EGGSHELLS AS AN INDEX OF AEDINE MOSQUITO PRODUCTION 2: RELATIONSHIP OF *AEDES TAENIORHYNCHUS* EGGSHELL DENSITY TO LARVAL PRODUCTION[1]

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ABSTRACT. To test if eggshell density could be used as an index of aedine mosquito production, we compared eggshell density with the larval production of *Aedes taeniorhynchus* in Florida mangrove basin forests. Quantitative ($n = 7$) and categorical ($n = 34$) estimates of annual larval production were regressed against the number of eggshells per cc of soil. Significant regressions were obtained in both instances. Larval production was concentrated in zones with the highest eggshell density. We suggest that eggshell density and distribution can be used to identify oviposition sites and the sequence of larval appearance.

INTRODUCTION

Eggshell sampling offers potential as an inexpensive method to quantify aedine mosquito production. Eggshells (the chorionic relic of hatched aedine mosquito eggs), accumulate in the soil (Lopp 1957, Scotton and Axtell 1979, Ritchie and Johnson 1989) and may reflect historic oviposition patterns (Lopp 1957, Ritchie and Johnson 1991a). In the preceding paper, Ritchie et al. (1992) demonstrated that in a single sampling session, eggshell density (number per cc of soil) could be used to identify oviposition sites and patterns of *Aedes taeniorhynchus* (Wied.) in mangrove forests. In this paper we attempt to prove that eggshell sampling can be used to quantify larval production.

However, 2 pieces of information are needed to validate this method. First, we must be able to estimate the age of eggshells. This would be used to differentiate active oviposition sites (oviposition within the last 2 years) from inactive sites and to measure how variability in the rate of eggshell decomposition affects eggshell density. This will be covered in a future paper. Second, the relationship of eggshell density to larval production must be established; this is the objective of this study.

Specifically, we compared quantitative and categorical assessments of larval production to eggshell density. Quantitative measurements allowed for a more precise assessment of larval production but limited the number of sites that could be studied. Categorical estimates, albeit less precise, allowed us to increase the number of sites 5-fold. Also, we tested the hypothesis that larval production is concentrated in mangrove strata with the highest eggshell densities.

MATERIALS AND METHODS

Quantitative relationship of eggshell density to larval production: Eggshell density and larval production were quantified in 7 mangrove basin forests on Marco Island ($n = 2$) and in the Rookery Bay Natural Estuarine Research Sanctuary ($n = 5$) in southwest Florida. Sampling was conducted within a 31 × 37 m grid. Dominant trees in the grid were black mangrove (*Avicennia germinans* Linn.; 3 sites), red mangrove (*Rhizophora mangle* Linn.; 3 sites) and white mangrove (*Laguncularia racemosa* Gaertn. f.; 1 site).

Eggshells were sampled from pond and plains strata (Ritchie et al. 1992) using a modified 6-cc (core volume = 3 cc) plastic syringe (Ritchie and Addison 1991). Pond soil cores (8/transect, total = 40) were systematically collected from 5 randomly selected transects (1/pond) that traversed from the pond center to the top of its bank. At each pond, a transect was located as follows: 1) the pond was arbitrarily divided into quadrants, one of which was selected using a random number from 1 to 4; and 2) this quadrant was subdivided into 4 quadrants, one of which was selected randomly. Plains samples ($n = 36$) were collected along 4 transects (9 samples/transect); location was determined using a random number table. Eggshells were isolated by water flotation (Ritchie and Addison 1991).

The mean number of eggshells/stratum was multiplied by the respective proportion of the grid that each stratum encompassed and summed to create an overall grid average. The stratum proportion was determined by dividing the number of elevation measurements that were within a stratum's elevational range by the total number of elevation measurements (240/grid). Water depth was used to measure relative

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elevation. Water depth was measured at 2 m intervals with a staff gage and set relative to the lowest elevation within the grid.

Larvae were sampled with a 300-ml dipper (50 randomly selected samples per session) after flooding events for at least 1 year at each site. Although the sampling period varied (ranging from 1 year at 4 sites to 2 years at 3 sites), each featured a dry winter–wet summer period characteristic of years with large populations of *Ae. taeniorhynchus* (Ritchie 1984). The mean number of larvae per dip was multiplied by the proportion of the grid that was flooded to produce a gridwise average. These values were summed then divided by the sampling period (in years) to estimate annual larval production.

We suspected that the thick detritus and peat accretion in mangrove basin forests (Twilley et al. 1986, Lynch et al. 1989) could affect eggshell density in 2 ways. First, a thick detritus layer would increase ovipositional area (*Ae. taeniorhynchus* will oviposit throughout thick detrital layers[4]), thereby diluting egg and eggshell density relative to sites with a thinner detrital layer for a fixed oviposition rate. Second, higher rates of litter fall could increase peat accretion, thereby diluting eggshell density. Litter standing crop is proportional to litterfall, most of which (80%) forms peat (Twilley et al. 1986). Thus, litter standing crop should be proportional to the rate of peat accretion and was used to calibrate eggshell density.

Litter was sampled at plains and pond strata using a 13-cm diameter plastic corer. Because litter standing crop does not change significantly from fall to spring (Twilley et al. 1986), sites were sampled only twice (April 1989 and 1990). Preliminary data indicated that 9 and 15 samples provided a precision level (SE/mean) of 0.10 for pond and plains strata, respectively. Sample location was determined randomly. Litter was collected by working the corer into the soil then removing litter (leaves, twigs, propagules and floral bracts) by hand until firm soil was exposed. Litter was placed in a paper sandwich bag then dried in a drying oven at 60°C for 48 h or until all litter was dry. The mean ovendry weight of litter from each stratum was used to obtain a gridwise average as described for eggshells. This value was divided by the average mean litter standing crop of all sites to determine the relative litter standing crop for the site. The relative litter standing crop was multiplied by the mean eggshell density to produce the litter-calibrated eggshell density.

The relationship of mean annual larval production to eggshell and litter-calibrated eggshell density was examined by regression analysis. All statistical analysis were performed using SYSTAT® (Wilkinson 1988).

Categorical relationship of eggshell density to larval production: Aedes taeniorhynchus larval production was assessed categorically to increase sample size. Categorical larval production data for 34 sites was obtained from historical data and observations made after flooding tides and rains from September 1989 to May 1991. This period was dominated by 2 distinctive regimes of weather and *Ae. taeniorhynchus* production; 1990 was characterized by a dry winter–wet summer pattern and widespread salt marsh mosquito production while 1991 featured a wet winter and spring with limited *Ae. taeniorhynchus* production. Larval density was assessed qualitatively and used to categorize site larval production (Table 1).

Eggshells and litter standing crop were sampled and used to calculate a grid average as described earlier. The relationship of eggshell and litter-calibrated eggshell densities to larval production was determined by regression analysis, with the larval production categories serving as indicator or "dummy" variables (Kleinbaum and Kupper 1978). Category means were separated with a Tukey-Kramer (Kramer 1956) test as suggested by Day and Quinn (1989).

Relationship of eggshell distribution to the location of larval hatches: The production of larvae following initial flooding of pond and upland strata was compared with the respective mean eggshell density for sites with type I and type II

Table 1. Larval population categories used to categorize production of *Aedes taeniorhynchus* larvae.

Larval population categories
  None = no larvae observed
  Light = less than 1 larvae per dip; larvae localized
  Moderate = 1 to 5 larvae per dip; larvae often localized
  Heavy = >5 larvae per dip; larvae widespread

These data were used to classify larval production as

1. Light broods, if any, upon initial flooding[a]
2. 1 to 2 moderate to heavy broods upon initial flooding[a]
3. Many broods/year; size, number, and timing relative to timing and frequency of oviposition/flooding events

[a] Initial flooding refers to flooding following prolonged drought that exposes the site (usually late winter–early summer).

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[4] Ritchie, S. A. 1988. A simulation model of the population dynamics of the black salt marsh mosquito (*Aedes taeniorhynchus*) in a Florida mangrove forest. Ph.D. dissertation, University of Florida, Gainesville, FL.

eggshell profiles (Ritchie et al. 1992). The proportional density of eggshells in pond and plains strata was calculated by dividing the mean eggshell density for a stratum by the sum of the mean eggshell density for both strata. Proportional larval production for each stratum was similarly calculated using the mean number of larvae per dip. The proportional eggshell density and larval production for the pond stratum were arcsine transformed then analyzed by Pearson correlation; unfortunately, only 4 sites with ponds produced sufficient larvae for analyses. Plains stratum data, being reciprocal to that for the pond stratum, were not included.

RESULTS

Quantitative relationship of eggshell density to larval production: Two series of regression analysis were performed to offset a potential error in larval production estimates. A Marco Island site produced suspiciously small larval broods despite high egg (Ritchie and Johnson 1991b) and eggshell densities (Fig. 1). During the larval sampling period (1985-87), mosquito control personnel initiated helicopter thermal fogging of the site with fenthion twice a week during periods of high *Ae. taeniorhynchus* activity, resulting in high mortality of larvae and adults. Oviposition may have also been affected. An egg population sampled in November 1984 was estimated to be 7,000/m^2 whereas 19 egg populations sampled from 1985 to 1987 produced a maximum of only 4,500/m^2. In an effort to restrict our estimate of oviposition to the spraying period, new eggshells were incorporated in the first series of regressions. Preliminary studies indicated that most eggshells flatten and fade to a moderate brown color within 2 years of hatching (Ritchie, unpublished data) and are distinguishable from new eggshells that are dark brown or black (Ritchie and Johnson 1989). In the second regression series, the Marco Island sites were excluded and both new and old eggshells were used.

Eggshells were significantly related to annual larval production (Table 2). In the first series of regressions, only new eggshells were significantly related to annual larval production. In series 2, significant regressions were obtained for eggshell and litter-calibrated eggshells. Litter calibration of eggshells had little effect on regression significance, suggesting that litter calibration of eggshells is unnecessary.

Categorical relationship of eggshell density to larval production: Most of the 34 sites (88.2%) did produce some *Ae. taeniorhynchus* larvae. Eggshell ($F = 35.2$; df = 1, 33; $P < 0.001$; $R^2 = 0.52$) and litter-calibrated eggshell ($F = 24.7$; df = 1, 33; $P < 0.001$; $R^2 = 0.43$) density were significantly related to larval production class (Table 3). Larvae were never observed in 4 (11.8%) sites, although one of these sites had a significant density of eggshells (0.09/cc soil). This site contained a permanent manmade pond that acted as a fish refugium during drought. Perhaps these fish ate mosquito larvae before we could detect them. Additionally, the observation period may have been insufficient to identify the sites' larval production potential.

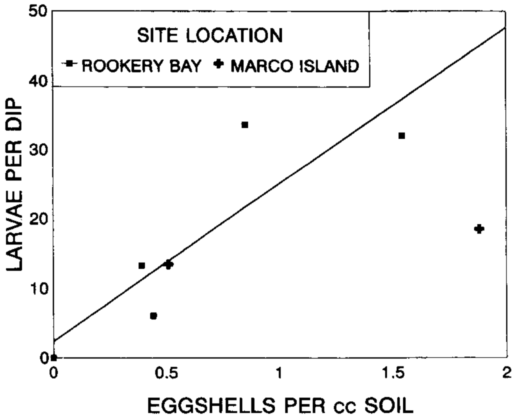

Fig. 1. Relationship of *Aedes taeniorhynchus* eggshell density to mean annual larval production (number/dip) in mangrove basin forests located on Marco Island and Rookery Bay Natural Estuarine Research Sanctuary. The regression line [Y = 2.39 + 22.70 (shell density)] is based on the Rookery Bay sites only.

Table 2. Regression analysis of the mean annual larval production of *Aedes taeniorhynchus* against eggshell and litter-calibrated eggshell density (see text for details).

	Shells		Shells + litter	
	R^2	P	R^2	P
Series I. Rookery Bay and Marco Island sites ($n = 7$)				
All eggshells	0.440	0.104	0.320	0.186
New eggshells[a]	0.783	0.008	0.729	0.015
Series II. Rookery Bay sites ($n = 5$)				
All eggshells	0.762	0.054	0.825	0.033

[a] New eggshells identified by dark brown to black color; older eggshells were moderate to light brown.

Relationship of eggshell distribution to the occurrence of larval hatches: Eggshell density coincided with the location of larval broods (Table 4). For sites with a type I eggshell profile, larvae were present in large numbers only after ponds had been flooded following exposure to ovipositing mosquitoes in late winter and spring. Sites with a type II eggshell profile hatched significant larval broods following flooding of both strata.

Table 3. *Aedes taeniorhynchus* eggshell densities (no./cc soil) for different larval production categories (see Table 1). Means followed by different letter are significantly different at $P < 0.05$ (Tukey-Kramer test).

Variable	Larval production category 1	2	3
No. (%)	10 (29%)	10 (29%)	14 (42%)
Eggshells Mean ± SE	0.02 ± 0.01a	0.16 ± 0.04a	0.80 ± 0.19b
Range	0–0.09	0.05–0.44	0.21–2.48

Table 4. Occurrence of *Aedes taeniorhynchus* eggshells[a] and larvae[b] in pond and plains strata[c] for type I and II eggshell profiles.[d] Parenthetical values are the respective percentage of total eggshells or larvae for each strata.

Eggshell profile and site	Eggshells			Larvae		
	Pond	Plains	% pond	Pond	Plains	% pond[e]
Type I						
A	0.71	0.15	82.6	24.7	0	100
B	2.77	0.27	91.1	70.6	0.1	99.8
Type II						
A	1.65	0.79	67.5	33.8	15.2	59.8
B	2.29	2.37	49.1	6.4	2.3	45.7

[a] Mean number of eggshells/cc of soil; based on 40 and 36 samples for pond and plains strata, respectively.

[b] Mean number of larvae per dip following flooding of previously dry stratum substrate.

[c] Ponds are low elevation depressions embedded within higher, relatively flat mangrove plains.

[d] Type II eggshell profile differs from a type I eggshell profile in having significant eggshell densities at high as well as low elevations (Ritchie et al. 1992).

[e] Percentage of larvae in pond based on annual larval production rather than mean no./dip.

The correlation between the proportion (arcsine transformed) of eggshells and larvae from the pond stratum was high ($R = 0.93$), although not significant ($P = 0.07$) due to the small number of sites ($n = 4$). The only site with a high eggshell density (4.9/cc) in the upland strata was also the only site to contain larvae following submergence of both pond and plains strata. Ritchie and Johnson (1991b) found that the transitional mangrove-tropical hammock soil within this zone serves as an oviposition site when mangrove plains are submerged. Conversely, sites with a type I eggshell profile produced broods only until ponds were submerged. Horsfall (1963) and Curtis (1985) found that the egg profile may be species specific. Divergent egg distribution may minimize overlap of broods and larval competition or may reflect the minimal flood level necessary before drydown strands larvae (Curtis 1985). The varied eggshell profiles of *Ae. taeniorhynchus* suggests that oviposition relative to elevation is not species-specific and is controlled by unknown site-specific factors.

DISCUSSION

Our results suggest that eggshell density can be used to estimate past production of *Ae. taeniorhynchus* in mangrove. While varying conditions (meteorological, hydrological and exposure to pesticides) make it difficult to compare the larval production of the sites, the concurrence of the results using both quantitative and categorical estimates of larval production strengthen this conclusion.

However, the relationship between eggshell density and larval production is complicated by characteristics of the soil. Eggshell distribution, particularly in compact soils, may be altered by runoff during heavy rain (Ritchie et al. 1992). Fortunately, eggshell flushing in mangrove peat, the dominant soil type of mosquito-producing mangrove basin forests (Ritchie, unpublished data), is not sufficient to significantly alter gross ovipositional patterns (Ritchie et al. 1991). The results also suggest that peat accretion, as estimated by litter standing crop, does not significantly affect eggshell density. However, this relationship will remain unknown until peat accretion rates in eggshell-bearing mangrove basin forests are determined. Finally, variability of the rate of eggshell breakdown could account for varying eggshell densities and deserves study.

Despite these sources of variability, we demonstrated a positive relationship between eggshell density and larval production. The range of eggshell densities associated with a category II larval producer (Table 4) suggests that an eggshell density of 0.05/cc of soil may be the minimal eggshell density for sites that produce significant broods. Higher eggshell densities may reflect an increase in brood frequency as much as brood size. This is exemplified by observations made in 1983 (Ritchie 1984) and 1990 (this study) suggesting that sites with 0.05–0.15 eggshells/cc do not produce significant larvae in years with wet winters.

Eggshell profiles can be used to identify the location and sequence of larval broods, as demonstrated by the correspondence between the location of peak eggshell density and larval production. Mangrove forests with a type I eggshell profile should produce most larvae only after incipient flooding of low lying areas (i.e., ponds), typically during late spring and early summer in south Florida (Ritchie 1990). However, sites with a type II eggshell profile may produce larvae upon flooding following any substantial period of high adult mosquito populations. When other sites are submerged, these sites may serve as foci of *Ae. taeniorhynchus* production.

However, eggshell data must be interpreted with caution. Unlike egg (Horsfall 1956) and larval surveys, it cannot predict brood size. And it cannot account for larval mortality due to predators, disease, flushing and stranding. Because the eggshell distribution reflects oviposition over a period of several years, the distribution of eggs from individual oviposition events may deviate from that of eggshells.

However, by integrating eggshell data with knowledge of site hydrology, topography and adult mosquito populations, eggshell data can be employed to predict larval broods. For example, one site was submerged for most of the period from 1983 to June 1985, only producing a small ~"puddle" brood in 1985. However, upon the discovery, in 1989, of eggshells in ponds (0.71/cc) and plains (0.15/cc), we felt that a large larval brood would hatch if ponds were exposed to ovipositing mosquitoes. The winter-spring of 1989 was dry, exposing ponds to large numbers of adult *Ae. taeniorhynchus*. A flooding tide and heavy rain in June hatched 2 large broods (mean of 18.3 and 45.8/dip). This example reiterates that eggshell sampling can potentially provide a quicker and, at times, more accurate assessment of aedine mosquito production than larval sampling.

Eggshell sampling has several potential applications. It can be used as an initial or backup surveillance system and to identify oviposition patterns and associated environmental cues. Because eggshells need to be sampled only once (Ritchie et al. 1992), many sites can be sampled, increasing the validity of the results. Ovipositional preference data can be applied to remote sensing technology (Welch et al. 1989) and in the search for oviposition repellants and attractants (Laurence and Pickett 1985). Eggshell profiles can differentiate sites that produce larvae upon initial flooding from those that produce under any water table regime.

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REFERENCES CITED

Curtis, G. A. 1985. Habitat selection strategies of mosquitoes inhabiting citrus irrigation furrows. J. Am. Mosq. Control Assoc. 1:169–173.

Day, R. W. and G. P. Quinn. 1989. Comparison of treatments after an analysis of variance in ecology. Ecol. Monog. 59:433–463.

Horsfall, W. R. 1956. A method for making a survey of floodwater mosquitoes. Mosq. News 16:66–71.

Horsfall, W. R. 1963. Eggs of floodwater mosquitoes (Diptera: Culicidae) IX. Local distribution. Ann. Entomol. Soc. Am. 56:426–441.

Kleinbaum, D. G. and L. L. Kupper. 1978. Applied regression and other multivariable methods. Duxbury, North Scituate, MA.

Kramer, C. Y. 1956. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics 12:307–310.

Laurence, B. R. and J. A. Pickett. 1985. An oviposition attractant pheromone in *Culex quinquefasciatus* Say (Diptera: Culicidae). Bull. Entomol. Res. 75:283–290.

Lopp, O. V. 1957. Egg sampling as an index of mosquito breeding. Proc. Annu. Meet. N. J. Mosq. Exterm. Assoc. 45:60–65.

Lynch, J. C., J. R. Meriwether, B. A. McKee, F. Vera-Herrera and R. R. Twilley. 1989. Recent accretion in mangrove ecosystems based on ^{137}Cs and ^{210}Pb. Estuaries 12:284–299.

Ritchie, S. A. 1984. Record winter rains and the minimal populations of *Aedes taeniorhynchus* (Wiedemann): cause and effect? J. Fla. Anti-Mosq. Assoc. 55:14–21.

Ritchie, S. A. 1990. A simulation model of water depth in mangrove basin forests. J. Am. Mosq. Control Assoc. 6:213–222.

Ritchie, S. A. and D. S. Addison. 1991. Collection and separation of *Aedes taeniorhynchus* eggshells from mangrove soil. J. Am. Mosq. Control Assoc. 7: 113–115.

Ritchie, S. A. and E. S. Johnson. 1989. Use of sodium hypochlorite to detect aedine mosquito eggs in mangrove soils and insect feces. J. Am. Mosq. Control Assoc. 5:612–613.

Ritchie, S. A. and E. S. Johnson. 1991a. *Aedes taeniorhynchus* (Diptera: Culicidae) oviposition patterns in a Florida mangrove forest. J. Med. Entomol. 28:496–500.

Ritchie, S. A. and E. S. Johnson. 1991b. Distribution and sampling of *Aedes taeniorhynchus* (Diptera: Culicidae) in a Florida mangrove forest. J. Med. Entomol. 28:270–274.

Ritchie, S. A., D. S. Addison and F. Van Essen. 1992. Eggshells as an index of aedine mosquito production: 1: Distribution, movement and sampling of *Aedes taeniorhynchus* eggshells. J. Am. Mosq. Control Assoc. 8:32–37.

Scotton, G. L. and R. C. Axtell. 1979. *Aedes taeniorhynchus* and *Ae. sollicitans* (Diptera: Culicidae) oviposition on coastal dredge spoil. Mosq. News 39:97–110.

Twilley, R. R., A. E. Lugo and C. Patterson-Zucca. 1986. Litter production and turnover in basin mangrove forests in southwest Florida. Ecology 67:670–683.

Welch, J. B., J. K. Olson, W. G. Hart, S. G. Ingle and M. R. Davis. 1989. Use of aerial color infrared photography as a survey technique for *Psorophora columbiae* oviposition habitats in Texas ricelands. J. Am. Mosq. Control Assoc. 5:147–160.

Wilkinson, L. 1988. SYSTAT: the system for statistics. SYSTAT, Inc., Evanston, IL.