Journal of the American Mosquito Control Association, 10(3):427–429, 1994

OPERATIONAL AND SCIENTIFIC NOTES

USE OF A MODIFIED CHEMICAL TRANSFER PUMP FOR SAMPLING *CULISETA MELANURA* LARVAE

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ABSTRACT. The cryptic nature of *Culiseta melanura* larval habitats has limited the use of the conventional one-pint dipper for enumerating the larval density of this species. A modified chemical transfer pump was used in a survey of a known breeding swamp. Field trials assessed the practicality and potential biases towards instars in open vs. closed crypts. From 148 samples taken at 37 sites, 631 *Cs. melanura* larvae were collected. Closed crypts accounted for 21% of the positive samples. The pump had utility in the field but a relative scarcity of 4th-instar larvae in the samples may indicate a source of bias toward younger instars.

The mosquito *Culiseta melanura* (Coq.) is recognized as the primary vector of eastern equine encephalitis virus (Morris 1988). The larval microhabitats (crypts) of *Cs. melanura* are typically shaded pools of water under stumps, root masses, fallen tree roots, and other overhanging earth (Lake et al. 1962), that is, conditions that satisfy the requirements of darkness and contact with soil (Siverly and Schoof 1962). Crypts can either be closed or open depending on the presence of entrances that allow light to enter the interior. The nature of closed breeding crypts results in a sampling bias toward open crypts with the standard one-pint (0.47-liter) dipper.

This report details the use of a chemical transfer pump as a collection device to sample *Cs. melanura* larval density in a breeding swamp. The pump was used successfully to sample larvae from both open and closed breeding crypts. Approximately 68% (n = 25) of the sample sites contained larvae. All larval instars were collected.

The sampling device was a modified 3.8 × 96.5-cm chemical transfer pump (Becksin Industrial Products, Inc., P. O. Box 468, Guilford, CT 06437) (Fig. 1). This was a device similar to the pump described by Walker and Crans (1986) for sampling *Coquillettidia perturbans* (Walker) larvae. The pump was modified by the addition of a screen consisting of a 15.9 × 13.9-cm piece of 0.6-cm (¼-in.) mesh hardware cloth that was wrapped around the inlet end of the pump and secured by a hose clamp. A plate (4.0-cm mailing tube cap) was fixed to the end of the screen with standard baling wire. A 5-link gap was left between the cap and the bottom of the pump inlet to allow the sample to enter through the screen without debris. At the outlet end of the hose, a 3.8–2.5-cm (1½–1-in.) reducing coupler was secured to allow samples to be transferred to one-liter narrow-mouthed Nalgene high-density polyethylene bottles.

Preliminary sampling was carried out on laboratory-reared *Aedes aegypti* (Linn.) larvae. Five successive samples were taken from a plastic washtub containing 100 mixed instars of *Ae. aegypti* larvae in 8 cm of distilled water. Visual observations were used to evaluate mortality, selectivity toward instars, and the ability of the pump to compensate for the escape potential of mosquito larvae.

Field sampling was carried out around the perimeter of Toad Harbor Swamp in Oswego County, NY (Morris et al. 1980) during August 1992. The swamp perimeter was chosen to take advantage of the edge affinity of *Cs. melanura* (Pierson and Morris 1982). Forty-three sites at 400-m intervals were established around the perimeter of the 1,012-ha swamp. At each site, a 70-m transect was constructed into the swamp. Four samples were taken at each site: one at 50 m and one at 70 m on the transect and 2 opposite each other 10 m from the 60-m point on the transect. Samples were taken at the first available crypt sites from the sample locations.

The pump was used to sample larval crypts by either pushing the inlet end of the pump into an available entrance or creating an entrance through the soil, roots, and moss with a 25.4-cm-long, folding pruning saw. The entrances were examined prior to inserting the pump by reaching down

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Table 1. Summary statistics for larval instars collected at sites in Toad Harbor Swamp (n = 148).

Instar	n	%	Mean ± SE	Range
1st	353	56.0	2.40 ± 0.60	0–53
2nd/3rd	267	42.3	1.82 ± 0.38	0–28
4th	11	1.7	0.07 ± 0.03	0–4
Total	631	—	4.29 ± 0.89	0–68

into the crypt to determine the presence of water. If water was present, then the pump would be inserted until the plate touched the substrate. A one-liter sample from each crypt sampled was pumped into a sample bottle within minutes after site preparation. Bottles were labeled by site and transported to a field laboratory where they were opened and left undisturbed for 2–24 h to allow larvae to surface. Larvae were pipetted from sample bottles and transferred to a spot plate. Samples were repeatedly checked until all larvae were removed. Larvae were identified to species and recorded as the number of 1st, 2nd/3rd, or 4th instars per sample.

Observations on laboratory-reared *Ae. aegypti* indicated that there was no direct mortality or noticeable impact on larval behavior even after numerous successive samples. Larval escape behavior did not influence sampling efficiency in artificial containers. The range sampled by the pump, approximately a 10-cm radius around the inlet, was sufficient to include escaping larvae.

The pump was durable, light, and easy to carry in the field. It could be used to sample water that contained large amounts of fine and coarse detritus without fouling. The pump was sufficiently durable to allow us to probe through dense material to get to closed crypts without damage to it. The weight of filled sampled bottles was at times burdensome. However, individual field personnel had no problem carrying up to 5 of the bottles through the swamp.

The pump easily sampled both open and closed crypts. With the dipper, Muul et al. (1975) indicated that they had to dig a substantial hole (40–80 cm in diameter and 30–50 cm deep), to get into the root mat when no sizeable hold was available. Our procedure required a much smaller hole (4 cm diam). This procedure required less time, which minimized vibration and light disturbance. Additionally, we were able to sample all possible crypt sites without bias towards those that were large enough to allow the entrance of a dipper. The percentage of positive samples taken from closed crypts (21%) indicates that sampling only open crypt sites could significantly bias collections when using the dipper for population estimates.

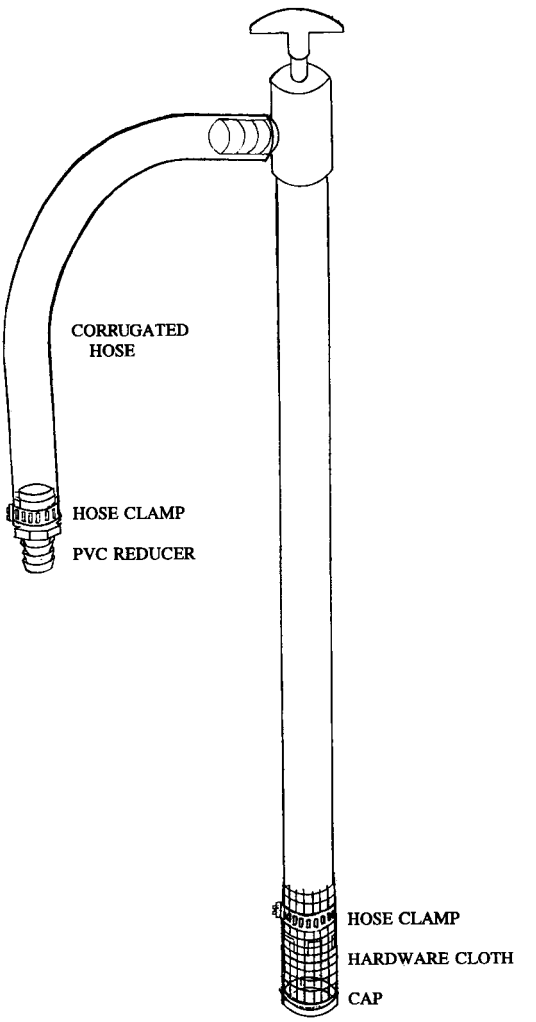

Fig. 1. Modified chemical transfer pump.

Summary statistics for instars in larval collections are presented in Table 1. The distribution of instars by sample was 28% (n = 16) only 1st, 24.5% (n = 14) only 2nd/3rd, 1.7% (n = 1) only 4th, and the rest (45.6%, n = 26) mixed instar combinations. The number of larvae per sample ranged from 1 to 68. The majority (56%, n = 32) of the positive samples contained 5 or fewer larvae. No pupae were collected in one-liter samples.

The low numbers of 4th-instar larvae (1.7%) collected and the absence of pupae in our samples suggests a sampling bias towards younger instars. Factors influencing this sampling bias could include differential behavior among instars. Thomas (1950) observed that larval instars of *Culex pipiens* Linn. reacted differently to both light

changes and vibration with 1st instars reacting most rapidly and staying submerged the longest. We observed the escape behavior of *Cs. melanura* larvae in our samples. The larger instars reacted to the slightest disturbance by rapidly submerging to the bottom of the bottles where they remained for a period of up to 5 min before returning to the surface. Because we had taken our samples immediately after site preparation, at least some of the larger instars could have been missed because of their escape behavior.

Although Hagstrum (1971) found that the standard one-pint dipper sampled all instars of *Cx. tarsalis* Coq. with no significant difference from the known proportion of instars in the study population, it is unlikely that the one-pint dipper would be able to sample those individuals that hide on the substrate. We initially assumed that we could overcome this problem because the pump could capture both surface and submerged larvae. Estimation of this bias requires the determination of the expected abundances of various instars in the field.

Additionally, the predominance of small samples (5 or less larvae) indicates a clumped distribution (Elliott 1977). It is possible that larvae are not evenly distributed throughout crypts, as was initially assumed. There may be groupings (or clumps) of larvae in crypts that may or may not come in contact with the pump during sampling. More accurate population data would result from an increase in the size or number of the samples obtained from a given crypt, or, transformation of the data to conform to a normal distribution.

This research was supported by grant number CO 10447 from the New York State Department of Health and was submitted by R. J. Woodrow in partial fulfillment of the requirements for the M.S. Degree from the State University of New York, College of Environmental Science and Forestry, Syracuse, NY 13210. We thank R. A. Wink and J. Oliver for field and laboratory assistance and C. D. Morris and D. J. White for reviewing this manuscript.

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