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Effectiveness of pyramidal emergence traps for estimating production of *Culex* mosquitoes (Diptera:Culicidae)

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Abstract. The percentage of emergent mosquitoes (*Culex* spp.) collected, temporal dynamics of collection by pyramidal emergence traps, and the effect of a chemical deterrent (pest control strips) for terrestrial scavengers, which remove emergent mosquitoes from collection jars, on the number of emergent mosquitoes collected were studied in ponds in southern California. On average, 75.5% of emergent mosquitoes were collected in jars at the apex of 0.25-m^2 emergence traps placed on the water surface. Recently emerged mosquitoes spent ~1 d in emergence traps before moving into the collection jars. Pest control strips (2.54 cm^2 of Vaportape II: 10% 2,2-dichlorovinyl dimethyl phosphate), which have been used in collection jars during previous studies to deter foraging on newly emerged mosquito adults by Argentine ants (*Linepithema humile* Mayr), did not significantly reduce the number of adult mosquitoes in collection jars and did not delay the movement of adult mosquitoes into collection jars. The cumulative proportion of adult mosquito production sampled by the pyramidal emergence traps increased at a decreasing rate across time (proportion of total production $= -0.314 + 1.021(1 - e^{-0.370t})$ where t is days of trapping). The proportion of adult mosquito production collected during 3- to 5-d collection periods ranged between 0.37 and 0.55 during periods that *Culex* emergence was continuous from aquatic habitats.

Key words: emergence trap, production, mosquitoes, *Culex*, wetlands, DVDP.

Emergence trap collections provide a measure of production for mosquitoes and other Diptera and emergent aquatic insects (Davies 1984). Emergence traps can be an important collection technique, especially in wetlands where immature mosquitoes are not readily sampled by standard collection methods. The sensitivity of immature mosquitoes to disturbance caused when sampling by standard methods, such as hand dipping, differs significantly among mosquito species inhabiting constructed wetlands (Workman and Walton 2003). For example, larvae of the tule mosquito, *Culex erythrothorax* Dyar, are more sensitive to small changes in ambient light level and to physical disturbance created by persons moving through the water and the emergent vegetation than are 3 congeners that co-occur in constructed treatment wetlands in southern California. Hand dipping of immature stages is an ineffective sampling method, even for qualitative assessment of abundance of this species (Walton and Workman 1998, Workman and Walton 2003). The intimate association of other mosquitoes, such as *Mansonia* spp. and *Coquillettidia* spp., with submerged vegetation (Carpenter and LaCasse 1955, Mulieri et al. 2005) often makes quantitative sampling of the immature stages difficult (Service 1993). Trapping emerging adult mosquitoes provides an alternative method to assess temporal and spatial differences in abundance and production for mosquito species that are not readily collected as immature stages (Bidlingmayer 1985, Service 1993, Walton et al. 1998).

Emergence traps do not collect aquatic insects with equal efficiency (Morgan et al. 1963, Kimerle and Anderson 1967, Southwood 1978) and understanding the trapping efficiency for the insect species of interest is critical for reliable estimates of production. Emergence traps effectively sample mosquitoes emerging from small, intermittently flooded habitats and fiberglass mesocosms (Lakhani and Service 1974, Service 1977, Castleberry 1986, Castleberry et al. 1989). Castleberry et al. (1989) found that the ratio of the number of mosquitoes entering collection jars of pyramidal emergence traps to the number of adults that emerged did not differ significantly across pupal densities stocked into fiberglass mesocosms containing wastewater. Trap efficiency was ~60% of emerged *Culex* spp. Service (1993) recommended that

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these results should not be extrapolated to field situations without further experiments.

An accurate assessment of the temporal and spatial differences of mosquito emergence from multipurpose constructed wetlands is important for determining whether mosquito production exceeds a threshold for the initiation of supplemental abatement activities and for evaluating the impact of wetland design features and operational procedures on mosquito production (Boyce and Brown 2003, Knight et al. 2003). Constructed treatment wetlands can provide a cost-effective, ecological alternative to conventional wastewater treatment and will fulfill an increasing role in water reclamation efforts in developed and developing countries (Kavisi 2001, Kadlec and Wallace 2009), especially as human populations and demand for water increase concomitantly. The necessity to situate constructed treatment wetlands in proximity to human populations creates public health concerns when conditions in a wetland are favorable for high levels of mosquito production (Walton 2002).

The use of emergence traps to estimate mosquito production from densely vegetated treatment wetlands requires that macrophytes, such as bulrush (*Schoenoplectus* spp.) and cattail (*Typha* spp.), are cut just above the water surface to maintain physical structure below the water surface and to facilitate placement of the traps on the water surface. Collection jars typically are retrieved after 3 to 5 d (Walton et al. 1998, Thullen et al. 2002, Sanford et al. 2005). If individuals do not enter collection jars soon after eclosion, then the accuracy of adult insect production estimates will vary as a function of the period of emergence trap deployment. The period that newly-eclosed mosquitoes spend in an emergence trap before entering the collection jar is unknown.

Colonization of emergence traps by predators (e.g., spiders, ants) of emerging insects during the deployment period also can be problematic (Southwood 1978, LeSage and Harrison 1979, Sanford et al. 2005). Sanford et al. (2005) added a small piece of pest control strip (10% 2,2-dichlorovinyl dimethyl phosphate [DVDP]) to each collection jar on emergence traps to deter foraging by ants (*Linepithema humile* Mayr) on emergent insects. The impact of this chemical deterrent for ants on collections of emergent mosquitoes has not been reported.

The objectives of my study were to: 1) determine the efficiency of collection of emergent mosquitoes by pyramidal emergence traps, 2) examine the temporal dynamics of the movement of emerged *Culex* mosquitoes into collection chambers, 3) assess the effect of a chemical deterrent for terrestrial scavengers on the number of emergent mosquitoes captured by pyramidal emergence traps, and 4) evaluate the effects of trap efficiency and temporal trends of collection on estimates of mosquito production.

Methods

Apparatus and procedures

The effectiveness of emergence traps placed on the water surface for collecting emerged mosquitoes was evaluated in 2 studies done between 16 October and 13 November 2003 (autumn study) and between 26 May and 11 June 2004 (spring study). For each study, a 4 × 7 m earthen pond at the University of California Riverside (UCR) Aquatic Research facility was filled over a period < 24 h to a depth ~0.3 m with water from an irrigation reservoir on the day that the first pupa was observed in laboratory cultures (see below). Water level in the pond was maintained by a float valve. Maximum and minimum water temperatures were measured daily with a recording thermometer (model 5458; Taylor Environmental Instruments, Fletcher, North Carolina). Air temperature was recorded at 60-s intervals at a weather station 0.32 km northeast of the study site on the UCR Agricultural Experiment Station, and hourly mean temperatures were downloaded from the California Irrigation Management Information System (CIMIS station 44, California Department of Water Resources; http://www.cimis.water.ca.gov).

Individuals used in the 2 studies were derived from field-collected egg rafts and reared to pupation in the laboratory. Egg rafts were collected daily from a plastic wash tub containing ~2.5 L of tap water and decaying vegetation (~25 g *Gazania* spp. [Asteraceae] clippings). Egg rafts were placed either individually in 350-mL waxed ice cream cups or in groups of 5 to 8 rafts in porcelain-coated metal rearing pans (22 × 35 cm) containing ~2 L of tap water. Larvae were fed daily a mixture of finely ground mouse chow and Brewer's yeast (3:1 volume:volume). Larvae were reared at 27 ± 1°C (SD) with a light:dark schedule of 16:8 h in the laboratory until pupation. Pupae were removed daily and transferred to the field.

Laboratory-reared pupae were transferred to the bottom section of replicate styrene rearing chambers (Mosquito Breeder, item no. 1425; BioQuip Products Inc., Rancho Dominguez, California). Each cylindrical rearing chamber (21 × 12 cm; diameter × height) consisted of 2 equally sized (volume ~1 L) styrene sections and a plastic collar that held a clear vinyl funnel and joined the top and bottom sections. Two 5.7-cm-diameter windows covered with amber lumite screen (mesh opening = 280 µm; BioQuip Products Inc., Rancho Dominguez, California) were added to the bottom section of each rearing chamber to permit water exchange. Each rearing chamber was suspended from a styrofoam float (30.5 × 30.5 cm) and placed beneath a floating 0.25-m^2 emergence trap (Walton et al. 1999). Each emergence trap had floats (FunNoodles®; Nomaco Inc., Zebulon, North Carolina) affixed on 2 sides to the base of the trap. Floats provided buoyancy but kept the bottom of each emergence trap submerged below the water surface. Traps were tethered to cinder blocks.

In both studies, adult mosquitoes that emerged were killed by freezing and identified to species with keys in Meyer and Durso (1998). After 7 d, the contents of each rearing chamber were filtered through a screen (mesh opening = 148 µm) to quantify pupal mortality.

Autumn study

During the autumn study, 100 pupae were placed in the bottom sections of each of 31 rearing chambers deployed in individual emergence traps. The mosquitoes were permitted to emerge into the emergence traps; the funnels and tops of the rearing chambers were not used. The proportion of emerged adult mosquitoes in collection jars on the traps was determined from daily collections. Replicate rearing chambers were deployed across a 12-d period. Controls (n = 5) for mosquito emergence from the ponds consisted of a styrofoam float without a rearing chamber placed beneath each emergence trap.

Spring study

During the spring study, 50 pupae were placed into the bottoms of each of 60 rearing chambers divided evenly among 3 treatments (jar, emerge, vapor tape; n = 20/treatment). In the jar treatment, mosquitoes were permitted to emerge directly into the emergence trap through a wide-mouth funnel (upper opening = 2.25 cm diameter). The tops of the rearing chambers were not used. Collection jars were replaced daily. In the emerge treatment, complete rearing chambers (i.e., collar, wide-mouth funnel, and both sections of the rearing chamber) were used, and mosquitoes emerged into the top sections of the rearing chambers. The top section of each rearing chamber was replaced daily. In the vapor tape treatment, mosquitoes were permitted to emerge directly into the emergence trap through a wide-mouth funnel (upper opening = 2.25 cm diameter). The tops of the rearing chambers were not used. A 2.54-cm^2 piece of pest control tape (DVDP; Vaportape II®; Hercon, Emigsville, Pennsylvania) was added to each of the 2 collection jars assigned to a vapor tape emergence trap on the day that pupae for a particular replicate trap were transferred to the field. Collection jars were replaced daily.

Replicate rearing chambers were deployed across a 14-d period. Controls (n = 20) for mosquito emergence from the ponds consisted of a styrofoam float with a complete chamber placed beneath each emergence trap.

The temporal pattern of movement of mosquitoes into collection jars was evaluated by comparing the trends for mosquito emergence into the top section of rearing chambers (treatment = emerge) to the trends for mosquito capture in the collection jars (treatment = jar). The effect of pest control tape on collection efficiency of emergence traps (treatment = vapor tape) was assessed by comparing the proportion of mosquitoes in collection jars of the vapor tape treatment to the proportion of mosquitoes in collection jars of the jar treatment.

Statistical analyses

The daily means for the number of adult mosquitoes in the tops of rearing chambers (emerge) or the number of adult mosquitoes in collection jars atop emergence traps (jar, vapor tape) were calculated for each trap and compared among treatments by analysis of variance (ANOVA) (Systat 9.01, SPSS, Inc., Chicago, Illinois). The proportion of individuals that emerged successfully into emergence traps or were collected in jars on emergence traps was arcsin(x)-transformed before ANOVA. Mosquitoes were absent from collection jars of the control treatment, so this treatment was excluded from the ANOVA.

The expected mean and variation of the proportion of emerging mosquitoes collected by emergence traps was estimated by bootstrap for 1000 collections of 75 emergence traps per collection using the distribution of emergence trap catches for the autumn and spring studies (Systat). The proportion of emerged mosquitoes collected in the jar and vapor tape treatments were used for the spring study.

The probability of individuals not emerging or not being captured in a collection jar was estimated daily for each trap in the emerge or the jar and vapor tape treatments, respectively, with the nonparametric Kaplan–Meier estimator for Type 1 data (exact failure, right censoring only) of the survival procedure of Systat. The mean and variation for the proportion of individuals that had not emerged or had not entered collection jars were calculated for traps in the emerge treatment and for traps in the jar and vapor tape treatments, respectively. The jar and vapor tape treatments were combined because statistical analyses indicated that the proportion of individuals collected in jars and the temporal trends of collection did not differ significantly between the 2 treatments.

Mosquito production

Modeling was used to determine the effects of trap efficiency and delayed entry into collection jars by recently emerged mosquitoes on the proportion of mosquito production collected daily and the cumulative proportion of mosquito production collected for different periods ($\leq$14 d) of deployment of emergence traps. Standard sampling procedures for the collection of wetland mosquitoes (Walton et al. 1998, Sanford et al. 2005) were assumed.

The model was set up such that, beginning on day 1, cohorts of 50 adult mosquitoes emerged following the time course for emergence of mosquitoes in the emerge treatment. A new cohort of 50 mosquitoes started emerging each day. Collection of adult mosquitoes in jars on the emergence traps followed the time course for collection in the jar treatment. The mean number of mosquitoes emerging and mean number of mosquitoes in collection jars were calculated per day by averaging adult mosquito collections across traps in the emerge and jar treatments, respectively, with day 1 as the day that the 1st adult emerged. The proportion of mosquitoes emerging or collected on each day of a 6-d period was calculated and applied to each cohort. No mosquitoes in a particular cohort were assumed to be captured after 6 d from the day that the 1st individual in the cohort emerged. The number of mosquitoes emerging or collected in jars on emergence traps each day was calculated as:

$$N_{Z_t} = p_{Z1}N_{0(x,1)} + p_{Z2}N_{0(x-1,2)} + p_{Z3}N_{0(x-2,3)} + p_{Z4}N_{0(x-3,4)} + p_{Z5}N_{0(x-4,5)} + p_{Z6}N_{0(x-5,6)} \quad [1]$$

where N is the number of individuals, Z is treatment (E = emerge, J = jar), t is day, p_Z is the proportion of mosquitoes emerged (or collected) from treatment Z on each day of the 6-d emergence period, and N_0 is the number of individuals (50) at the start of each cohort (x, …, $x - 5$; cohort $\geq$ 1) of emerging mosquitoes on each day of the 6-d emergence period.

The proportion of mosquito production collected daily (P_t) in the jars of the emergence traps was calculated as the ratio of the number of mosquitoes in the jars (N_J) to the number of mosquitoes emerging successfully (N_E),

$$P_t = \frac{N_{J_t}}{N_{E_t}} \quad [2]$$

where t is day from 1 to 14.

The cumulative proportion of mosquito production (P_T) collected by jars on the emergence traps was calculated as

$$P_T = \frac{\sum_{1}^{T} N_{J_t}}{\sum_{1}^{T} N_{J_t}} \quad [3]$$

where T is the trapping period in days (t). The cumulative proportion of mosquito production collected daily (P_T) by emergence trapping across a 14-d trapping period was fitted as a 3-parameter exponential rise to a maximum

$$P_T = y_0 + a\left(1 - e^{-bt}\right) \quad [4]$$

with the global curve-fitting routine in SigmaPlot (version 11; Systat Software, Inc., San Jose, California), where a is the amplitude of the exponential, b is the rate constant, and y_0 is the 0 intercept.

Results and Discussion

Trap collection efficiency

Culex quinquefasciatus Say was the predominant species (99.4% of individuals) during the autumn study. *Culex stigmatosoma* Dyar and *Culiseta incidens* (Thomson) were <1% of emergent adults. During the spring study, *C. quinquefasciatus* was again the predominant mosquito (62.6%) collected in emergence traps. *Culex tarsalis* Coq. (35.0%) and *C. stigmatosoma* (2.4%) included comparatively smaller proportions of the emergent mosquitoes. In both studies, mosquitoes were never collected by emergence traps in the control treatment.

Emergence traps collected 75.5 ± 18.9% (SD; n = 71) of emerged mosquitoes for both experiments. A significantly greater proportion of emerging mosquitoes was collected in October (back-transformed mean = 0.809, 95% CI: 0.752–0.866) than in June (jar treatment: mean = 0.70, 95% CI: 0.57–0.81) ($F_{2,57}$ = 19.38, $p < 0.001$; Fig. 1A, B). The bootstrap estimate of the mean proportion of mosquitoes collected by emergence traps was 0.756 (95% CI: 0.713–0.796; Fig. 1C). The bootstrap estimate for central tendency was the same as the mean proportion of emerged *Culex* collected for both studies.

Water temperature was similar in the 2 studies and did not affect the proportion of mosquitoes collected by emergence traps (covariate effect not significant).

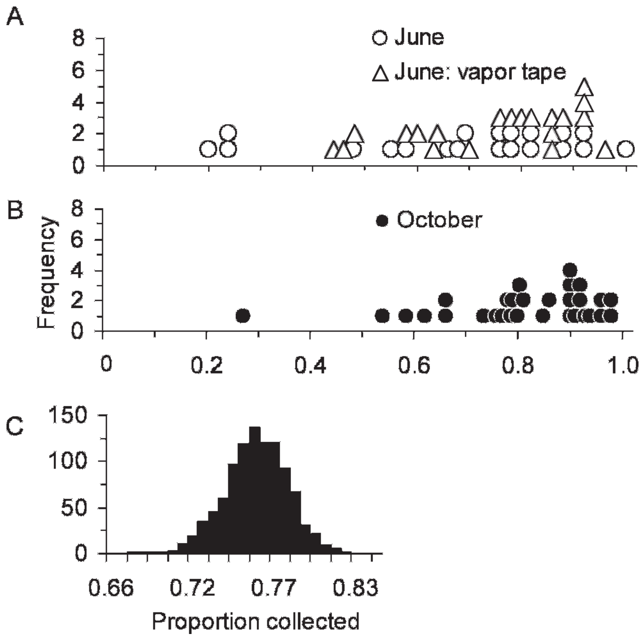

FIG. 1. Frequency distribution of the proportion of *Culex* spp. pupae added to rearing chambers that were collected as adults in collection jars in pyramidal emergence traps during studies in June (A) and October (B) and of the mean proportion of emerged mosquitoes collected in 1000 collections of 75 emergence traps per collection bootstrapped from the distribution of catches illustrated in panels A and B (C). Vapor tape refers to traps in which collection jars each contained a 2.54-cm^2 piece of 10% 2,2-dichlorovinyl dimethyl phosphate (DVDP) pest control tape.

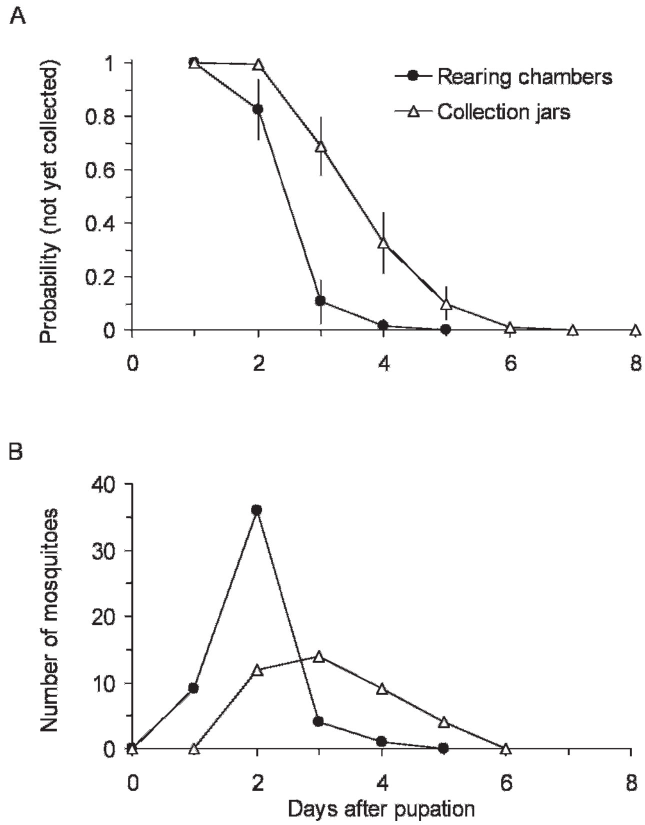

FIG. 2. Temporal trends of probability (± 95% CI) of not being collected in the top of a rearing chamber or in the collection jar (with or without a 2.54-cm^2 piece of 10% 2,2-dichlorovinyl dimethyl phosphate pest control tape) of an emergence trap (A) and the number emerging adults and adult *Culex* spp. trapped in collection jars from cohorts of 50 pupae placed in a rearing chamber beneath an emergence trap (B) as functions of days since pupation.

Maximum water temperatures ranged between 28°C and 31°C in autumn and between 27°C and 31°C in spring. The range of minimum water temperatures (19–24°C) was the same in both studies.

Mosquito activity during crepuscular periods probably was not limited by air temperature, especially in the enclosed space above the comparatively warm water. Maximum air temperatures (measured above turf) were >15°C during the autumn study on all dates except one (3 November: maximum hourly mean air temperature = 12.7°C) and 19°C during the spring study. Minimum daily air temperature ranged between 10°C and 18°C during the 2-wk period at the beginning of the autumn study but declined to between 5°C and 10°C from 31 October to 8 November. During the spring study, minimum daily air temperature ranged from 10°C to 16°C.

Temporal dynamics of collection and effects of a chemical deterrent for scavengers

Recently emerged mosquitoes spent ~1 d in an emergence trap before moving into the collection jar. The mean number of days after pupation that mosquitoes in each treatment entered either the top section of the rearing chamber or the collection jar differed significantly ($F_{2,57} = 16.13$, $p < 0.001$). Mosquitoes entered the top of the rearing chamber on day 3.0 (± 0.2) after pupation, significantly earlier than they entered collection jars (mean ± 95% CI, jar: 4.2 ± 0.3 d; vapor tape: 4.1 ± 0.4 d; Fig. 2A). The proportions of mosquitoes of a particular cohort that emerged or were collected daily over a 6-d period were: emerge 0.180, 0.720, 0.080, 0.020, 0, 0; jar 0, 0.308, 0.359, 0.231, 0.102, 0 (Fig. 2B).

Emergence traps without (jar) and with (vapor tape) a chemical deterrent for scavengers in the collection jar collected statistically similar proportions of emerged mosquitoes (jar: mean = 0.70, 95% CI: 0.57–0.81; vapor tape mean = 0.76, 95% CI: 0.68–0.83). About 3% of individuals did not pass through the funnel in the emerge treatment (mean = 0.97; 95% CI: 0.94–0.98).

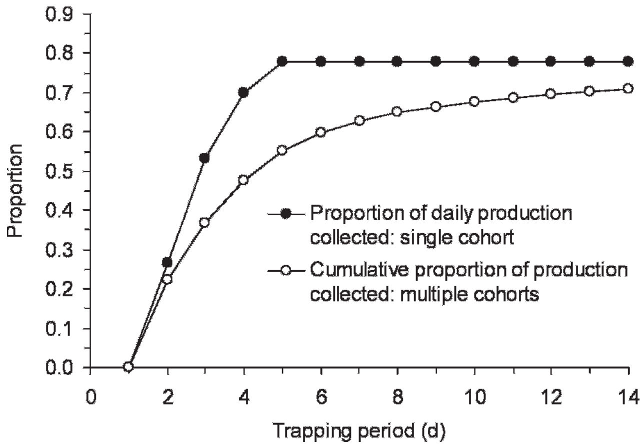

FIG. 3. Proportion of *Culex* spp. production from emergence of a single cohort of individuals that pupated during 24 h and from continuous adult mosquito emergence during the period over which the emergence trap was deployed.

Mosquito production

The proportion of mosquito production collected by emergence traps increased at a decreasing rate across time (Fig. 3). Approximately 37%, 55%, and 63% of emerged *Culex* spp. were estimated to be collected during 3-, 5-, and 7-d trapping periods, respectively. The percentage of mosquito production collected in emergence traps increased to 71% by day 14. For trapping periods $\leq$ 14 d (t), the empirical relationship of the cumulative proportion of production collected (P_T) with time was described well by an exponential rise to a maximum (coefficient $\pm$ 1 SE: $P_T = -0.314\ [\pm 0.009] + 1.021\ [\pm 0.008](1 - e^{[-0.370 \pm 0.005]t})$, $R^2 = 0.99$, $n = 14$).

Emergence traps have been used successfully to quantify mosquito production, especially from comparatively small (<0.1 ha) developmental sites. Lakhani and Service (1974) used egg densities and the probabilities of immature survival to estimate the number of emergent adults and concluded that emergence traps provide reasonable estimates of the total emergent population of *Aedes cantans* (Meigen). Similar survival rates of eggs to adults were observed when an emergence cage was erected over an entire pond to estimate emergence (Service 1977). Approximately 67% of emerging *C. tarsalis* and *Culex pipiens* (L.) adults were collected by emergence traps (Castleberry 1986, Castleberry et al. 1989).

Corbet (1965) cautioned that bias in sampling by emergence traps will greatly influence the accuracy of estimates of aquatic insect production from shallow ponds. In addition to differential selectivity of emergence traps among insect species (Morgan et al. 1963, Kimerle and Anderson 1967, Southwood 1978), sampling biases for emergence traps used to estimate aquatic insect production have been discussed by several authors (Mundie 1956, Morgan et al. 1963, Corbet 1965, Southwood 1978, Davies 1984, Service 1993). Potential biases include: 1) avoidance of the trap by pupae or the final nymphal instar caused by changes in illumination or the discontinuity of physical structure created by the trap, especially shading over open water habitats; 2) congregation of immature stages below emergence traps, particularly in situations where the edges of the trap provide protection from physical disturbance or predators; 3) effects of trap placement because of spatial differences in adult insect production, which is not always limited to standing water; 4) changes in habitat size that affect estimates of the proportion of a population emerging and production; 5) degradation of the catch in the collection chamber; 6) inconstant trapping efficiency caused by tipping of traps; and 7) predators and scavengers that either intercept emerging insects before collection or remove individuals from collection chambers (spiders: Bradley 1926, LeSage and Harrison 1979, WEW, personal observation; ants: Sanford et al. 2005).

Placement of emergence traps in sites where maximum emergence is likely to occur (Corbet 1965) might be appropriate for studies that focus on the phenology of life histories or for surveillance of production of species of public health importance but is not always the best strategy, especially if the goal is assessment of the overall insect production from a wetland or another type of ecosystem. Sampling effort should be stratified across habitat types within the ecosystem of interest. Moreover, understanding the trapping efficiency for the species of interest is critical for reliable estimates of production. My results indicate that, on average, emergence traps intercept ~76% of emerging *Culex* mosquitoes. The use of a small piece of pest control strip (10% DVDP) in the collection jar did not significantly reduce the abundance of the *Culex* species studied here and effectively deterred ants from foraging on insects in the collection chamber (Sanford et al. 2005). Recently emerged adults of the species studied here spent ~1 d in the emergence trap before entering the collection jar. When adult emergence is continuous during the period when emergence traps are deployed (usually 3–5 d; Walton et al. 1998, Thullen et al. 2002, Sanford et al. 2005), production of *Culex* mosquitoes might be underestimated by 63 to 37%, depending on the period of deployment.

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