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Relative Abundance and Gonotrophic Status of *Aedes albifasciatus* (Diptera: Culicidae) During the Autumn–Winter Period in Córdoba Province, Argentina

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ABSTRACT Variations in the abundance of larvae and females of *Aedes albifasciatus* (Macquart) were monitored biweekly during the autumn–winter periods of 1996 and 1997 on the southern edge of the Mar Chiquita Lake in Córdoba Province. The degree of development of the primary ovarian follicles also was determined in host-seeking females. Abundance of the larvae and the females were noticeably different during the 2 yr of the study and matched changes in the pattern of precipitation. Although most females had ovarian follicles in Christophers stages Ib–IIa, 14% and 4% in 1996 and 1997, respectively, were in stages III–V (i.e., exhibited gonotrophic discordance). Females appeared to continue taking blood meals and laying eggs even during the autumn–winter period.

KEY WORDS *Aedes albifasciatus*, abundance variation, gonotrophic status, autumn–winter

Aedes albifasciatus (Macquart) is a Neotropical species that is widespread in Argentina (Prosen et al. 1960, Forattini 1965). This species attacks humans and domestic mammals and is a pest in vast zones of Argentina (Del Ponte 1958; Prosen et al. 1960; Forattini 1965; Ludueña Almeida and Gorla 1995a, b). Females of this floodwater mosquito affect dairy production on the southern edge of Mar Chiquita Lake (northeast Córdoba Province) (Raña et al. 1971). Several viruses have been isolated from female *Ae. albifasciatus* captured in Argentina (Bianchini et al. 1968, Mitchell et al. 1985, Sabattini et al. 1985), including western equine encephalitis virus (Avilés et al. 1992).

In the subtropical zones of Argentina, *Ae. albifasciatus* females have been captured throughout the year, although in Corrientes Province they predominated in autumn and winter (Hack et al. 1978) and in late summer and autumn in Punta Lara (Buenos Aires Province) (Ronderos et al. 1992, Maciá et al. 1995). In temperate areas seasonality is more marked, so that females are captured mainly during the spring–summer period in La Plata (Buenos Aires Province) (Campos et al. 1993) and during summer and early autumn in Córdoba (central Argentina) (Almirón and Brewer 1995, Gleiser and Gorla 1997, Gleiser et al. 1997, 1999). Studies on the biology of this species have been carried out mostly during the warm seasons in central Argentina, but very little information is available on its winter biology. The persistence host-seeking by the vector during the cold months could have significance in persistence of pathogens between transmission seasons. Almirón and Brewer (1994, 1995) and Ludueña Almeida and Gorla (1995a, b) collected the larvae of *Ae. albifasciatus* during both autumn and winter in

different areas of Córdoba Province. Almirón and Brewer (1994) also captured females host-seeking at human bait. Given that larvae and adult females of this species have been found during the cold, dry period in Córdoba, the aim of our study was to describe the temporal variation and magnitude of abundance during the winter period and to determine the state of development of the ovarian follicles in the females.

Materials and Methods

Area of Study. The field work was done at the southern edge of Mar Chiquita Lake. The lake surface has varied significantly in size and was estimated to cover 1,850 km² in 1984 (Vázquez et al. 1979). Lake surface variations affect the water table and upland flooding, and vast areas may be transformed into larval habitat suitable for *Ae. albifasciatus*. Mosquitoes were captured 7 km northeast of La Para (30° 91' [min] S, 63° W) at a representative larval habitat ≈50 m in diameter. After heavy rain this habitat may double its size, but in the autumn–winter period it may dry completely. It is covered with grasses and surrounded by trees, *Geoffroea decorticans* and *Prosopis* spp.

The study area is mesothermal, with a period of frosts that may last from the 2nd half of May until the 2nd half of September, although most occur in July. Average annual temperature is 18°C (maximum–minimum of 26–11°C). The absolute maximum temperature is 42.6°C (January), whereas the absolute minimum is –6°C (July). Precipitation ranges from 800 to 900 mm annually (Capitanelli 1979).

Sampling of Larvae and Adult Females. During the autumn–winter periods of 1996 and 1997, samples

were taken every 2 wk. On each sampling date, larvae were collected by 20 dips with a 300-ml plastic dipper and then transported to the laboratory for identification and age-grading by instars. First and 2nd instars were reared to 4th instar for identification.

Females landing on 2 collectors were collected with mechanical aspirators during three 20-min periods. According to Ludueña Almeida and Gorla (1995b), the females of *Ae. albifasciatus* are active when ambient temperatures exceed 6°C, and our collections were made at 1.5-h intervals before, during, and after midday. Females were placed in 1-liter plastic flasks, covered inside with brown paper and closed with netting, and transported alive to the laboratory where specific determinations were made and females were dissected to determine ovariole maturation.

A continuous record of precipitation and temperature in the study area was kept with a rain gauge and a thermograph. Environmental conditions were summarized using the remaining water in the breeding site index, where remaining water in the breeding site = $\Sigma(P/t)T$ and P is precipitation in mm, t is time between precipitation and the date of calculation of the remaining water in the breeding site, and T is the average temperature during this period. This index is an adaptation of the Ch (Hydric Content) proposed by Ludueña Almeida (1994). The weather data used for the calculation of the remaining water in the breeding site for each sampling date, corresponded to 100 d before the date of sampling, because Ludueña Almeida (1994) obtained the best fit using this time period. The abundance of adult females was expressed as daily relative density or the estimated number of females that would be captured during 24 h, taking into account the daily pattern of female flight activity (Ludueña Almeida and Gorla 1995b). The daily relative density obtained was correlated with the remaining water in the breeding site recorded 15 d before the corresponding capture date.

State of Development of Ovarian Follicles. A maximum of 30 females was dissected from each sample. Five primary and secondary follicles were measured for each ovary using an ocular micrometer, and the mean ratio of 10 primary follicles/secondary follicles was calculated for each female. Observations also were made on the presence of yolk granules. The stage of ovarian development was based on Christophers stages (Clements 1992).

Results

Larvae. Precipitation differed markedly between the 2 sampling periods (Table 1). In 1996, rains were recorded at the beginning of the autumn-winter period, whereas in 1997 rain took place only after the middle of this period. Consequently, the abundance of larvae and adult females of *Ae. albifasciatus* differed noticeably between years (Table 2, 3). During 1996, larvae were collected in April and then in September (Table 2). At the beginning of April 1996, precipitation presumably stimulated the hatching of eggs, and the subsequent cohort of emerging adults were incorpo-

Table 1. Rainfall (mm) recorded during the autumn-winter periods of 1996 and 1997 in La Para, Córdoba Province

Month	1996	1997
Mar.	195	51
April	112	—
May	—	—
June	—	25
July	—	25
Aug.	—	15
Sept.	33	48
Total	340	164

rated into the existing population. Because the autumn-winter period in Córdoba is marked by low temperatures and little rain, the breeding site under study gradually reduced in size, drying completely by June. During September, larvae were collected again, 5 d following a 30-mm rainfall event that presumably stimulated egg hatch. The period between rainfall and sampling was sufficient for larvae to reach 3rd instar (Table 2).

The summer and autumn of 1997 were dry (Table 1), so that the breeding site which had been sampled in 1996 was dry when the 2nd sampling period began. Rainfall was recorded from June to September 1997; consequently, larvae of mixed ages were collected during June, August, and September (Table 2).

Adult Females. In total, 1,113 females were captured from April to August 1996 (Fig. 1). The correlation between the biweekly daily relative density and remaining water in the breeding site was highly significant ($r = 0.92$, $df = 8$, $P < 0.05$). On the 2nd sample of April, the maximum of abundance (daily relative density = 618) was recorded, matching a considerable increase in the remaining water in the breeding site as a result of the rains at the beginning of the month (Fig. 1). The number of females decreased after this peak in association with the slow drying of the larval habitat.

In total, 762 females were collected during 1997. When autumn began, female abundance was lower than observed during the same period of 1996 (Fig. 1) and continued decreasing until rainfall commenced in June (Fig. 1). The correlation between the daily relative density and the remaining water in the breeding site again was highly significant ($r = 0.88$, $df = 10$, $P < 0.05$). In spite of the low winter temperature, new adult females were added to the population. Whereas only 1% of the females were captured between the months of July and September 1996, 38% of the total females were collected during the succeeding 3 mo of 1997 (Fig. 1).

State of Development of the Ovarian Follicles. During 1996 and 1997, the majority (86% and 96%, respectively) of the females dissected had primary follicles at stages Ib-IIb (Table 3). In general, the number of individuals in stage Ib was not greater than the number in IIa, except for the 2nd capture of April and the 1st of July 1996 and the 1st capture of September 1997. The 1st and 3rd of these occasions corresponded to population increases (Fig. 1) related to the emergence of new individuals. In 1997 a progressive population

Table 2. Number of *Ae. albifasciatus* larvae collected during the autumn-winter periods of 1996 and 1997 in La Para, Córdoba Province

Year	Instar	Sample												Total	
		April		May		June		July		Aug.		Sept.			
		1	2	1	2	1	2	1	2	1	2	1	2		
1996	I-II	15	—	—	—	—	—	—	—	—	—	—	—	121	136
	III	2	1	—	—	—	—	—	—	—	—	—	—	227	230
	IV	14	44	—	—	—	—	—	—	—	—	—	—	—	58
1997	I-II	—	—	—	—	—	36	—	—	—	14	—	—	—	50
	III	—	—	—	—	—	17	—	—	—	38	31	—	—	86
	IV	—	—	—	—	—	1	—	—	—	17	59	—	—	77

reduction was observed, and in June the number of females with follicles in stage IIa also decreased. The trend was reversed after the rains (Table 3). Overall, 14% and 4% of the females dissected in 1996 and 1997, respectively, presented follicles at stages III-V, indicating gonotrophic discordance (i.e., the ingestion of >1 blood meal during a gonotrophic cycle).

Discussion

Larvae were collected during April, June, August, and September, months whose temperatures averaged 17.2, 10.6, 12.0, and 15.0°C, respectively. Precipitation was recorded between 4 and 10 April, ≈10 d before the 1st sample of that month, when 48% of 1st and 2nd instars were collected, and some 25 d before the 2nd sampling when 98% of the larvae were captured in 4th instar. In June, 2 wk passed between rainfall and the 2nd sample of the month, when 67% of larvae were in 1st and 2nd instars. Precipitation during August also induced the hatching of eggs ≈10 d before the 2nd sample of the month when larvae were collected in 1st-4th instars and 23 d before the 1st sampling of September when larvae were captured only in the 3rd and 4th instars. These data indicated that eggs will hatch and larvae will develop even during winter, although not as rapidly as the 7 d needed from the 2nd to the 4th instar at 23 ± 1.7°C (Ludueña Almeida and Gorla 1995a).

In the current study, adults were last added to the existing population during the 2nd half of April 1996, because subsequently there was not sufficient rain to stimulate egg hatch. This indicated that if the cold period is very dry, as was the case in 1996, the population decreases steadily until individuals that emerged at the end of summer and beginning of autumn die out. Even though no females were collected in the 2nd half of July 1996, this was not caused by low temperature, because the temperature of 17°C during the capture period was above the 6°C threshold for female flight activity (Ludueña Almeida and Gorla 1995b). Possibly the wind (15 km/h) added to our low estimate of population abundance. Nonetheless, if there was sufficient rain during the winter, as occurred in 1997, egg hatch occurred and new adults were added to the existing population.

In the subtropical forest of Punta Lara, variation in the level of the water in the breeding site in combination with low temperatures (winter: average maximum and minimum of 15 and 6°C, respectively) did not allow the hatching of the eggs (Maciá et al. 1995). This would explain the seasonal distribution of this species being restricted to spring and autumn, periods in which mild temperatures would permit development. In temperate areas like Córdoba, Almirón and Brewer (1994) and Ludueña Almeida and Gorla

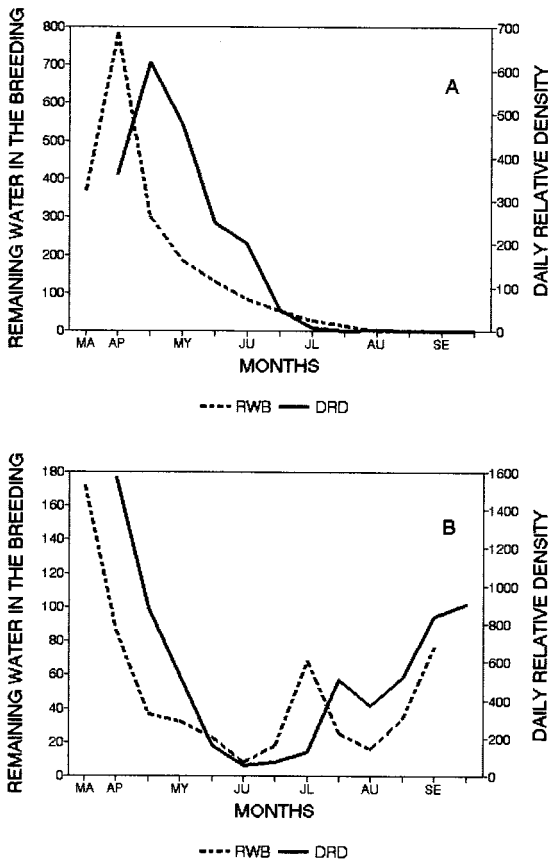


Fig. 1. Correlation between remaining water in the breeding site index and the daily relative density of *Ae. albifasciatus* females recorded during the autumn-winter periods of 1996 (A) and 1997 (B) in La Para, Córdoba Province.

Table 3. Number of *Ae. albifasciatus* females scored according to ovarian stage, collected during the autumn–winter periods of 1996 and 1997 in La Para, Córdoba Province

Year	Follicle stage	Sample												Total
		April		May		June		July		Aug.		Sept.		
		1	2	1	2	1	2	1	2	1	2	1	2	
1996	Ib	3	17	2	—	—	4	4	—	1	—	—	—	31
	IIa	25	10	20	16	28	12	3	—	1	—	—	—	115
	IIb	2	—	1	2	2	5	1	—	1	—	—	—	14
	IIIb	—	1	—	1	—	—	—	—	—	—	—	1	2
	IVa	—	—	—	4	—	—	—	—	—	—	—	—	4
1997	V	—	2	2	7	—	9	—	—	—	—	—	—	20
	Ib	5	3	3	—	—	—	—	5	—	—	21	3	40
	IIa	19	23	22	9	2	4	10	22	24	19	7	26	187
	IIb	4	2	4	—	2	1	—	1	2	1	—	—	17
	IIIa	1	1	—	—	—	—	—	—	—	—	—	1	3
	IIIb	—	—	1	1	—	—	—	—	—	1	—	—	3
V	—	1	—	—	1	—	—	2	—	—	—	—	4	

(1995a, b) collected the larvae of *Ae. albifasciatus* during the autumn–winter period. It would be useful to determine the lowest minimum temperature that permits egg hatch, because during the current study, eggs hatched during July with an average minimum temperature of 3.5°C. Therefore, scarce rain recorded in the autumn–winter period in Córdoba appears to be more important than temperature in limiting egg hatch.

In autumn the eggs of floodwater *Aedes* at temperate latitudes typically undergo diapause with eclosion limited by both temperature and photoperiod (Mitchell 1988). Our recovery of 1st instars during April after the autumnal equinox and during June at the winter solstice indicates that eclosion of *Ae. albifasciatus* eggs may not be limited by photoperiod, a topic deserving additional investigation.

In floodwater mosquitoes, larval eclosion depends upon increases in water depth inundating eggs oviposited at the habitat margin and increases in the number of adults on the permanence of the water for the completion of development. The high significant correlation obtained between the daily relative density and remaining water in the breeding site indices indicated that this variable is a very useful tool to predict the timing of adult emergence.

Overall, 17% and 16% of females dissected in 1996 and 1997, respectively, presented follicles at stage Ib. In 1996 53% of these females were collected in the 2nd 2 wk of April, coinciding with peak population abundance. Something similar occurred in 1997, because 73% of the females with follicles in stage Ib were collected during July and September, when the population abundance of females was increasing. Because we sampled only host-seeking females, we expected that most individuals would present follicles at stage Ib–IIb; however, some females also presented follicles at stage III–V. Therefore, we concluded that even during the autumn–winter period, *Ae. albifasciatus* females continue gonotrophic activity and that some females may take >1 blood meal during each gonotrophic cycle, thereby increasing their frequency of host contact.

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