The Biology of *Aedes (Ochlerotatus) albifasciatus* Macquart, 1838 (Diptera: Culicidae) in Central Argentina

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Aedes albifasciatus *is a flood water mosquito occurring in the southern countries of South America. It is a competent vector of the Western Equine Encephalitis (WEE) and causes important losses on milk and beef production in central Argentina. Field work was carried out from December 1990 to March 1993, on a monthly basis during the dry season and biweekly during the rainy season. Larvae were collected using the 'dipping' technique and females with CDC traps baited with CO_2. Field collected larvae were used to build laboratory cohorts, from which basic population parameters were estimated. Eggs survived up to six months on dry soil, although there was a linear decrease of viability with time. At 23°C, larval development time was around nine days, and all adults emerged within one week. The estimation of larval development in the laboratory seems to be very near the development on the field, as larvae have been collected on average eight days after a rainfall. Egg to adult survival was 83%, with the highest mortality on fourth larval instar (6%). In the laboratory studies, sex proportion among the adults was 1:1, females lived longer than males (median 13 and five days, respectively), and adult survival pattern showed a constant number of individuals dying per unit time. Field collected females layed an average of 84 eggs per batch, and completing up to five gonotrophic cycles, suggesting an estimated survival of up to 35-50 days.*

Key words: *Aedes albifasciatus* - Culicidae - population biology - Argentina

Aedes albifasciatus is a neotropical species, occurring in the southern cone of South America. It is present from Santa Cruz de la Sierra and Cochabamba (Bolivia), through southern Brazil, Uruguay, Chile, reaching southern Patagonia down to Tierra del Fuego (Argentina) (Prosen et al. 1960, Forattini 1965).

The breeding sites of this species are temporary shallow water ponds, variable in size, mainly on flat terrains. They might be natural or artificial, with or without vegetation. Immature stages occur during summer and autumn months (Prosen et al. 1960). In the Andean valleys, breeding sites were found as high as 2300 m, in places protected from the cold winds (Duret 1954), and even in water with high salinity (5%) (Bachman & Casal 1962). Females lay eggs on muddy soils, surrounding the ponds. The eggs can enter diapause or produce first instar larvae immediatly after flooding.

Ae. albifasciatus is a serious problem in plain regions where, small ground "depressions" flood periodically. In the Mar Chiquita region (NW Córdoba province, see Fig. 1a), rainfall and river overflows cover periodically large ground extensions, causing eventually the explosive emergence of adult mosquitos.

Ae. albifasciatus has semi-domestic habits, insistently attacking humans and domestic mammals. In some places of North and Western Argentina, field workers refuse to work during this mosquito explosions (Forattini 1965), and in the Mar Chiquita region it causes important losses in milk and beef production (Raña et al. 1971). The species has been found infected by Bunyamwera virus group (Bianchini et al. 1968) and the WEE virus (Mitchell et al. 1987). The species was especially abundant during the WEE outbreaks in Argentina, and laboratory work showed that it is a competent vector of the WEE virus (Avilés et al. 1990).

This paper reports on field and laboratory studies on the biology of *Ae. albifasciatus*, aiming at a better understanding of field populations of the species, to improve the efficiency of the ongoing mosquito control programme in central Argentina.

MATERIALS AND METHODS

The field studies were carried out in the southern coast of the Mar Chiquita Lake, in the NE of Córdoba province, central Argentina (30° S, 63°

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W) (Fig. 1). The lake has shown long term oscillation cycles. In 1964, its surface was estimated as 1850 km^2 (Vázquez et al. 1979), but in 1984 reached about 5250 km^2 (Jiménez & Sedrán, pers. com.). At present, the lake is approximately 80 km E-W and 70 km N-S.

Four mosquito sampling stations were located on the southern border of the Lake, near La Para (Fig. 1b). Station 1 was 20 km E of La Para, and 300 m from the place where the Suquía River flows into the Lake. The breeding site was 50 x 150 m, had a clay surface and frequently dried out totally during the dry season. The ground was covered with salt marsh vegetation (*Allenrolfea* sp., *Spirostachys* sp., *Atriplex* sp., *Salicornia* sp., and *Heterostachys* spp.). Station 2 was 14 km E of La Para. It was an approximately circular breeding site, 200 m in diameter, surrounded by trees (*Geoffroea decorticans* and *Prosopis* spp.) and always covered with abundant floating vegetation (mainly *Marsilea azolla* spp. and *Marsilea* spp.). Station 3 was 7 km NE of La Para, had a diameter of around 50 m; the water of this breeding site sometimes mixed with the rainfall water running along the local road beside it; and as station 2, it was surrounded by trees (*G. decorticans* and *Prosopis* spp.). Station 4 was 6 km NE from La Para and about 1 km from Station 3. It was a small lagoon, with the ground covered by grass fluctuating between 40-240 ha, depending on rainfall, and surrounded by a few trees. Another sampling site, mainly for adult collection was located in San Carlos, 10 km south of Córdoba city.

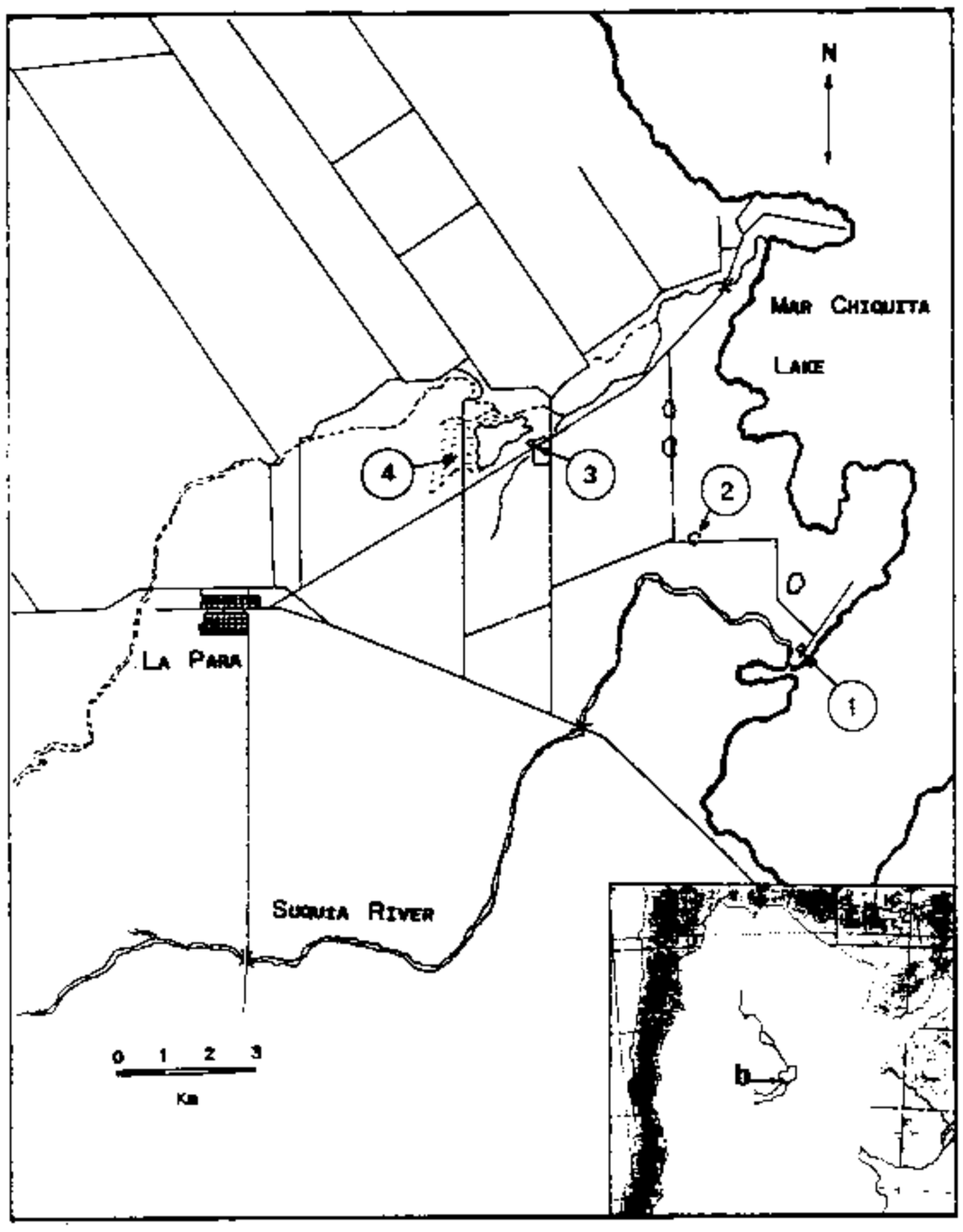

Fig. 1: a - location of the Mar Chiquita Lake within Argentina; b - location of the four sampling sites within the study area.

Adults and immature stages of *Ae. albifasciatus* were sampled monthly between December 1990 and September 1991, biweekly between October 1991 and April 1992, monthly between May and September 1992, and biweekly between October 1992 and March 1993. Larvae and pupae were collected with a dipper, either in lotic or lentic habitats. Eggs were sampled from ground samples collected around the breeding sites and studied by the flooding technique (Service 1976). The square ground samples (10 x 10 cm) and 5 cm depth were transported to the laboratory in plastic trays. Females were caught with CDC traps baited with CO_2 or mechanical aspirators. They can be captured by CDC traps only when environmental temperatures are higher than 6°C. From September to April (warmest period) there are two activity peaks, at dawn and at dusk; the rest of the year (coldest period) there is only one peak during hours of maximum illumination. Considering these previous findings, the CDC traps were operated at dusk (Ludueña Almeida & Gorla, in press). The collected mosquitoes were identified to species and counted at the laboratory. As the activity of *Ae. albifasciatus* females changes during the day in different ways throughout the year, the observed daily mosquito density was estimated as the number of females actually captured within 1 hr divided by the expected proportion of active females within the hour, according to Ludueña Almeida and Gorla (in press).

Considering the importance of the water table fluctuation for the species, studies in the laboratory were carried out to estimate the time to complete the embryogenesis under humid conditions, and to study the effect of drought on egg viability. The eggs used to estimate the time to complete embryogenesis were laid by field collected females, fed every other day on a rat. Females were maintained in cages and laid eggs on a Petri dish with a humid filter paper. A new Petri dish was added daily and the old one kept at 20-22°C outside the cage. Daily, 10 eggs were treated with sodium hypochlorite to transparent the corium, and study the mosquito embryo development status under a stereoscopic microscope (Morterson 1950, Trpis 1970). The procedure was repeated between 0 and 14 days, and the embryo development phases were analyzed according to Christophers (1960).

To estimate the effect of drought on viability, eggs from 94 field collected females were used. Females were individually maintained in a plastic cylinder (9x6 cm) with a bottom covered by a filter paper above a piece of humid cotton. Eggs of a particular day were maintained in the humid en-

vironment of the cylinder up to 12 days, and the female transferred to another cylinder. After the 12th day, the filter papers with the eggs were transferred to open Petri dishes where it progressively dried out. During the first week from drought, the eggs were observed daily, then weekly until 30 days, and biweekly from the second to the fourth month. The number of embryonated and collapsed eggs were recorded. Multiple regressions of the number of embryonated eggs (dependent variable) on the number of days under humid and drought conditions (independent variables) were estimated. The treated eggs were flooded with previously boiled water (low-level oxygen concentration) to measure larval emergence.

Field collected first and second larval stages of *Ae. albifasciatus* were used to build 10 cohorts of 50 second instar larvae. Each cohort was arranged in a plastic tray filled with water from the field breeding site (pH 7.0-7.2). Powdered rabbit balanced food (350 mg) was added every other day to each tray until adult emergence. Cohorts were maintained at 23±1.7°C. The number of individuals in each age class was recorded daily until adult emergence. Age-specific survival and development time were calculated from these records.

The number of emerging adult males and females were daily recorded and fed with sugar solution. Fecundity was estimated in 24 field collected females, caged individually, maintained at 20±2°C and blood fed on a rat. The number of eggs laid by each female was recorded. After death, each female was dissected to count the number of developed eggs within the ovarioles and the number of gonotrophic cycles estimated as the number of follicular relics (Detinova 1962).

RESULTS

Ae. albifasciatus larvae were found in the field only within eight days after a rainfall (Fig. 2). No larvae occurred in places with running water, but only in quiet and shallow water. They were always collected along the borders of the pools, independently of the presence of vegetation. Females were collected during all seasons, although the highest densities occurred during the spring, after rains following a long drought.

The fluctuation of adult number - In December 1990, site 1 showed a high number of pupae and adults, compared with the other sites (2-4) (Fig. 3). Between January and November 1991 the sampling stations showed three small adult peaks. In December, an outbreak of adult females occurred. This peak followed a series of rainfall days (20-40 mm rainfall each day) separated by 2-3 days of good weather (Fig. 4). The peak ended towards mid January 1992, coinciding with the beginning of a dry period. Adult population decreased, reaching a minimum in July 1992. At the beginning of

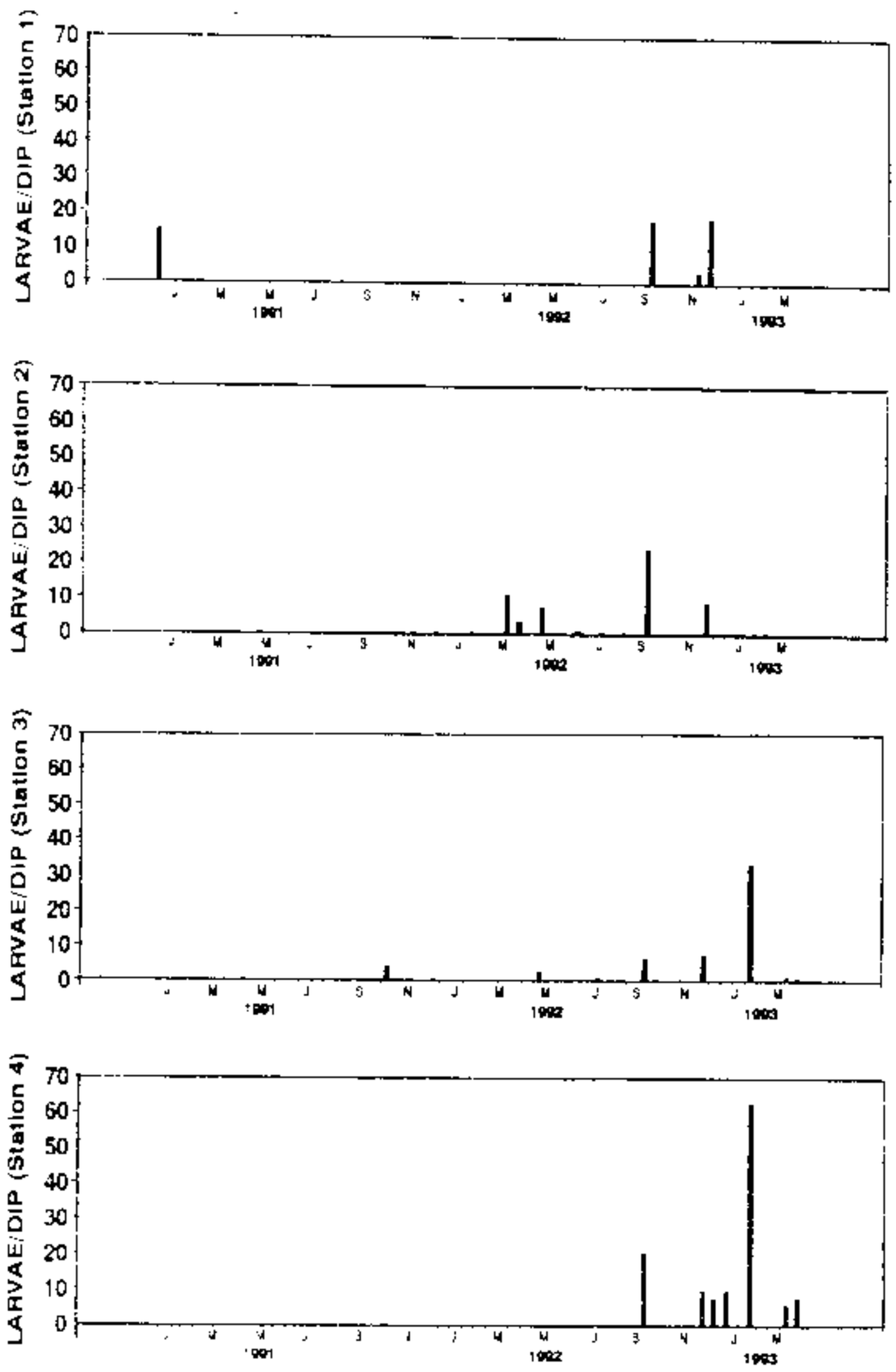

Fig. 2: larval occurrence on each sampling site along the period of study.

September (days 8 and 9), an unusual rainfall occurred (118 mm). This promoted an increase of the sampling sites surfaces (3-4 times), and the occurrence of larvae in all sites. Towards 15 September all sites had fourth instar *Ae. albifasciatus* larvae, on 17 September the first adult males appeared and towards October 3 a high number of females occurred. On 2 December another peak of larvae occurred, because of the rainfall on 28 November. During December, the rainy days were more frequent than average and as the surface of the sampling sites did not decrease, *Ae. albifasciatus* females were not able to lay eggs in the study areas. Although larvae of this species did not appear again during the rest of the warm period, larvae of other species (especially *Culex* spp.) increased their numbers, because of the different requirements for females of the last species for egg laying.

Preadult survival and development time - The embryo completed development in four days in a humid environment. The developed embryo was able to live up to six months over a dry filter paper. However, there was a decrease in egg viability (y) according to a significant linear function ($r = -0.81$, $p<0.0001$, $n = 9$ time intervals group-

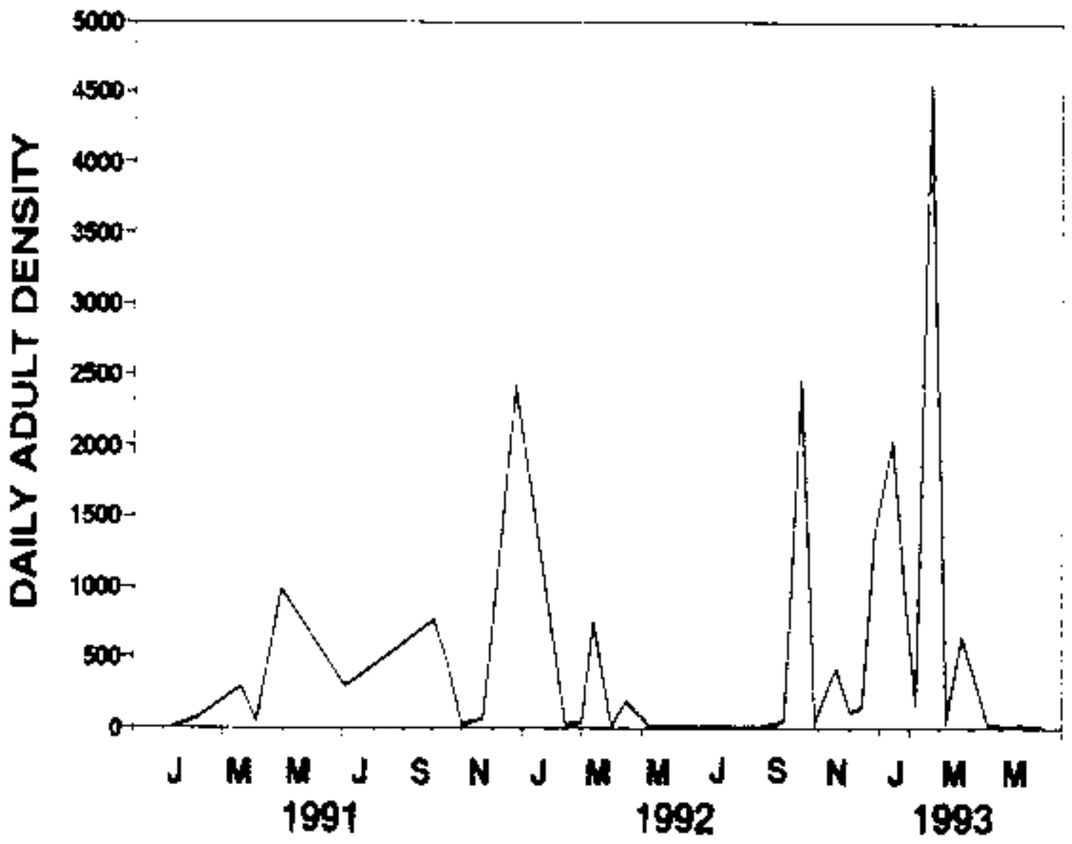

Fig. 3: corrected daily density of *Aedes albifasciatus* collected with CDC traps baited with CO_2 during the period of study. Average over the four sampling sites.

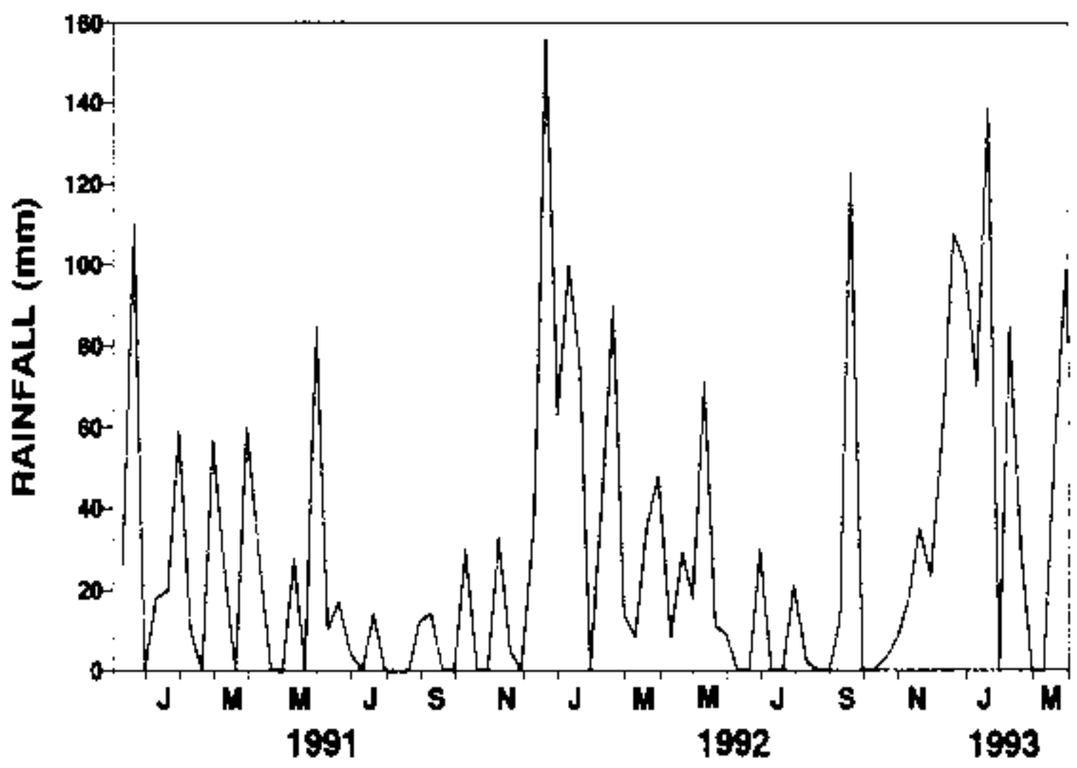

Fig. 4: rainfall accumulated in periods of ten days at the study area during the study period (values were represented as a polygon for better reading, although rainfall is an intrinsically discret event).

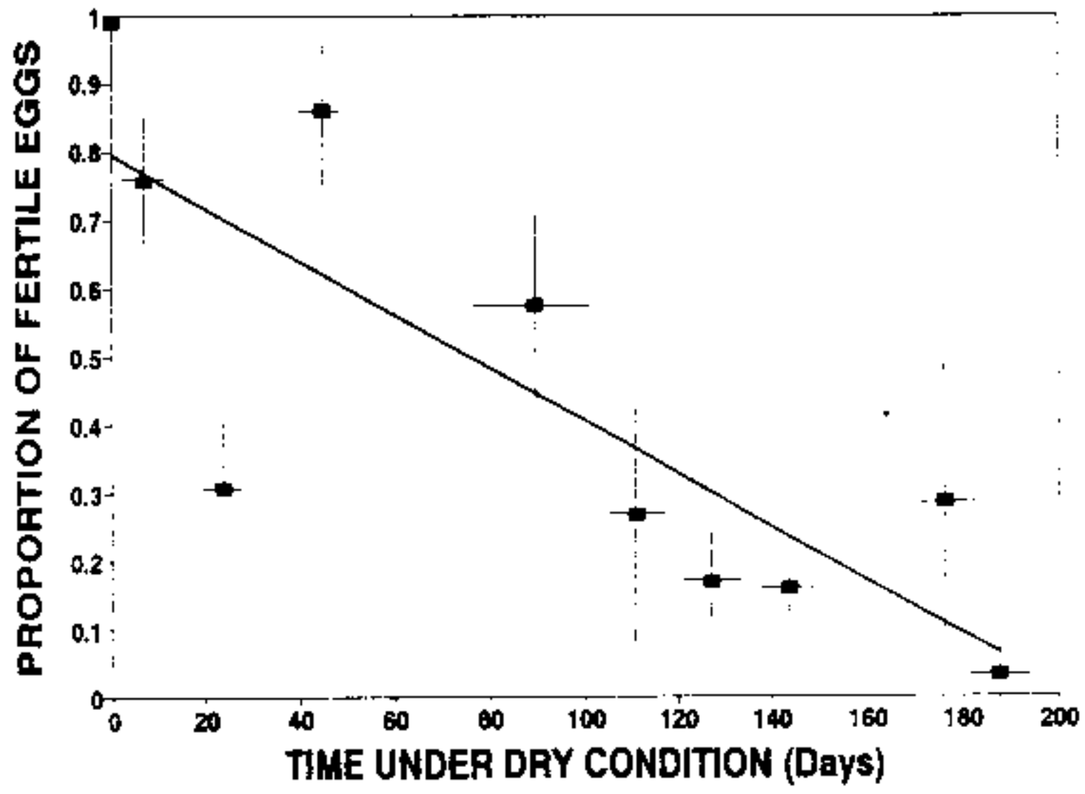

Fig. 5: viability of *Aedes albifasciatus* eggs laid by field collected females after increasing time (days) under dry conditions. Each point represents the average viability of eggs within time intervals of 20 days. Vertical and horizontal bars represent one standard deviation.

ing 186 egg batchs) y = 0.79 - 0.0039 x (where x is the period during which the egg lived in a dry environment) (Fig. 5).

On average, larvae reached the third instar in 3 days and the fourth in 4 days. Pupae appeared in 7 days and 50% of the adult emerged in 9 days. The last adults emerged 15 days after egg flooding. Under the laboratory breeding conditions, egg to adult proportional survival was relatively high: 0.83 (s = 0.074). The fourth larval instar showed the highest proportional mortality: 0.06 (s = 0.047) (Table).

From the larvae collected at San Carlos a higher proportion of females (61%) than males (39%) (P<0.01) was obtained. Adult males and females appeared at the same time (about 9 days). Females lived longer (median = 13 days) than males (median = 5). Under laboratory conditions, the survival pattern of all the age classes follows a Type 2 curve (Deevey 1947) (Fig. 6), showing a constant number of individuals dying per unit time. Larvae from La Para produced the same proportion of both sexes, but in the laboratory males emerged in average 1.6 days before females (t test, p<0.001).

Efforts to obtain copulations in the laboratory failed, so that no blood meal volume or fecundity estimations were obtained from the laboratory hatched and emerged females. The mean number of eggs (either laid or remaining within the ovarioles) developed per gonotrophic cycle in field fe-

TABLE

Development time (in days) and survival rates of larvae and pupae of *Aedes albifasciatus* (mean and s.d. [in brackets]), based on 10 cohorts of 50 individuals. Absolute survival of age class i is the number of individuals surviving until the age class i, divided by the initial number of second instar larvae in the cohort. Relative survival in age class i is the number of individuals leaving the age class i divided by the number that entered the age class i

	Age class			
	Larval instar			Pupae
	Second	Third	Fourth	
Development time	1.04 (0.046)	1.05 (0.086)	3.44 (0.269)	1.65 (0.142)
Absolute survival	0.98 (0.019)	0.95 (0.033)	0.90 (0.047)	0.83 (0.074)
Relative survival	0.99 (0.019)	0.96 (0.041)	0.94 (0.047)	0.92 (0.065)

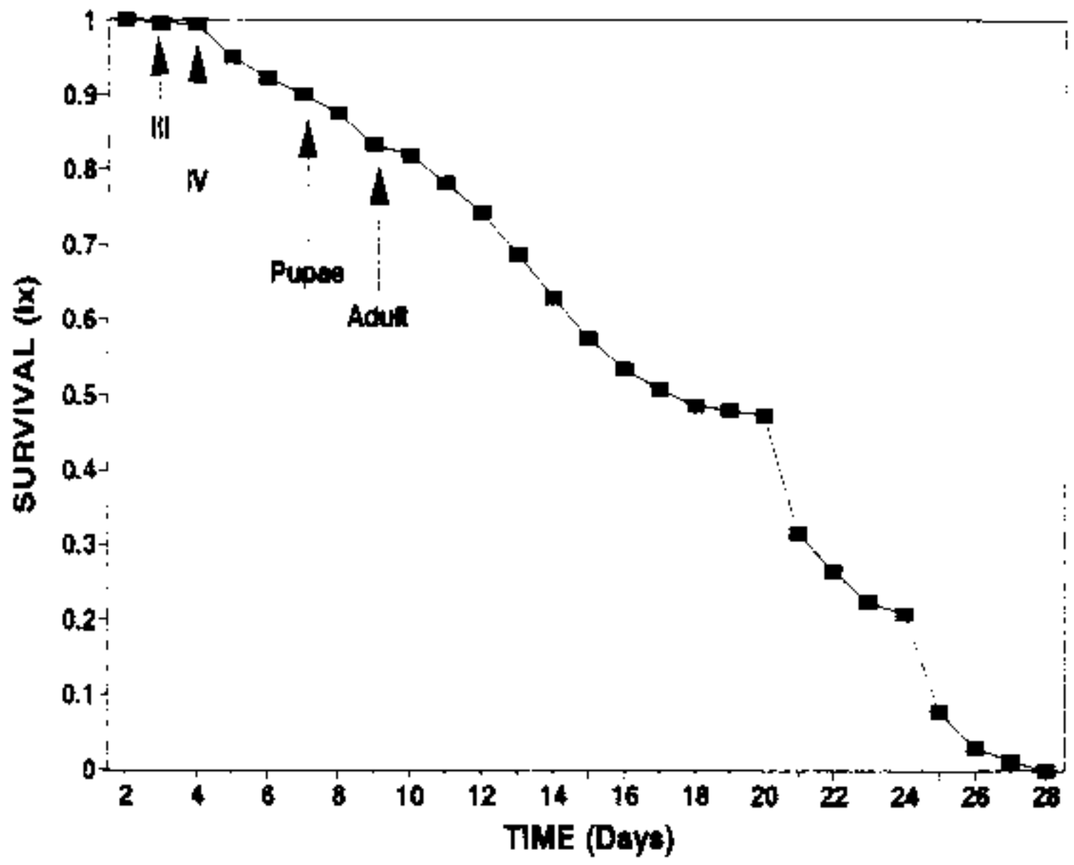

Fig. 6: survival curve of *Aedes albifasciatus* under laboratory conditions, based on 500 initial first and second instar larvae developed in 10 cohorts. Arrows mark 50% molting to third (III), fourth (IV) instar larvae, pupae and adults.

males was 110 (s = 39.65), and relatively less variable (CV = 36%) than the mean number of eggs effectively laid by the same females of 83.8 (CV = 67%). A maximum number of five gonotrophic cycles was recorded among the dissected females from the field (average = 3.8, sd = 0.98). Egg laying began 3 days after the blood meal. Most of these females (58%) layed eggs between 5-8 days after a blood meal, although some females layed eggs after 19 days (average = 8.2 days, CV = 49.2%).

DISCUSSION

As in other *Aedes* species (*Ae. taenyorhyncus*, *Ae. sollicitans*), the embryogenesis in *Ae. albifasciatus* takes nearly four days to be completed (Nayar 1985). The larvae hatched after the egg was covered by water for at least 24 hr, either immediately after the embryo has developed or after a dry period of up to 4-6 months. The observed variability of the hatching rate, may have occurred because of a mixture of physiological ages of field captured females, which may have produced eggs of different capabilities of resisting dry periods according to the environment affecting the development of larvae, pupae and adults, as was shown for *Psorophora columbiae* (Focks et al. 1988) and *Ae. albopictus* (Toma & Miyagi 1990). A field study reporting *Ae. albifasciatus* first instar larvae within a pool flooded during the winter months showed that low temperatures do not constrain larval development (Almirón 1993), coinciding with similar findings for *Ae. taeniorhyncus* and *Ae. sollicitans* (Nayar 1985).

Under laboratory conditions, preadult survival was 82.9%, similar to the 83.4% for *Ae. vexans* (Trpis & Shemanchuck 1970) and the 88.3% for *Ps. columbiae* (Andis & Meek 1985). Preadult developmental time, under temperatures between 23-25°C was nine days, the fourth instar larvae being the longest lasting (four days). The occurrence of *Ae. albifasciatus* in the field samples, depended strongly on the number of days after a rainfall. Only samples taken up before eight days after a rain yielded larvae. This clearly suggests that larval development time under field conditions is less than eight days. Field collected *Ae. albifasciatus* larvae developed under laboratory conditions without additional food supply (other than that included in the original water sample), showed lower development rate, higher mortality and male emergence before females, as compared with those larvae developed with additional food supply. This is similar to the results found for *Cx. pipiens* (Gorla et al. 1992).

Considering the time between emergence and copulation, time to get a blood meal reported by Forattini (1962) for different species of Culicidae, time for egg laying and that a female can lay five egg batches (this work) an estimation of the potential maximum longevity of field females could be calculated as 35-50 days.

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REFERENCES

Almirón W 1993. *Bionomía y taxonomía de los principales mosquitos (Diptera, Culicidae) recolectados en Córdoba, con particular énfasis en el género* Culex *L. 1758*. Thesis. Universidad Nacional de Córdoba, 221 pp.

Andis MD, Meek CL 1985. Mortality and survival patterns for the immature stages of *Psorophora columbiae*. *J Am Mosq Control Assoc 1*: 357-362.

Avilés G, Sabattini MS, Mitchell JC 1990. Peroral susceptibility of *Aedes albifasciatus* and *Culex pipiens* complex mosquitoes (Diptera: Culicidae) from Argentina to Western Equine Encephalitis Virus. *Rev Saúde Púb São Paulo 24*: 265-269.

Bachman A, Casal O 1962. Mosquitos argentinos que crían en aguas salobres y saladas. *Rev Soc Entomol Argentina 25*: 21-27.

Bianchini NR, Sabattini MS, Bianchini JP, González LE 1968. Aislamiento de arbovirus del grupo Bunyamwera de mosquitos *Aedes (O.) albifasciatus* en la Argentina. *Ciencia e Investigación 24*: 463-468.

Christophers SR 1960. Aedes aegypti (L.). *The Yellow Fever Mosquito*. Cambridge University Press. 739 pp.

Deevey ESJ 1947. Life tables for natural populations of Animals. *The Quarterly Rev Biol 22*: 283-314.

Detinova TS 1962. Age-grouping methods in Diptera of medical importance. *WHO, monograph series 47*: 215 pp.

Duret J 1954. Datos sobre culícidos de la región cuyana (Diptera: Culicidae). *Rev Sanidad Militar Argentina 53*: 12-13.

Focks DA, McLaughlin RE, Smith BM 1988. A Dynamic Life Table Model of *Psorophora columbiae* in Southern Louisiana Rice Agroecosystem with Supporting Hydrological Submodel. Part 1. Analysis of Literature and Model Development. *J Am Mosq Control Assoc 4*: 266-281.

Forattini OP 1962. *Entomologia Médica. Vol. I. Parte Geral, Diptera, Anophelini.* Ed. Univ. São Paulo. São Paulo. 662 pp.

Forattini OP 1965. *Entomologia Médica. Vol. II. Culicini:* Culex, Aedes, Psorophora. Ed Univ São Paulo. São Paulo. 506 pp.

Gorla D, Ludueña Almeida F, Serra G 1992. Effect of food competition on *Culex pipiens quinquefasciatus* under laboratory conditions. *Ecol Austral 2*: 57-61.

Ludueña Almeida F, Gorla D 1995. Daily pattern of flight activity of *Aedes albifasciatus* in Central Argentina. *Mem Inst Oswaldo Cruz*, in press.

Mitchell JC, Monath TP, Sabattini MS, Daffner J, Crop CB, Calisher CH, Darsie RF, Jakob WL 1987. Arbovirus isolation from mosquitoes collected during and after the 1982-1983 epizootic of western equine encephalitis in Argentina. *Am J Trop Med Hyg 36*: 107-113.

Morterson EW 1950. The use of sodium hypoclorite to study *Aedes nigromaculis* (Ludlow) embryos (Diptera: Culicidae). *Mosq News 10*: 211-212.

Nayar JK 1985. Development of Immature Stages and Larval Excretion. In JK Nayar, *Bionomics and Physiology of* Aedes Taeniorhynchus *and* Aedes sollicitans, *The Salt Marsh Mosquitoes of Florida 852*: 18-39.

Prosen AF, Martínez A, Carcavallo R 1960. La Familia Culicidae (Diptera) de la rivera fluvial de la Provincia de Buenos Aires. *An Inst Med Reg 6*: 59-124.

Raña JC, Quaino OR, Paterno DH 1971. Informe sobre daños provocados por mosquitos en la zona del Departamento San Cristobal, limítrofe con Córdoba y Santiago del Estero. *Tech Rep INTA EERA-Rafaela.*

Service MW 1976. *Mosquito Ecology, Field Sampling Methods*. Applied Science Publishers Ltd. London. 583 pp.

Toma T, Miyagi I 1990. Seasonal Changes in the Hatchability of *Aedes albopictus* (Diptera: Culicidae) eggs in Okinawajima, Ryukyu Archipelago, Japan. *Jap J Sanitary Zool 41*: 195-204.

Trpis M 1970. A new bleaching and decalcifying method for general use in Zoology. *Can J Zool 48*: 892-893.

Trpis M, Shemanchuk JA 1970. Effect of constant temperature on the larval development of *Aedes vexans* (Diptera: Culicidae). *Can Ent 102*: 1048-1051.

Vázquez JB, Miatello RA, Roqué ME 1979. *Geografia Física de la Provincia de Córdoba*. Ed Boldt. Córdoba. 464 pp.