LIMNOLOGICAL AND BOTANICAL CHARACTERIZATION OF LARVAL HABITATS FOR TWO PRIMARY MALARIAL VECTORS, *ANOPHELES ALBIMANUS* AND *ANOPHELES PSEUDOPUNCTIPENNIS*, IN COASTAL AREAS OF CHIAPAS STATE, MEXICO

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ABSTRACT. Field surveys of mosquito breeding sites on the Pacific coastal plain and foothill regions of southern Chiapas, Mexico, were carried out in the dry and wet seasons of 1988. At each site, selected environmental variables were measured or estimated, presence and percent cover of aquatic plants recorded, a water sample collected for subsequent analyses, and 10–30 dips made for mosquito larvae. Logistic regression and discriminant analyses revealed that the occurrence of *Anopheles albimanus* larvae in both the wet and dry seasons was positively associated with planktonic algae and negatively associated with altitude. In the dry season, *An. albimanus* larvae were largely restricted to the margins of permanent water bodies and were associated with the presence of floating plants, particularly *Eichhornia crassipes*. During the wet season *An. albimanus* larvae were positively associated with emergent plants, particularly seasonally flooded Cyperaceae, and phosphorus (PO_4) concentrations, and were negatively associated with abundant filamentous algae, high levels of total suspended solids (TSS) and *Salvinia*. In the dry season, *An. pseudopunctipennis* larvae were positively associated with filamentous algae, altitude and the presence of *Heteranthera* if encountered in a riverine setting, and were negatively associated with water depth. During the wet season, flooding eliminated typical floodplain *An. pseudopunctipennis* habitats, and larvae were rarely encountered.

INTRODUCTION

Malaria is an important public health problem in the coastal and foothill regions of Chiapas State, Mexico. The Tapachula area was selected from this malaria endemic region as the site of a long-term project, sponsored by the National Aeronautics and Space Administration (NASA), to develop a predictive model of *Anopheles* abundance and the potential for malaria transmission that may be driven by remotely sensed data. Project activities in Mexico were launched with an extensive mosquito faunal survey of the study area that will be published elsewhere. The faunal survey was followed by initial efforts to characterize the larval habitats for the 2 primary vector species, *An. albimanus* Wiedemann and *An. pseudopunctipennis* Theobald, which is the subject of this report.

The study area includes Pacific coastal plain and foothill sites at elevations of 0–850 m in southern Chiapas (Fig. 1). The natural vegetation of the area, as characterized by Miranda (1952) and Breedlove (1981), includes a coastal zone of mangrove swamps, a narrow band of deciduous coastal forest, palm forest and semi-evergreen seasonal forest on the coastal plain, and tropical deciduous forest in the foothills. However, much of the natural vegetation has been modified for, or replaced by, agricultural crops including banana, soybean, tobacco, mangos, coconut and pastures on the coastal plain, and coffee in the foothill region.

Field surveys of possible mosquito breeding sites were carried out during one dry season trip, May 1988, and one wet season trip, September 1988. Our primary objectives were to determine if habitats positive or negative for larvae of each vector species could be correctly categorized based upon knowledge of selected botanical and limnological variables, and to investigate seasonal differences in habitat availability and utilization. At each of 35 dry season and 38 wet season sites (Fig. 1), data on selected environmental variables were measured or estimated, the presence and percent coverage of semiaquatic and aquatic plants recorded, a water sample taken and preserved for subsequent analyses, and 10–30 dips made for mosquito larvae. Mosquito larvae were slide-mounted and identified to species, and water samples were analyzed for a variety of constituents.

MATERIALS AND METHODS

At each site a collection form was completed which included data on the following: average water depth in the habitat sampled (WD), surface area of the water body, water temperature, amount of shade, current speed and altitude (ALT). Conductivity (COND) and pH values were determined with portable field meters. The presence and percent cover in the area sampled by emergent plants, floating plants, filamentous algae (FILAMALG) and detritus were estimated

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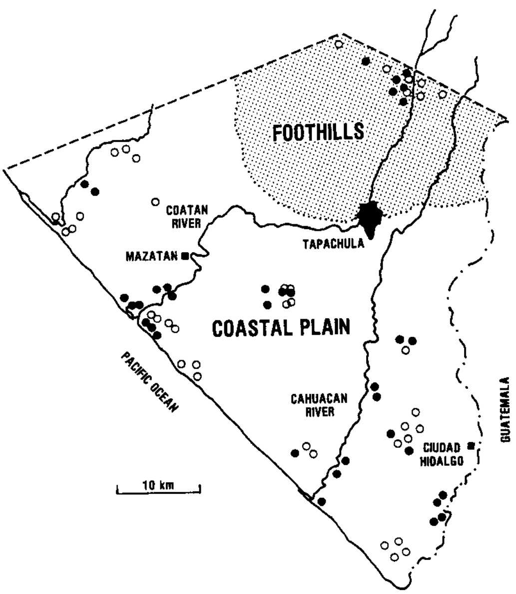

Fig. 1. Location of sampling sites in the dry (solid circles) and wet (open circles) seasons of 1988 in southern Chiapas, Mexico.

along with presence and percent cover for each of 29 genera and 4 families of aquatic and semi-aquatic plants. Plants whose identifications were in question were collected and pressed for subsequent identification. Ten dips for mosquito larvae were taken at each site during the dry season, and 30 dips were taken at each site during the wet season. Mosquito larvae were slide-mounted, and second to fourth instar *Anopheles* larvae were identified to species.

Water analyses for total suspended solids (TSS), particulate organic matter (POM), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), nitrate nitrogen (NO_3), ammonium nitrogen (NH_4), orthophosphate phosphorus (PO_4) and biological oxygen demand (BOD) followed standard limnological methods (American Public Health Association 1985, Janik and Byron 1987, Strickland and Parsons 1972). The cations, sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg), were analyzed by atomic absorption spectrophotometry. Planktonic algae (PLANKALG) were estimated by fluorometric measurements of the amount of chlorophyll a. Water color was colorimetrically determined. Water color, BOD, DOC and DON values were determined only for sites sampled during the wet season trip.

Many plant groups occurred in 2 or fewer of the surveyed habitats in either the dry or wet season, and were eliminated from their respective data sets. This left the following core group of 12 plant variables common to both data sets: *Avicennia*; *Eichhornia* (EICH); *Cyperus*; *Lemna*; *Ludwigia*; *Mimosa*; *Pistia*; *Salvinia*; *Typha*; semi-aquatic and aquatic grasses including the common genera *Echinochloa, Panicum, Paspalum, Jouvea* and others; tall grasses such as *Pennisetum*; and the Cyperaceae including the common genera *Cyperus, Fimbristylis, Eleocharis, Scirpus* and others. In addition to the core plant variables, *Heteranthera* occurred in 5 dry season sites and was included in the dry season analyses. The following plant taxa occurred in 3 or more wet season sites and were retained as separate variables in the wet season data set: *Fimbristylis, Jouvea, Laguncularia, Nymphaea, Rhizophora* and the Malvaceae.

Among the water chemistry variables, the natural logarithmic (ln) transformation of POM and TSS were highly correlated in both data sets ($r > 0.91$, $P < 0.001$), and as expected a number of cations, Mg, Ca, and K, were highly intercorrelated and correlated with conductivity (for each ln transformed comparison, $r > 0.83$, $P < 0.001$). Additionally, ln altitude was negatively correlated with the ln of conductivity, Na, K, Ca, Mg and PO_4 in both the wet and dry season data (for each ln transformed comparison with ln altitude, $r < -0.403$, $P \leq 0.01$). Despite the high correlations among these variables and their correlation with altitude, all water chemistry parameters were retained for further analyses.

After elimination of rarely encountered plants as indicated above, 35 independent environmental variables remained in the dry season data set, whereas the wet season data set contained 42 independent variables. Univariate logistic regression (Hosmer and Lemeshow 1989) was employed to screen the data sets for variables that appeared to be associated with the presence/absence of larvae of each vector species, *viz. An. albimanus* and *An. pseudopunctipennis*. Logistic regression is particularly suited for such analyses because in logistic regression, unlike linear regression, the dependent variable is binary or dichotomous (Hosmer and Lemeshow 1989). In our study, the dependent variable is the presence or absence of larvae of a particular vector species. Additionally, logistic regression allows one to use the raw data and to mix variables with different distributions because logistic regression does not require the assumptions of normally distributed sets of independent variables with identical covariances (Udevitz et al. 1987) as does linear regression.

As an initial screening procedure, a univariate logistic regression equation in the form $Y = B_o + B_1 x$ was calculated for each independent variable, x, and tested for significance against a model fitted with only the respective constant, or $Y = B_o$, using the likelihood ratio test as outlined by Schlesselman (1982) and Hosmer and Lemeshow (1989). Environmental variables significantly associated ($a = 0.05$) with the presence/absence of either *An. albimanus* or *An. pseudopunctipennis* in either the dry or wet seasons were retained for further analyses (Tables 1 and 2). The goal of our initial screening procedure was to produce a list of all variables that might be associated with the presence or absence of a vector species. Therefore, environmental variables with P values between 0.1 and 0.05 for the above univariate tests were subjected to transformation and retested. Continuous variables such as Ca concentration were ln (x + 1) transformed. Plant variables that were originally expressed as percent cover were subjected to angular transformation (Sokal and Rohlf 1969) and also recoded as presence/absence variables. If after transformation a variable was significantly associated ($a = 0.05$) with the presence/absence of a vector species, it was also retained for further analyses. This transformation step allowed three additional variables, Na, water depth and the presence/absence of *Heteranthera*, to be retained for further analyses (Tables 1 and 2).

Relationships between the presence/absence of each vector species and the selected variables (Tables 1 and 2) were further explored with multiple logistic regression and discriminant analyses in order to select a reduced set of variables that appear to be most important for understanding the distribution of each vector species in each season. However, because *An. pseudopunctipennis* was encountered in only 2 of the 38 wet season sites, no multivariate analyses were conducted on the *An. pseudopunctipennis*-wet season data.

For each vector species a multiple logistic regression model using the respective selected dry season variables (Tables 1 and 2) was fitted. The significance of each variable and various combinations of variables were tested by comparison to regression models without these variables employing the likelihood ratio test as outlined in Hosmer and Lemeshow (1989). Nonsignificant ($a = 0.05$) variables were removed from the equation until a reduced logistic regression equation containing only significant variables was obtained for each vector species. Due to the large number of variables that appear associated with the distribution of *An. albimanus* in the wet season (Table 1) and the difficulty of deciding which variables to remove in the initial steps, a different approach was used to analyze the *An. albimanus*-wet season variables. In this case, we started by selecting one variable to produce a simple regression equation and tested each of the remaining variables for significance if added. The most significant variable as measured by the size of the corresponding likelihood ratio test statistic was then added to the equation. The original variable(s) was then retested for significance and eliminated or retained.

Discriminant analysis was conducted on the transformed variables selected by the univariate logistic analysis to select a reduced set of important variables, and to investigate from a purely heuristic point of view the correct assignment of sites as positive or negative for a particular vector species. Variables in Tables 1 and 2 were subjected to either ln (x + 1) or angular transformation (arcsine $\sqrt{p}$), with the exception of *Heteranthera* P/A, which was replaced with the angular transformation of the percent cover data. Initially, a discriminant function including all variables was generated for each species and season. Variables not significantly affecting correct site assignment were individually removed until a reduced discriminant function was obtained.

RESULTS AND DISCUSSION

Multiple logistic regression and discriminant analyses between the presence/absence of *An. albimanus* larvae and various combinations of the 4 dry season variables retained from the univariate logistic analyses (Table 1) were conducted to ascertain the importance and predictive ability of these variables with respect to *An. albimanus* larval distributions. Both methods revealed that planktonic algae, altitude and the presence of the floating macrophyte *Eichhornia crassipes* are important in understanding the distribution of *An. albimanus* larvae in the dry season.

Multiple logistic regression analysis suggests that 3 variables, planktonic algae, altitude and *Eichhornia*, are of primary importance in understanding the distribution of *An. albimanus* larvae in the dry season ($Y_{alb-dry} = -0.87 + 69.02$ PLANKALG + 0.16 EICH − 0.04 ALT, $\chi^2_{d.f.=3} = 29.82$, $P < 0.005$). Removal of ln Na from the 4-variable model barely changed the log likelihood and the null hypothesis, H_o:$B_{Na} = 0$, could not be rejected ($P > 0.50$). However, the separate removal of any one of the 3 remaining variables from the above model resulted in significant likelihood ratio test statistics ($P < 0.01$ for each test), indicating that each of the 3 included variables was important to understanding the distribution of *An. albimanus* larvae in the dry season.

Two discriminant functions, one with 3 variables, and the other with 2 variables, were able to classify *An. albimanus* positive and negative habitats with approximately equal success (Fig. 2). A discriminant function with 3 variables, ln planktonic algae, ln altitude and arcsine

Table 1. Variables determined by univariate logistic regression analyses to be significantly associated with the presence/absence of *Anopheles albimanus* larvae in the dry and wet seasons of 1988. Analyses conducted on raw data except ln (x + 1) transformation used for sodium (Na) in dry season analysis; chi-square value = −2 ln (likelihood ratio), d.f. = 1 for each comparison; type of association indicates whether variable pairs are negatively or positively associated, see Methods section for details.

Variable	Chi-square	*P*	Association
Dry season (*n* = 35)			
Planktonic algae (μg chlorophyll a/liter)	11.52	<0.005	positive
Eichhornia (% cover)	9.43	<0.005	positive
Na (ln(ppm + 1))	5.16	<0.025	positive
Altitude (m)	6.05	<0.025	negative
Wet season (*n* = 38)			
Planktonic algae (μg chlorophyll a/liter)	4.03	<0.050	positive
Cyperaceae (% cover)	8.03	<0.005	positive
Fimbristylis (% cover)	4.14	<0.050	positive
Avicennia (% cover)	4.14	<0.050	positive
Mimosa (% cover)	6.02	<0.025	positive
Conductivity (μS)	4.06	<0.050	positive
Ca (ppm)	6.85	<0.010	positive
K (ppm)	14.43	<0.005	positive
Mg (ppm)	4.62	<0.050	positive
Na (ppm)	4.15	<0.050	positive
PO4 (ppb)	12.64	<0.005	positive
Dissolved organic nitrogen (ppb)	6.60	<0.010	positive
Dissolved organic carbon (ppm)	4.31	<0.050	positive
Water color (colorimetric units)	9.50	<0.005	positive
Water temperature (°C)	8.12	<0.005	positive
Altitude (m)	5.25	<0.025	negative
Filamentous algae (% cover)	7.79	<0.010	negative
Salvinia (% cover)	4.69	<0.050	negative
Rhizophora (% cover)	3.88	<0.050	negative
TSS (ppm)	4.10	<0.050	negative

Table 2. Variables determined by univariate logistic regression analyses to be significantly associated with the presence/absence of *Anopheles pseudopunctipennis* larvae in the dry and wet seasons of 1988. Analyses conducted on raw data except presence/absence data used for *Heteranthera* and ln (x + 1) transformation used for water depth; chi-square value = −2 ln (likelihood ratio), d.f. = 1 for each comparison; type of association indicates whether variable pairs are negatively or positively associated, see Methods section for details.

Variable	Chi-square	*P*	Association
Dry season (*n* = 35)			
Altitude (m)	5.72	<0.025	positive
Filamentous algae (% cover)	6.65	<0.010	positive
Heteranthera (P/A)	10.52	<0.005	positive
Conductivity (μS)	5.95	<0.025	negative
K (ppm)	7.36	<0.010	negative
Mg (ppm)	4.56	<0.050	negative
Na (ppm)	7.69	<0.010	negative
Water depth (ln(cm + 1))	4.43	<0.050	negative
Wet season (*n* = 38)			
Filamentous algae (% cover)	5.24	<0.025	positive
Ludwigia (% cover)	6.74	<0.025	positive

Eichhornia, successfully classified 68.8% (11 of 16) of the *An. albimanus* positive sites, and 100% (19 of 19) of the negative sites (Fig. 2, top). A second discriminant function (Fig. 2, bottom) composed of the variables ln planktonic algae and arcsine *Eichhornia* correctly classified an equal number of positive sites (68.8%), but failed to correctly classify all negative sites (94.7% or 18 of 19). The inclusion of ln Na to produce a discriminant function including all 4 variables

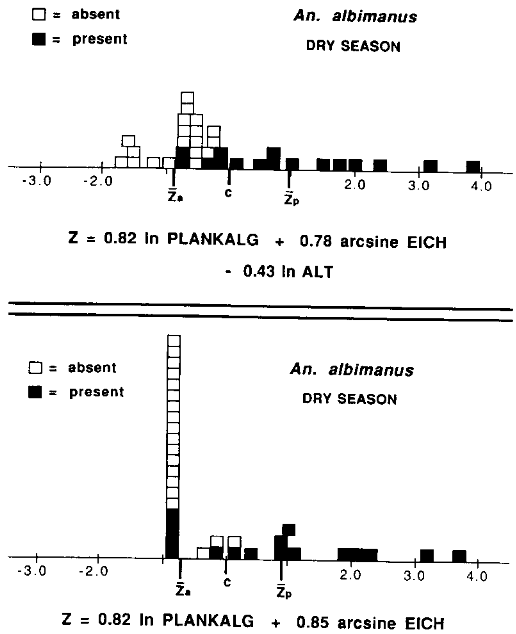

Fig. 2. Two discriminant functions that predict the absence/presence of *Anopheles albimanus* larvae with approximately equal success in the dry season, see results and discussion section for details. $\bar{Z}a$, $\bar{Z}p$ = respective group centroids for habitats in which *An. albimanus* was absent or present, c = cut off value for classification of sites. For upper function, F = 8.507, $P < 0.01$; for lower function, F = 10.837, $P < 0.01$.

(Table 1) did not improve the predictive ability for positive (68.8%) or negative (94.7%) sites over that of the 2-variable model.

In the discriminant analyses, ln Na was not predictive of *An. albimanus* sites if 2 or 3 of the other variables (Table 1) were also included in the function. Similarly, the addition of ln Na to either the 2- or 3-variable logistic regression models did not significantly ($a = 0.05$) alter the log likelihood for those equations. The positive correlation between *An. albimanus* and ln Na suggested in Table 1 stems from strong negative correlation between ln altitude and ln Na ($r = -0.735$, $P < 0.001$), and the common occurrence of *An. albimanus* in a wide range of coastal plain sites and its rarity in the more dilute waters of the foothill region in the dry season. The generally higher Na levels of coastal sites result from the mixing of brackish and freshwaters in coastal lagoons, aerosol inputs and resolution of evaporites by isolated or early rains. *Anopheles albimanus* is an established euryhalinic species, and we collected larvae in water with conductivity values ranging between 50–18,800 μS.

To evaluate whether *Eichhornia* could be replaced by the more general variable floating plants that include the common macrophytes *Eichhornia*, *Pistia*, *Salvinia* and *Lemna*, we replaced *Eichhornia* with arcsine floating plants in the 3-variable discriminant function including ln planktonic algae and ln altitude. This function correctly classified the same number of *An. albimanus* positive sites (68.8%) but misclassified 2 negative sites (89.5%, or 17 of 19) that had been correctly classified by the function with arcsine *Eichhornia*. Both misclassified sites had nearly total coverage by floating plants, in one case 95% by *Salvinia*, and in the other 100% by *Lemna*. We then formed a new variable including only the percent cover by *Eichhornia* and *Pistia*, and placed arcsine *Eichhornia* + *Pistia* in the discriminant function in place of floating plants. This new function also performed poorly, correctly classifying only 10 of 16 positive sites (62.5%), and 17 of 19 negative sites (89.5%). Based upon our preliminary data, use of the general variable, floating plants, is not justified as the included genera are biologically very different with respect to providing suitable habitat for *An. albimanus* larvae. Dense cover by *Salvinia* and *Lemna* apparently inhibits oviposition, and interferes with larval respiration and maintenance of surface position during feeding. In addition, dense mats of floating plants may be detrimental to *An. albimanus* larvae via their inhibitory effect on planktonic algae levels due to shading (Wetzel 1975), particularly as our dry and wet season data indicate that planktonic algae is the single best predictor of *An. albimanus* positive sites (Table 1).

The distribution of *An. albimanus* larvae in the wet season is correlated with a larger number of variables than in the dry season (Table 1). This correlation with a variety of variables reflects the wide diversity of habitats available to *An. albimanus* in the wet season and the propensity of *An. albimanus* to colonize seasonally flooded areas such as pastures. However, multiple logistic regression and discriminant analyses reveal that the distribution of *An. albimanus* larvae may be understood by employing combinations of 4 or 5 of the following 5 variables: planktonic algae, the Cyperaceae, PO_4, TSS and filamentous algae. *Anopheles albimanus* larvae are positively associated with planktonic algae, PO_4 and emergent plants of the family Cyperaceae, indicating that the larvae are typically encountered in sunlit, productive water bodies (higher planktonic algae and PO_4 levels) and in association with seasonally flooded emergent plants particularly the Cyperaceae. Larvae are negatively associated with total suspended solids (TSS), suggesting a preference for relatively undisturbed areas, for example, sites with some protection from heavy rains, or animal and auto traffic. *Anopheles albimanus* larvae were also negatively associated with filamentous algae and were rarely encountered in association with abundant filamentous algae, primarily *Spirogyra* spp. and *Cladophora* spp.

Multiple logistic regression selected 4 variables, planktonic algae, the Cyperaceae, PO_4 and TSS, as being most important in understanding the distribution of *An. albimanus* positive and negative sites during the wet season ($Y_{alb-wet}$ = 0.61 + 5.34 PLANKALG + 1.36 CYPERACEAE + 0.01 PO_4 − 0.16 TSS, $\chi^2_{d.f.=4} = 29.90$, $P < 0.005$). The separate addition of each remaining wet season variable (Table 1) to the above 4-variable equation produced no significant test statistics ($a = 0.05$), indicating that none of the remaining variables contributed significant independent information regarding the distribution of *An. albimanus* larvae. Indeed, only one variable, filamentous algae ($\chi^2_{d.f.=1} = 2.60$, $0.2 > P < 0.1$), was associated with a likelihood ratio test statistic that suggested contribution of some independent information, although this change was not significant at the $a = 0.05$ level. In contrast, the separate removal of each variable from the above 4-variable model resulted in significant likelihood ratio test statistics ($P < 0.02$ for each test), indicating that each of the 4 included variables is important in understanding the distribution of *An. albimanus* larvae in the wet season.

The most efficient discriminant function for classifying *An. albimanus* positive and negative sites in the wet season included the 4 variables, ln planktonic algae, arcsine Cyperaceae, ln PO_4 and ln TSS. These 4 variables correctly classified 88.5% (23 of 26) of the *An. albimanus* positive sites, and 83.3% (10 of 12) of the negative sites (Fig. 3). Addition of filamentous algae as suggested by the multiple logistic regression analysis to the above 4-variable discriminant function resulted in one additional correct classification of a positive site (92.3% or 24 of 26), but the same correct classification rate for negative sites (83.3%). In contrast, removal of any single variable from the 4-variable function resulted in marked decreases in correct site classification.

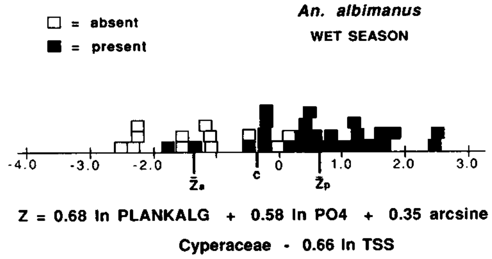

Fig. 3. Most efficient discriminant function for predicting the absence/presence of *Anopheles albimanus* larvae in the wet season. $\bar{Z}a$, $\bar{Z}p$ and c as in Fig. 2. F = 7.332, $P < 0.01$.

The 15 remaining variables selected by the univariate logistic analysis (Table 1) did not enter the discriminant or multiple regression equations largely because the associations among these variables and *An. albimanus* were parceled out or removed by one or more of the 4–5 selected variables. Additionally, due to the large number of univariate tests conducted, we should expect a couple of biologically nonsignificant variables to be found to be statistically significant by chance alone (1 of 20 at $a = 0.05$). Despite the possibility of chance significance, the association between *An. albimanus* and certain remaining variables merits comment. *Fimbristylis* was excluded from the multivariate analyses because this genus is a member of the Cyperaceae, which is more highly correlated with *An. albimanus* than *Fimbristylis* alone. The positive associations between *An. albimanus* and Ca, K, Mg, Na, and conductivity and the negative association with altitude are indicative of the common occurrence of *An. albimanus* on the coastal plain, and the higher levels of these elements in waters at lower elevations (for each comparison between ln ALT and ln Ca, ln K, ln Mg, ln Na, ln COND, $r < -0.47$, $P < 0.01$). The positive associations between *An. albimanus* larvae and dissolved organic nitrogen and carbon likely stem from the preference of *An. albimanus* for productive water bodies with higher levels of planktonic algae and microbes. The negative effect of dense cover by *Salvinia, S. minima* and *S. auriculata* were present in our study area, on *An. albimanus* populations apparent in our data (Table 1) has also been observed by other authors. Hobbs and Molina (1983) report that dense cover by *S. auriculata* significantly depressed *An. albimanus* larval populations in experimental ponds in Guatemala, and that *An. albimanus* females did not oviposit in trays covered with *Salvinia* under laboratory conditions. Abundant growths of *Salvinia* appear to provide a mechanical barrier by reducing or eliminating open areas for larval respiration and adult oviposition.

The variables determined to be most important for understanding the distribution of *An. albimanus* larvae in the wet and dry seasons reflect seasonal differences in habitat availability and the ecological preferences of this species (Table 1, Figs. 2 and 3). The only variables significantly associated with the presence/absence of *An. albimanus* larvae in both seasons are planktonic algae and altitude. Planktonic algae were the only variable positively correlated with *An. albimanus* sites in both data sets. High planktonic algae levels are indicative of sunlit, productive water bodies with sufficient food resources to support rapid growth and development of *An. albimanus* larvae; although, *An. albimanus* larvae do not appear to use significant amounts of planktonic algae as a food source. Altitude was negatively correlated with *An. albimanus* sites in both seasons, reflecting the common association between *An. albimanus* and low coastal areas, although this correlation is weaker in the wet season. The remaining variables differ sharply by season. In the dry season, flooded pastures and other temporary and seasonally flooded habitats are largely absent, and *An. albimanus* is restricted to the margins of permanent lakes, ponds and ditches where floating macrophytes (Orr and Resh 1989), particularly *Eichhornia*, provide refuge from predators. When the wet season rains come, immense areas of flooded pasture and other seasonal habitats become available. *Anopheles albimanus* females readily oviposit and colonize these productive temporary habitats, producing significant associations between *An. albimanus* larvae and emergent plants, particularly the Cyperaceae.

Eight dry season variables selected by univariate logistic regression analyses (Table 2) were evaluated to ascertain their importance in understanding the distribution of *An. pseudopunctipennis* larvae in the dry season. Multiple logistic regression on these 8 variables revealed that the best combination of variables for understanding *An. pseudopunctipennis* distributions in the dry season included only 2 variables, percent cover by filamentous algae, primarily *Spirogyra* spp. and *Cladophora* spp., and the presence/absence of *Heteranthera* ($Y_{pseudo\text{-}dry} = -3.49 + 0.05$ FILAMALG + 4.21 HETERANTHERA P/A, $\chi^2_{d.f.=2} = 17.51$, $P < 0.005$). However, when the variable, presence/absence of *Heteranthera*, was replaced with the variable, percent cover by *Heteranthera*, *Heteranthera* was not significantly associated with *An. pseudopunctipennis* larval distributions and was deleted from the equation. Reanalysis employing the percent cover of *Heteranthera* variable and the remaining 7 variables in Table 2 suggests that filamentous algae, conductivity and water depth are of prime importance in understanding the distribution of *An. pseudopunctipennis* larvae in the dry season ($Y_{pseudo\text{-}dry} = 3.46 + 0.05$ FILAMALG − 0.01 COND − 1.68 ln WD, $\chi^2_{d.f.=3} = 17.71$, $P < 0.005$).

Discriminant analyses on the 8 transformed variables, including arcsine percent cover by *Heteranthera*, revealed that the best combination of variables for predicting *An. pseudopunctipennis* positive and negative sites in the dry season included the 3 variables, arcsine filamentous algae, ln altitude and ln water depth. The discriminant function including these 3 variables correctly classified 71.4% (5 of 7) of the *An. pseudopunctipennis* positive sites, and 89.3% (25 of 28) of the negative sites in the dry season (Fig. 4). The addition of any 2, including arcsine *Heteranthera* and ln conductivity, of the remaining 5 variables to the discriminant function did not increase the number of correct classifications.

Each of the 4 variables, ln conductivity, ln Na, ln Mg and ln K, is negatively correlated with ln altitude (for all comparisons with ln altitude, $r < -0.68$, $P < 0.001$), and hence also negatively correlated with *An. pseudopunctipennis* (Table 2). The negative correlations between *An. pseudopunctipennis* and conductivity, Na, Mg and K (Table 2) stem from the preference of *An. pseudopunctipennis* for river floodplain sites and higher elevations. When present at lower elevations, *An. pseudopunctipennis* is nearly always encountered in a riverine setting, and never in brackish or mixed brackish-freshwater sites of elevated conductivity and salinity. In this respect, simple conductivity measurements could be used to define sites that are unsuitable for *An. pseudopunctipennis* larvae. During our limited survey we collected *An. pseudopunctipennis* larvae only from waters with conductivity values of less than 450 μS. When sites with conductivity values of 2,000 μS and greater are eliminated from the dry season data set, none of these four variables is significantly correlated with *An. pseudopunctipennis* ($P > 0.05$). Therefore, within the normal range of freshwater habitats, none of these 4 variables appears important in understanding the distribution of *An. pseudopunctipennis* larvae.

The plant genus *Heteranthera* (in our study area *H. reniformis* was the most common species) does appear to be associated with *An. pseudopunctipennis* positive sites in the dry season as suggested by the multiple logistic regression analysis. These plants typically occur on moist soil along the margins of streams and lakes (Beal 1977) and in shallow water, particularly seasonally or temporarily flooded sites. In our dry

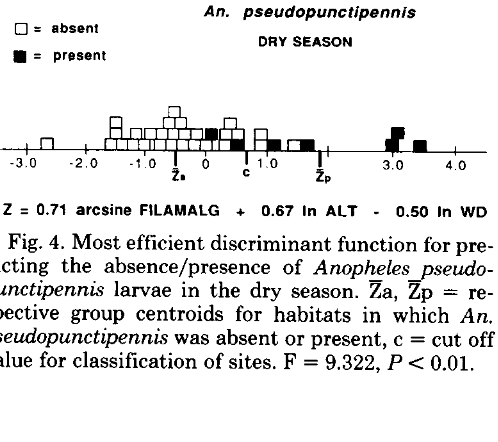

Fig. 4. Most efficient discriminant function for predicting the absence/presence of *Anopheles pseudopunctipennis* larvae in the dry season. $\overline{Z}a$, $\overline{Z}p$ = respective group centroids for habitats in which *An. pseudopunctipennis* was absent or present, c = cut off value for classification of sites. F = 9.322, $P < 0.01$.

season survey, we encountered *Heteranthera* only at elevation of 58 m and above. Due to its common occurrence in floodplain pools and along stream margins at higher elevations, *Heteranthera* was encountered in 57% (4 of 7) of the *An. pseudopunctipennis* positive sites. However, *Heteranthera* was not significantly associated with *An. pseudopunctipennis* if the percent cover data were employed, and the inclusion of arcsine *Heteranthera* did not improve the predictive ability of the discriminant function (Fig. 4) because the variables ln water depth and ln altitude parceled out much of the correlation between *Heteranthera* and *An. pseudopunctipennis*. However, if one approaches the definition of *An. pseudopunctipennis* habitats from a purely botanical perspective, the discriminant function including only the variables arcsine filamentous algae and arcsine *Heteranthera* classifies *An. pseudopunctipennis* positive (71.4%) sites as well as the function employing the variables arcsine filamentous algae, ln altitude and ln water water depth (Fig. 4), and misclassifies only one additional negative (85.7% or 24 of 28) site. The caveats that keeps us from recommending the purely botanical approach are of 2 types. The first is that we have observed *Heteranthera* at lowland sites (10 m) on other field trips. Therefore, the correlation between *Heteranthera* and altitude that increases the predictive ability of *Heteranthera* could be an artifact of our dry season data set. Secondly, the addition of *Heteranthera* as a fourth variable in the discriminant function in Fig. 4 results in one additional misclassification of a negative site as a positive site and thereby decreases the predictive ability for negative sites (85.7%). The misclassification results from the presence of *Heteranthera* in a nontypical *An. pseudopunctipennis* habitat. *Heteranthera* is likely only a good indicator of the presence of *An. pseudopunctipennis* when encountered along a stream margin or in a floodplain pool setting. Therefore, the presence of *Heteranthera* along a lake or pond margin, particularly at low elevation, would not be indicative of a likely *An. pseudopunctipennis* positive site.

In summary, the dry season data indicate that *An. pseudopunctipennis* positive sites are typically shallow, freshwater sites with abundant filamentous algae, located more commonly at higher elevations, or more commonly in the foothills than on the coastal plain, and by the presence of *Heteranthera* if in a stream setting.

In the wet season, *An. pseudopunctipennis* was encountered in only 2 of 38 sites. Typical *An. pseudopunctipennis* larval habitats, that is floodplain pools, stream margins and small streams and ditches in the foothills, are destroyed by flooding associated with the heavy wet season rains. A similar seasonal pattern in the abundance of suitable *An. pseudopunctipennis* habitats has been documented in other areas with similar wet and dry seasons (Kumm and Zuniga 1942, De Zulueta and Garrett-Jones 1965). Due to the rarity with which *An. pseudopunctipennis* was encountered in the wet season, the univariate logistic regression data (Table 2) must be interpreted with caution and further statistical analyses are not justified. The association between *An. pseudopunctipennis* and filamentous algae suggested by the wet season data follows a pattern similar to that observed in our dry season data and by other workers (Hoffmann and Samano 1938, Kumm and Zuniga 1942). The association between *An. pseudopunctipennis* and *Ludwigia* is probably spurious and an artifact of the small number of *An. pseudopunctipennis* positive sites, and this association needs to be tested with a larger data set. In our wet season data, *An. pseudopunctipennis* larvae were encountered in 33% (2 of 6) of the *Ludwigia* positive sites.

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REFERENCES CITED

American Public Health Association. 1985. Standard methods for the examination of water and waste water. 16th ed. APHA, Washington, DC.

Beal, E. O. 1977. A manual of marsh and aquatic vascular plants of North Carolina with habitat data. N. C. Agric. Exp. Sta. Tech. Bull. 247:1–298.

Breedlove, D. E. 1981. Flora of Chiapas. Part 1. Calif. Acad. Sci., San Francisco.

De Zulueta, J. and C. Garrett-Jones. 1965. An investigation of the persistence of malaria transmission in Mexico. Am. J. Trop. Med. Hyg. 14:63–77.

Hobbs, J. and P. Molina. 1983. The influence of aquatic fern *Salvinia auriculata* on the breeding of *Anopheles albimanus* in coastal Guatemala. Mosq. News 43:456–459.

Hoffmann, C. C. and A. Samano B. 1938. Los criaderos invernales de *Anopheles pseudopunctipennis* en el estado de Oaxaca. An. Inst. Biol. Univ. Nac. Auton. Mex. 9:182–192.

Hosmer, D. W. and S. Lemeshow. 1989. Applied logistic regression. J. Wiley, New York.

Janik, M. and E. Byron. 1987. Lake Tahoe interagency monitoring program. Quality assurance manual. Dept. Environ. Studies, Univ. Calif., Davis.

Kumm, H. W. and H. Zuniga. 1942. The mosquitoes of El Salvador. Am. J. Trop. Med. 22:399–415.

Miranda, F. 1952. La vegetation de Chiapas. Tuxtla Gutierrez, Mexico.

Orr, B. K. and V. H. Resh. 1989. Experimental test of the influence of aquatic macrophyte cover on the survival of *Anopheles* larvae. J. Am. Mosq. Control Assoc. 5:579–585.

Schlesselman, J. J. 1982. Case-control studies. Oxford Univ. Press, New York.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. Freeman, San Francisco.

Strickland, J. D. H. and T. R. Parsons. 1972. A practical handbook of seawater analysis. Bull. 167. Fish. Res. Board Canada, Ottawa, Ontario, Canada.

Udevitz, M. S., P. Bloomfield and C. S. Apperson. 1987. Prediction of the occurrence of four species of mosquito larvae with logistic regression on water-chemistry variables. Environ. Entomol. 16:281–285.

Wetzel, R. G. 1975. Limnology. Saunders, Philadelphia.