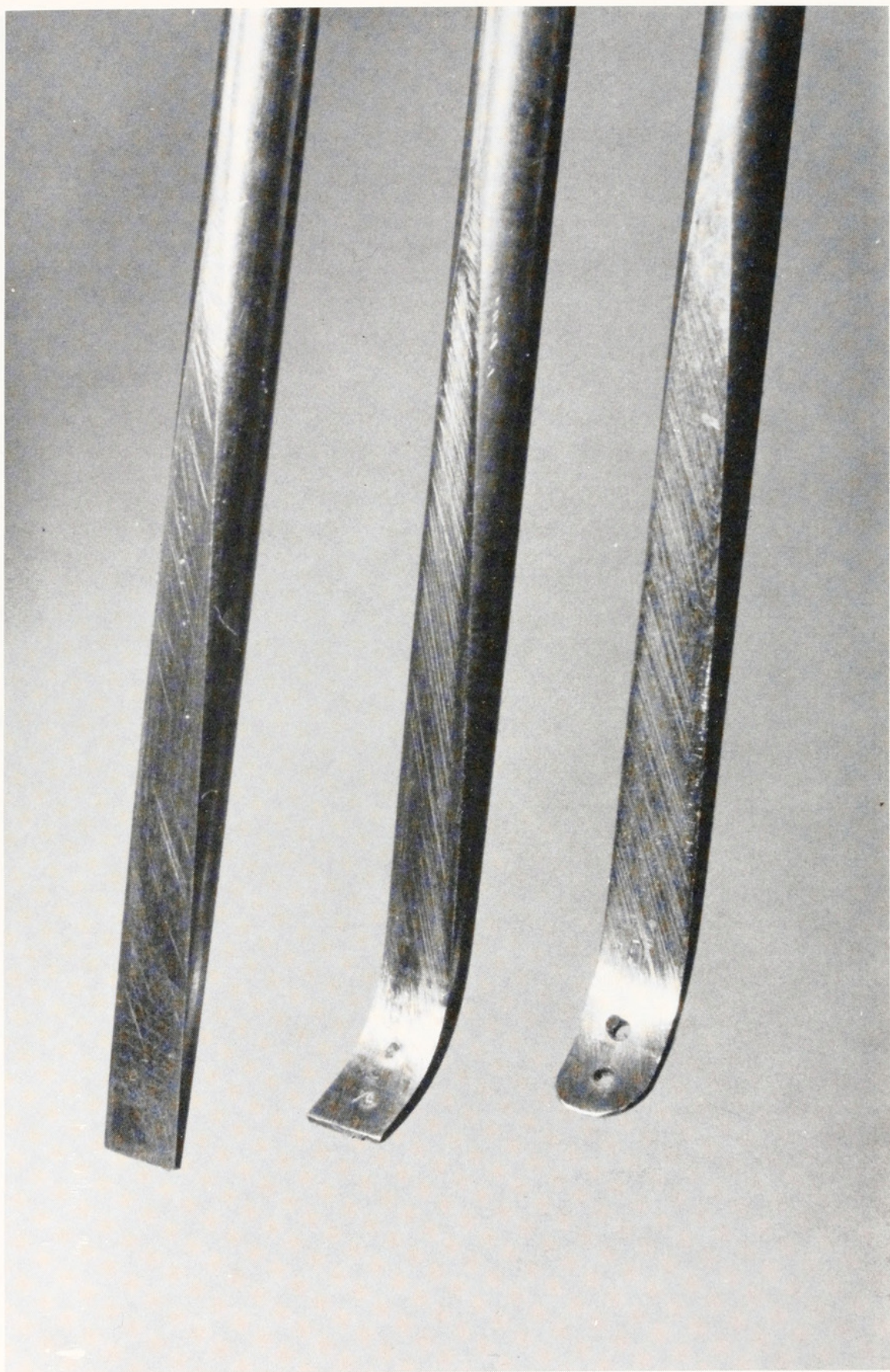


Figure 2. The three stages in making a raft spoon. Left to right: the taper has been filed; tip has been bent and punched; holes have been bored at punch marks and tip rounded.

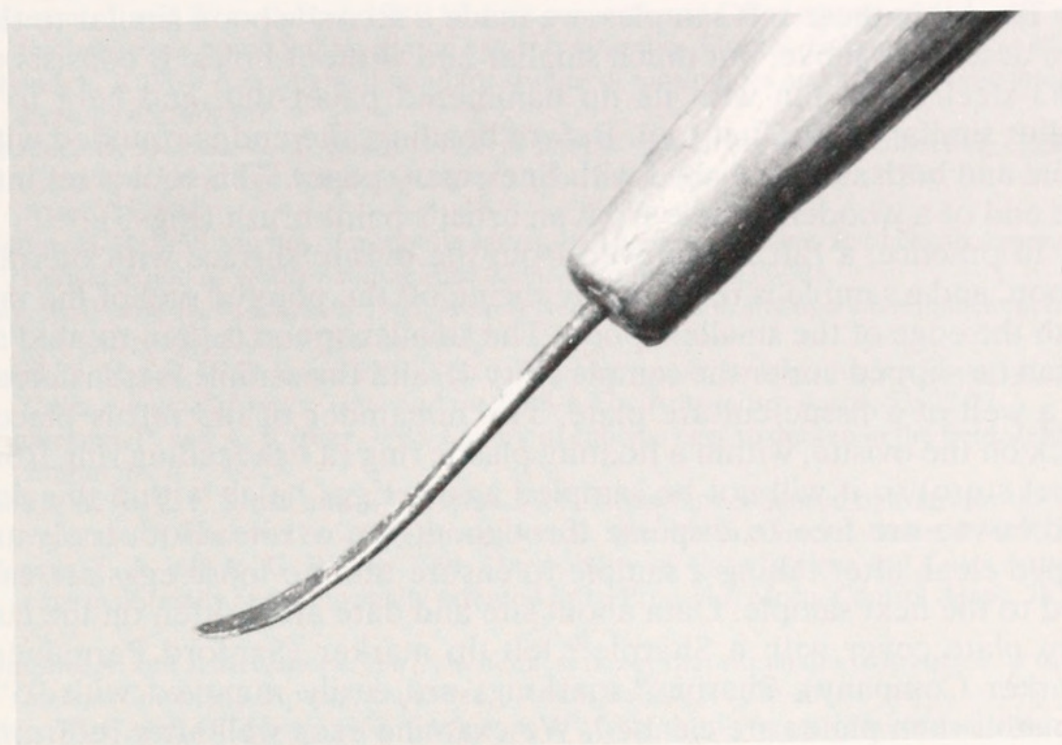


Figure 3. Small spoon for taking samples from egg rafts.

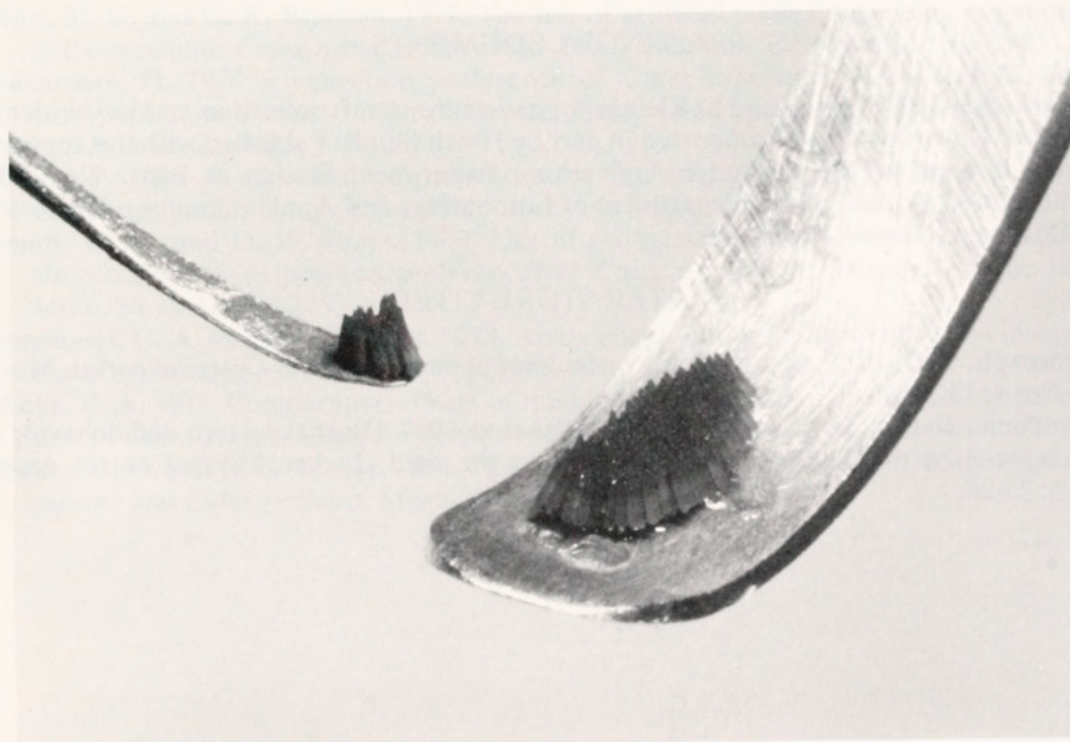


Figure 4. Using the small spoon to obtain a sample of eggs from an egg raft.

To obtain these raft samples, we made a second spoon similar to the one described above, but much smaller and without holes. It consists of a #3 steel insect pin with its tip hammered paper-thin and bent to a radius similar to the first tool. Before bending, the end is rounded with a file and both sides polished with fine emery paper. This tool is set into the end of a wooden handle from an artist's paint brush (Fig. 3).

In practice, a raft is removed from the ovisite surface with the raft spoon, and a sample is removed by slicing off the pointed end of the raft with the edge of the smaller spoon. The smaller spoon is then rotated so it can be slipped under the sample (Fig. 4), and the sample is transferred to a well of a tissue culture plate. The remainder of the raft is placed back on the ovisite, within a floating plastic ring (a fish feeding ring from a pet store) so it will not be sampled again. Eggs hatch within the ring and larvae are free to disperse throughout the ovisite. Both tools are wiped clean after taking a sample to ensure that no loose eggs are carried to the next sample. Data about site and date are written on the culture plate cover with a Sharpie® felt-tip marker (Sanford Permanent Marker Company). Sharpie® markings are easily removed with 95% ethanol when plates are cleaned. We examine each well after returning plates to the laboratory to be sure all samples are upright, which helps ensure hatching (Horner and Weber 1991). It is also necessary to rest one end of the cover on the upper surface of the plate itself, with the other end resting on the table so the two don't seal together from condensation and smother developing embryos (Reiter 1986). For larval identification, the entire culture plate (minus lid) is placed on the stage of a binocular microscope and moved cell-by-cell under the lens.

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LITERATURE CITED

- Andreadis, T. G.** 1977. An oviposition attractant of pupal origin in *Culex salinarius*. Mosq. News. 37: 53-56.
- Arredondo-Bernal, H. C. and F. Reyes-Villaneuva** 1989. Diurnal pattern and behavior of oviposition of *Toxorhynchites theobaldi* in the field. J. Am. Mosq. Control Assoc. 5: 25-28.

- Beament, J. and S. A. Corbet.** 1981. Surface properties of *Culex pipiens pipiens* eggs and the behaviour of the female during egg-raft assembly. *Physiol. Entomol.* 6: 135-148.
- Briand, L. J.** 1964. A permanent pond for studies of mosquitoes and other aquatic insects. *Ecology* 45: 365-367.
- Chadee, D. D. and G. J. Small.** 1988. A simple spoon device for collecting eggs of *Toxorhynchites* from small containers in the laboratory and field. *J. Fla. Anti-Mosq. Assoc.* 59: 5-6.
- Dodge, H. R.** 1966. Studies of mosquito larvae II. The first-stage larvae of North American Culicidae and of world Anophelinae. *Can. Entomol.* 98: 337-393.
- Fay, R. W. and D. A. Eliason.** 1966. A preferred oviposition site as a surveillance method for *Aedes aegypti*. *Mosq. News* 26: 531-535.
- Frank, J. H. and H. C. Lynn.** 1982. Standardizing oviposition traps for *Aedes aegypti* and *Culex quinquefasciatus*: Time and medium. *J. Fla. Anti-mosq. Assoc.* 53: 22-27.
- Guptavanij, P. and A. R. Barr.** 1985. Failure of culicine eggs to darken in the field. *J. Med. Entomol.* 22: 228-229.
- Holck, A. R., C. L. Meek and J. C. Holck.** 1988. Attractant enhanced ovitraps for the surveillance of container breeding mosquitoes. *J. Am. Mosq. Control Assoc.* 4: 97-98.
- Horner, T. A. and R. G. Weber.** 1991. Hatchability of *Culex pipiens* and *Culex restuans* eggs in normally and abnormally oriented rafts. *Proc. N.J. Mosq. Control Assoc.* 78: 77-82.
- Hudson, A. and J. McLintock.** 1967. A chemical factor that stimulates oviposition by *Culex tarsalis* Coquillett (Diptera, Culicidae). *Anim. Behav.* 15: 336-341.
- Illitis, W. G. and G. Zweig.** 1962. Surfactant in apical drop of eggs of some culicine mosquitoes. *Ann. Entomol. Soc. Am.* 55: 409-415.
- Jakob, W. L. and G. A. Bevier.** 1969. Application of ovitraps in the U. S. *Aedes aegypti* eradication program. *Mosq. News* 29: 55-62.
- Lowe, R. E., H. R. Ford, B. J. Smittle and D. E. Weidhass.** 1973. Reproductive behavior of *Culex pipiens quinquefasciatus* released into a natural population. *Mosq. News* 33: 221-227.
- Madder, D. J., R. S. MacDonald, G. A. Surgeoner and B. V. Helson.** 1980. The use of oviposition activity to monitor populations of *Culex pipiens* and *Culex restuans* (Diptera: Culicidae). *Can. Entomol.* 112: 1013-1017.
- Maw, M. G. and G. K. Bracken.** 1971. The use of artificial pools in assessing populations of the mosquito *Culex restuans* Theobald. *Proc. Entomol. Soc. Ontario* 102: 78-83.
- Nakamura, H.** 1978. Oviposition preference of *Culex pipiens molestus* and *C. tritaeniorhynchus summorosus* onto the waters conditioned by the egg rafts or the larvae. *Jap. J. Sanit. Zool.* 29: 117-123.
- Reiter, P.** 1986. A standardized procedure for the quantitative surveillance of certain *Culex* mosquitoes by egg raft collection. *J. Am. Mosq. Control Assoc.* 2: 219-221.
- Smith, W. W. and D. W. Jones.** 1972. Use of artificial pools for determining presence, abundance, and oviposition preferences of *Culex nigripalpus* Theobald in the field. *Mosq. News* 32: 244-245.
- Surgeoner, G. A. and B. V. Helson.** 1978. An oviposition trap for arbovirus surveillance in *Culex* sp. mosquitoes (Diptera: Culicidae). *Can. Entomol.* 110: 1049-1052.
- Woke, P. A.** 1937. Comparative effects of the blood of man and of canary on egg production of *Culex pipiens* Linn. *J. Parasitol.* 23: 311-313.
- Weber, R. M. and R. G. Weber.** 1985. The egg raft seam as an indicator of species in *Culex pipiens* and *Culex restuans*. *Mosq. Syst.* 17: 363



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