# DISTRIBUTION OF *ANOPHELES QUADRIMACULATUS* AND *AN. CRUCIANS* LARVAE WITHIN RICE FIELD HABITATS IN SOUTHWESTERN LOUISIANA

R. E. MCLAUGHLIN,[1] M. F. VIDRINE[2] AND O. R. WILLIS[1]

*U. S. Department of Agriculture, Agricultural Research Service, Insects Affecting Man and Animals Research Laboratory, P. O. Box 14565, Gainesville, FL 32604*

ABSTRACT. This study, conducted in 3 parishes of Louisiana, documented the distribution pattern of *Anopheles quadrimaculatus* and *An. crucians* during the first crop season. Ratios of numbers of 3rd and 4th instar larvae of *An. quadrimaculatus* to the number of *An. crucians* 3rd and 4th instar larvae (Q/C), varied both spatially and temporally. The mean Q/C ratio during 1984 was 1.37; during 1985 it was 1.40. The ratios changed from week to week during each year, and increases or decreases in a particular week of 1984 were paralleled by increases or decreases during that same week of 1985. The changes in relative abundance of the 2 species were strongly related to the timing of irrigation practices and to a succession of habitat conditions. *Anopheles quadrimaculatus* larvae were dominant or exclusively present for several days after fresh water was added to the field. *Anopheles crucians* larvae then became dominant or exclusively present as the habitat progressed towards a eutrophic condition. Irrigation influenced the conditions of the habitat by freshening and deepening the water, causing a reversion towards a more oligotrophic condition.

## INTRODUCTION

Larvae of *Anopheles crucians* Wiedemann and *An. quadrimaculatus* Say normally are found in semi-permanent or permanent bodies of water. Carpenter and LeCasse (1955) reported that *An. crucians* larvae are found in ponds, lakes, swamps and semi-permanent and permanent pools, whereas *An. quadrimaculatus* larvae are found in permanent fresh water in sluggish streams, canals, ponds, lakes, and only occasionally in pools of a temporary nature. Breeland et al. (1961) reported both species in Tennessee and pointed out the occurrence of *An. crucians* in woodland acidic waters. Thus, historically the habitats have been reported as predominantly non-agricultural land use areas. Manipulation of arable land for rice production has created another environment for anophelines.

*Anopheles quadrimaculatus* adults were reported in the rice growing area around Stuttgart, Arkansas (Gahan et al. 1969), and in Brazoria County, Texas (Kuntz et al. 1982). Larvae of *An. crucians* were reported from 2 rice fields in Louisana (Andis and Meek 1984). Steelman et al. (1973) reported adults of both species around cattle herds and rice fields in Acadia Parish, Louisiana, and Lacey and Inman (1985) reported larvae of both species in Louisiana rice fields. Chambers et al. (1979) reported *An. crucians* present throughout Louisiana, but did not report *An. quadrimaculatus* larvae from rice fields in the central Louisiana region of Calcasieu, Jefferson Davis, and Acadia parishes. The occurrence of larvae is, of course, direct evidence of oviposition by adults of either species, and thus their relative distribution depicts the behavioral differences between the females of each species. The original aim of this study was to describe differences in larval distribution as a vital first step toward gaining an understanding of the basic patterns of utilization of the riceland habitat by these species.

Research was undertaken to determine the spatiotemporal occurrence of larvae of each species in southwestern Louisiana rice fields. The study was to determine the ratio of: (1) the number of 3rd and 4th instar larvae of *An. quadrimaculatus* to the number of 3rd and 4th instar larvae of *An. crucians* (the Q/C ratio) throughout the rice production season and; (2) the 2 species in relation to habitat changes in rice fields brought about by climatic and agricultural water management events.

## MATERIALS AND METHODS

The study area included 2300 km$^2$ (900 mi$^2$) in 3 southwestern Louisiana parishes in 1984 and 220 km$^2$ (85 mi$^2$) in 1985. The rice-soybean crop system interspersed with cattle, wheat and sorghum involves irrigation, drainage and reflooding of about 45% of the land each year.

The rationale for selection of rice fields to be sampled was different for each year. In 1984 sampling was based upon the concept of widely dispersed random sampling to provide a seasonal phenology of the occurrence of each species. Thus, collections were made only once from any particular field. Fields were selected each

[1] U. S. Department of Agriculture, Agricultural Research Service, Insects Affecting Man and Animals Research Laboratory, P.O. Box 14565, Gainesville, FL 32604.

[2] Department of Biological Sciences, Louisiana State University, Eunice, LA 70535.

week from a table of random numbers. Ten samples, each consisting of the larvae collected in 2 dips from each side of an earthen levee that subdivided the field into smaller areas (pans), were taken with a 400-ml dipper at 10-step intervals. Two levees were routinely transversed to sample 4 pans in each field. Occasionally, because of changes in personnel availability and work schedules, collections were made from either one or 4 levees. The 3rd- and 4th-instar larvae in each sample were identified in the laboratory.

The next year, sampling procedures were altered in order to study changes in the Q/C at specific sites as the season progressed and also to obtain larger and more consistent sample sizes. Collections in 1985 consisted of repetitive sampling of 24 sites, commencing May 31 and continuing once each week until they had all been drained for harvest on about July 26. The sampling site was always at the same location in a field and usually at the edge. A relatively small and uniform area was sampled to eliminate within-field variability encountered by traversing long distances of widely separated levees as had been done in 1984. Dippers were used but when water levels were low, larvae were collected with a pipette after detection in water that had been deliberately muddied by stepping around in the area to disturb the plant/water interface and to float the larvae away from the vegetation. Samples of at least thirty 3rd and 4th instar larvae were collected from each of these locations each week when possible.

Some exceptions were made to this sampling procedure. One field (#219) was sampled every Monday, Wednesday and Friday because of its unique location adjacent to a pen of sheep, several sheds housing chickens and pigeons, and the presence of cows, goats, mules and dogs. Large populations of adults of both species were observed in the sheds. Another site (field #61) was sampled 5 days/week along a levee.

A habitat description of each collection site in 1985 included the general appearance of the water, its depth, clarity or turbidity, and the interval since new water had been added. Interval estimates were usually accurate within 3–4 days prior to the sampling date and were obtained by observing water being added at the inlet into the field or from the farmer. The presence and abundance of surface coverage of aquatic plants such as *Heteranthera limnosa* (Sw.) Willd., *Spirodella polyrhiza* Linn., and/or *Lemna* spp. was noted. Submerged vegetation, including algal mats of *Spirogyra* spp. and other algae, was noted in addition to flowering plants. A subjective assessment of abundance of the aquatic fauna was also included. The observed fauna consisted of ostracods, beetles, dragonfly and damselfly nymphs, true bugs, mayflies, mites, snails, turbellarians and free-swimming colonial rotifers.

Habitat variability was assessed on the basis of: (1) interval between floodings and, (2) progression from oligotrophic to eutrophic conditions. The changes in the rice field habitat formed a continuum that we divided into 5 successional seres. The categories were established on the basis of our experience with the 37 fields in 1985 and from more than 238 first-crop fields in 1984 as follows:

Sere I. Fresh clear water fully flooding the pan; immature rice; water free of submerged or surface vegetation; and soil barren.

Sere II. Fresh clear water fully flooding the pan; immature rice; some algae obvious and young submerged plants present; no blue green algal aggregations present; and, moderate vegetation on soil.

Sere III. Water clear but more than 1 week old; algal growth modest; and submerged plants have emerged above water surface.

Sere IV. Water shallow, barely covering the soil and pan surface; algal mats obvious and abundant; submerged vegetation well above water surface; and soil obscured by abundant vegetation.

Sere V. Climax community in some fields; water usually clear, but colored and sometimes turbid; water level low and present only in ruts and other field depressions; algal mats prolific and well developed, and mature to aged or dying; and, surface of water covered by floating vegetation.

Another condition was noted in which the water became turbid or remained clear, but had noticeable quantities of blue-green algal aggregations, e.g., *Anabaena* spp. Another variable affecting the water state was the addition of water by the farmer or from rainstorms. Such events usually resulted in regression to an earlier sere.

## RESULTS

*Distribution of the species among fields during the season.* During the first crop rice season of 1984, 238 collections from as many fields contained 973 3rd and 4th instar *Anopheles* spp. larvae. Of these, 562 were *An. quadrimaculatus* for a Q/C = 1.37, or 42.2% *An. crucians.* During the first crop season of 1985, 153 collections from 37 fields contained 4,146 larvae. Of these, 2,416 were *An. quadrimaculatus* (Q/C = 1.4), or 41.7% *An. crucians.* The weekly results are presented in Table 1.

The seasonal averages and weekly ratios were similar each year. An increase or decrease in the ratios for any specific week in 1984 were paralleled by a similar change in that week in 1985. The only exception to this trend occurred in mid-July of each year when the ratio value decreased from 1.72 the week of 7/9–12 to 1.58 the next week in 1984 but increased from 1.05 the week of 7/8–12 to 1.66 the next week in 1985. *Anopheles quadrimaculatus* outnumbered *An. crucians* in all weeks except July 1–5, 1985.

The changes in the relative abundance of each species at a specific site as the season progressed was examined by intensive sampling (3–5 days/week) of fields 61 and 219, by comparison of the Q/C ratios to time since irrigation and by comparison of Q/C ratios to habitat classifications.

*Distribution of the species within fields as the season progressed.* The relation of the Q/C to water addition in field #61 is shown in Fig. 1. The field was initially fully flooded on May 31. Larval populations were detected on June 3. The ratio that week was 2.125, but declined steadily until 4 days after initiation of the next reflooding event, at which time the ratio was 0.11 (or 9 times as many *An. crucians* as *An. quadrimaculatus*). The ratio then increased after the irrigation, but did not exceed 1.00 until after the next flooding. The sample taken 4 days after start of the irrigation contained young 3rd instar larvae, and the ratio was again high. The developmental rate for anopheline larvae at this time of the year is about an instar a day until the 3rd instar. Thus, the data points in Fig. 1 show that *An. quadrimaculatus* adults probably commenced ovipositing very soon after the addition of fresh water to the field.

The consistent relationship of species dominance to habitat conditions as influenced by irrigation was also noted in field #219 in 1985. The observations from this habitat were particularly significant because of the presence of a large population of adults of both species across the road in the sheds containing chickens and pigeons. Irrigation in this field was such that the habitat never became freshened. Eutrophic conditions with shallow water and copious growth of algae and small plants persisted throughout the season. *Anopheles crucians* was dominant or exclusively present in all but one collection, which was obtained after a shallow irrigation. The ratio reverted to *An. crucians* dominance at the next collection.

*Changes of Q/C in relation to the time since irrigation.* The Q/C values decreased as time after flooding increased. Larval samples in 9 of the 95 (9.5%) collections were exclusively one species or the other. There were 4 of 23 collections at 3 days and 1 of 34 collections at 7 days that were 100% *An. quadrimaculatus*. There were 2 of 27 collections at 13 days and 2 of 11

Table 1. Ratios (Q/C) of the number of 3rd and 4th instar larvae of *Anopheles quadrimaculatus* to the number of 3rd and 4th instar larvae of *An. crucians* in first crop rice fields in southwestern Louisiana in 1984 and 1985.

| Dates 1984 | No. of coll/ no. larvae | Ratio (Q/C) | Dates 1985 | No. of coll/ no. larvae | Ratio (Q/C) |
|---|---|---|---|---|---|
| June 1–4 | 5/31 | 1.21 | June 3–7 | 24/555 | 1.77 |
| June 12–15 | 26/114 | 1.59 | June 10–14 | 20/570 | 1.89 |
| June 18–21 | 43/160 | 1.05 | June 17–21 | 21/636 | 1.03 |
| June 25–29 | 41/137 | 1.26 | June 24–28 | 24/634 | 1.83 |
| July 2–5 | 31/112 | 1.04 | July 1–5 | 17/466 | 0.75 |
| July 9–12 | 41/239 | 1.72 | July 8–12 | 23/668 | 1.05 |
| July 16–19 | 37/116 | 1.58 | July 15–19 | 16/412 | 1.66 |
| July 23–25 | 14/64 | 1.46 | | | |
| Total | 238/973 | 1.37 | Total | 149/3,994 | 1.32 |

Table 2. Mean (± SD) of the ratio (Q/C) of the number of 3rd and 4th instar *Anopheles quadrimaculatus* larvae to the number of 3rd and 4th instar *An. crucians* larvae in relation to the successional sere of the rice field, Acadia Parish, southwestern Louisiana, 1985.

| | | | | | No. collections with 100% | |
|---|---|---|---|---|---|---|
| Sere* | No. | Mean | (SD) | Range | *An. quadrimaculatus* | *An. crucians* |
| I | 28 | 7.7 | (6.3) | 1.7–29.0 | 4 | 0 |
| II | 28 | 3.6 | (3.0) | 1.0–14.0 | 2 | 0 |
| III | 26 | 2.2 | (1.5) | 0.2–6.5 | 0 | 0 |
| IV | 16 | 1.1 | (1.3) | 0.1–3.7 | 0 | 2 |
| V | 21 | 0.6 | (0.7) | 0.1–2.8 | 0 | 4 |

* See Materials and Methods section.

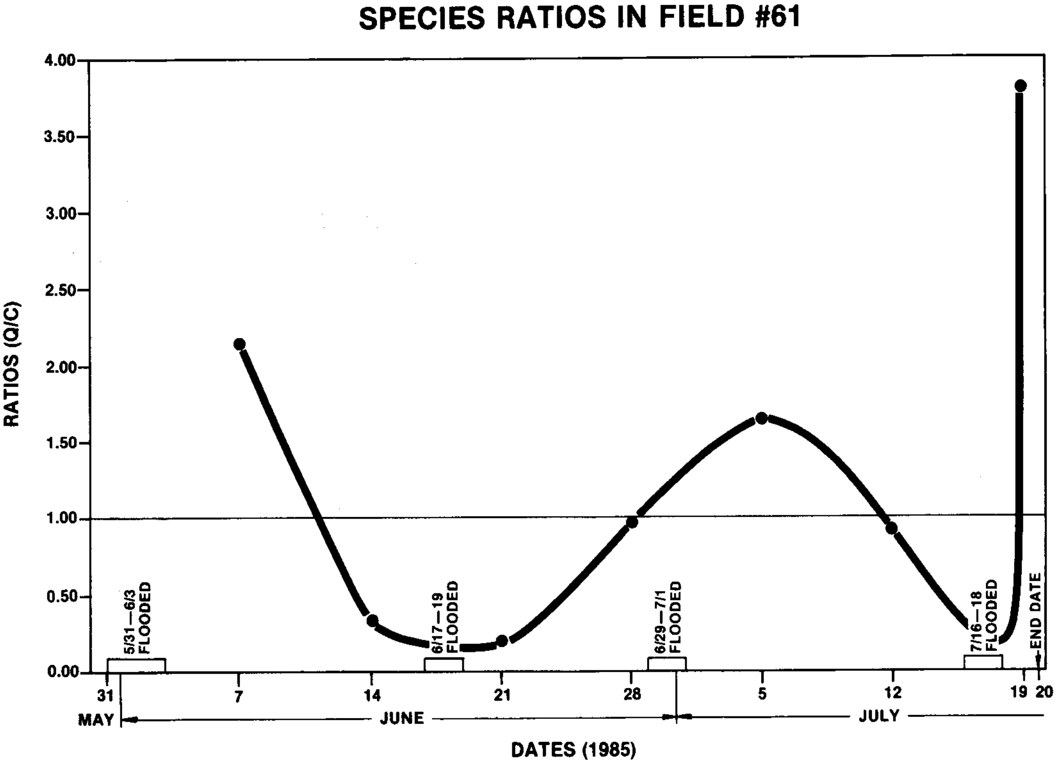


Fig. 1. Ratio (Q/C) of 3rd and 4th instar larvae of *Anopheles quadrimaculatus/An. crucians* collected in rice field #61 in Acadia Parish, Louisiana, near the town of Iota, 1985. A ratio of 1.0 indicates equal numbers of each species; a ratio >1 signifies dominance by *An. quadrimaculatus*; a ratio of <1 signifies dominance by *An. crucians*. Days during which water was being added to the field are shown as arrows.

collections at 20 days that were 100% *An. crucians*. The progression was from totally *An. quadrimaculatus* in the 3-day or 7-day period postirrigation to totally *An. crucians* in the 13- and 20-day postirrigation periods. Although sampling limitations may well have resulted in failure to detect extremely low numbers of one species in the overwhelming presence of the other, recognition of the existence of populations that are composed of nearly 100% of one species adds to the understanding of the phenomena. The mean Q/C ratios, standard deviation, and ranges for the 4 time periods postirrigation were: 3 days (8.7 ± 6.6, 0.4–15.7); 7 days (3.2 ± 3.1, 0.2–6.0); 13 days (1.3 ± 1.2, 0.2–3.3); and 20 days (0.8 ± 1.1, 0.1–1.1).

A linear regression (excluding the 9 occasions when the samples were 100% of one species) of the Q/C against the number of days after flooding showed a highly significant correlation, ($P > F = 0.001$). The estimate of the intercept was 10.72 and the slope (B-value) was −0.64. The r-square value showed that 72% of the variation in the ratios was associated with number of days after flooding.

Although these data showed that the Q/C changed with time, some sites were dominated by one species over the majority of the season. Of the 24 sites studied, 15 had a seasonal mean ratio in favor of *An. quadrimaculatus*. Of these 15 sites, 9 were always dominant for *An. quadrimaculatus*, and 2 were dominant for *An. crucians* for only a single sample period. Of the 9 sites with a seasonal mean ratio in favor of *An. crucians*, only one field was always dominated by *An. crucians* and 4 additional fields were dominated by *An. quadrimaculatus* in one sampling period. Eleven sites exhibited a reversal of species dominance at least once during the season.

*Changes of Q/C in relation to habitat classification:* The ratios in sites categorized into seres I–V are shown in Table 2. Each specific site usually progressed through a continuum of successional changes. The decision as to whether a site was sere I or II, or whether it was sere II or III was sometimes difficult. Seres I, IV, or V were always definitive. No difficulty was encountered in deciding between seres that were 2 categories apart from each other in the scale, such as I and III or II and IV. The number of collections was reasonably well distributed among seres (Table 2) *Anopheles quadrimaculatus* larvae were dominant in the more oligotrophic sites, and *An. crucians* was dominant in the more eutrophic sites. At no time did we observe a marked shift from one dominant species to the other without the occurrence of an appropriate change in the habitat.

Inspection of the observations on flora and fauna at the fixed sites revealed no indication that the abundance or type of invertebrates or vegetation serve as indicators of the presence of anopheline species. However, 8 sites that were excluded from these data because they had very few or no anophelines did have noticeable quantities of blue-green algae. The blue-green algal clumps in these fields occurred as small (less than 5 cm) discrete masses sparsely developed and separated by clear water areas 10 times greater than the diameter of the algal clump.

## DISCUSSION

Changes occurred in the habitat in relation to irrigation. Newly flooded fields were oligotrophic early in the season. The flora and fauna changed as time increased following the addition of water. Lowered water levels (due to transpiration, evaporation, and soil absorption) were followed by additional irrigation. Later in the season eutrophic fields never became as oligotrophic after irrigation as they originally were. Management practices varied, and some fields became almost dry before water was added. Other fields were irrigated after relatively small water level decreases. These events resulted in extreme habitat variation from site to site (spatial) and within each site (temporal). Some habitats seemed to maintain a more stable condition because of consistently high or low water level maintenance practices.

This study suggests that adult ovipositional behavior tends to separate the immature stages of *An. quadrimaculatus* and *An. crucians* both spatially and temporally in the riceland ecosystem. The same selection process may have resulted in the historical habitat categorization between fresh water ponds and woodland pools (Carpenter and LeCasse 1955). Extensive literature exists regarding selective ovipositional behavior and larval mortality regulating factors in the habitat. One specific report established precedent for successional mosquito species occurrence in rice fields. Sen (1948) reported 11 anopheline species occurred selectively in rice fields of lower Bengal according to the successive changes in the normal maturity of the crop and aging of the water. He further observed that these normally occurring species successions were modified according to irrigation and heavy rains. Further study is needed to detect and define the more specific factors influencing ovipositional behavior and to determine whether the larvae are selectively influenced by increased mortality factors. Such basic information will lead to improved understanding of the population dynamics of anopheline mosquitoes in the riceland ecosystem.

## ACKNOWLEDGMENTS

The authors acknowledge the assistance of John Billodeaux, Director, Jefferson Davis Mosquito Control Unit No. 1, Jennings, LA, and his staff for their cooperation in the conduct of this study.

## REFERENCES CITED

Andis, M. D. and C. L. Meek. 1984. Bionomics of Louisiana riceland mosquito larvae. II. Spatial dispersion patterns. Mosq. News 44:371–376.

Breeland, S. G., W. E. Snow and E. Pickard. 1961. Mosquitoes of the Tennessee Valley. J. Tennessee Acad. Sci. 36:249–319.

Carpenter, S. J. and W. J. LaCasse. 1955. Mosquitoes of North America (north of Mexico). Univ. of Calif. Press, Berkeley and Los Angeles, CA.

Chambers, D. M., C. D. Steelman and P. E. Schilling. 1979. Mosquito species and densities in Louisiana ricelands. Mosq. News 39:658–668.

Gahan, J. B., H. G. Wilson and D. E. Weidhaas. 1969. Behavior of *Psorophoro* spp. and *Anopheles quadrimaculatus* inside and around untreated farm buildings in a rice growing area. Mosq. News 29:574–582.

Kuntz, K. J., J. K. Olson and B. J. Rade. 1982. Role of domestic animals as hosts for blood-seeking females of *Psorophora columbiae* and other mosquito species in Texas ricelands. Mosq. News 42:202–210.

Lacey, L. A. and A. Inman. 1985. Efficacy of granular formulations of *Bacillus thuringiensis* (H-14) for the control of *Anopheles* larvae in rice fields. J. Am. Mosq. Control Assoc. 1:38–42.

Sen, P. 1948. Anopheles breeding in the rice fields of lower Bengal: Its relation with the cultural practices and with the growth of rice plants. Indian J. Malariol. 2:221–237.

Steelman, C. D., T. W. White and P. E. Schilling. 1973. Effects of mosquitoes on the average daily gain of Hereford and Brahman breed steers in southern Louisiana. J. Econ. Entomol. 66:1081–1083.