

## FECUNDITY OF *OCHLEROTATUS ALBIFASCIATUS* FROM CÓRDOBA, ARGENTINA

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**ABSTRACT.** Seasonal differences in the number and length of gonotrophic cycles of *Ochlerotatus albifasciatus* were determined in a temperate area of Argentina, in addition to analysis on number of eggs laid by females corresponding to the number of bloodfeedings per gonotrophic cycle throughout the year. Landing females were collected by using human bait and mechanical aspirators along the southwestern coast of Mar Chiquita Lake (in northeastern Córdoba Province) from February to November 2000. Collected females were kept in captivity under natural weather conditions, fed on a sugar solution (10%), and provided blood via a human host by different methods (treatment A: 1 bloodfeeding/gonotrophic cycle and treatment B: 2 or 3 bloodfeedings/gonotrophic cycle). A maximum of 5 gonotrophic cycles (average  $2.24 \pm 1.11$ ) were determined by dissecting ovarioles. The gonotrophic cycles were longer in females of treatment B ( $11.23 \pm 4.06$  days) than those of treatment A ( $9.02 \pm 5$  days). Females that completed the highest number of cycles were collected in winter and during the beginning of spring. The average number of eggs laid in each cycle was 90 (SD = 37.85) for treatment B females and 80 (SD = 29.99) for those of treatment A, with significant differences between treatments. Considering both treatments, the number of eggs laid was significantly higher after 3 blood meals than with 1 or 2 feedings. These results suggest that an increase in the number of feedings would lengthen the cycle and increase the production of eggs. No correlation was found relating to temperature and the length of the cycles, which suggests that the number of blood meals may be the determining factor in seasonal variations in egg production.

**KEY WORDS** *Ochlerotatus albifasciatus*, fecundity, Córdoba, Argentina, gonotrophic cycle

### INTRODUCTION

*Ochlerotatus albifasciatus* (Macquart) is widely distributed in Argentina, and is remarkable in its medical and economic importance (Brewer et al. 1991, Ludueña Almeida and Gorla 1995a). Along the southwestern coast of Mar Chiquita Lake (in northeastern Córdoba Province) cattle breeding for milk and beef production is the main economic activity. During the warm season, mosquito attacks can cause economic decreases of up to 22.2% (Raña et al. 1971). *Ochlerotatus albifasciatus* is the most important pest species in the area (Ludueña Almeida and Gorla 1995a, 1995b).

This mosquito has public health importance because western equine encephalitis (WEE) virus has been isolated from female *Oc. albifasciatus* in Argentina (Mitchell et al. 1987) and laboratory studies have concluded that this species is the primary vector for the WEE virus in the southern hemisphere (Avilés et al. 1992).

According to available literature, *Oc. albifasciatus* is found in a wide variety of breeding places (Del Ponte and Blaksley 1945/48, Bachmann and Casal 1962, Almirón and Brewer 1996), with larvae being found throughout the year in temperate Córdoba Province (Almirón and Brewer 1994, Ludueña Almeida and Gorla 1995a). Females are commonly found attempting to feed on humans in autumn and winter (Almirón and Brewer 1994). Almirón et al. (2000) suggested that even during these colder periods, females continue to feed on blood and lay eggs. They also suggest that these females require more than 1 blood meal for each gonotrophic

ic cycle. Fava et al. (2001) observed that a high percentage of bloodfed females (42.9%) completed 1 gonotrophic cycle during the autumn-winter period in Córdoba Province, and 14.6% had completed a 2nd cycle. A gonotrophic cycle starts with the 1st blood meal, involves the maturation of a batch of oocytes, and ends when oviposition occurs (Clements 1992).

Fecundity is one of several aspects of the biology of *Oc. albifasciatus* that has not been thoroughly studied. This knowledge is considered essential for the application of effective control measures (Ludueña Almeida 1994). The objectives of this work are to determine the number and length of the gonotrophic cycles during different times of the year, and to ascertain the number of eggs laid as related to the number of bloodfeedings per gonotrophic cycle throughout the year.

### MATERIALS AND METHODS

**Study site:** Female *Oc. albifasciatus* were captured on the southern edge of Mar Chiquita Lake (between 29°30' and 31°S, 62°10' and 63°15'W), in northeastern Córdoba Province. Females were collected 14 km east of La Para (30°91'S, 63°W), near a representative larval habitat approximately 150 m in diameter. This habitat is a small lagoon formed after heavy rains, surrounded by trees (*Geoffroea decorticans*, *Prosopis alba*, *Prosopis nigra*, and *Prosopis ruscifolia*), and with abundant aquatic vegetation (*Azolla* spp. and *Marsilea* spp.). During the autumn and winter, this breeding source may

completely dry out and is then covered by grasses (Ludueña Almeida and Gorla 1995a).

The study area has periods of frosts occurring mostly in July, but that may occur from the 2nd half of May to the 2nd half of September. Average annual temperature is 18°C (maximum average of 26° and minimum average of 11°C). The overall maximum temperature is 42.6°C (January), and the overall minimum is -6°C (July). Precipitation ranges from 800 to 900 mm annually (Capitanelli 1979).

*Sampling adult female Oc. albifasciatus:* Because this species has not been cultured in the laboratory, this work was carried out with females collected in the field. Sixteen collections were made between February and November 2000. Females landing on 2 human-bait collectors were captured with mechanical aspirators (Tonn et al. 1973, Service 1993). Collections were made at midday during autumn and winter, and during the morning and afternoon in the spring and summer, because of different adult flight activity during different seasons, according to Ludueña Almeida and Gorla (1995b). Female mosquitoes were transferred to 1-liter plastic flasks covered inside with brown absorbent paper and closed with netting tops. The mosquitoes were transported live to Villa del Totoral (120 km west of La Para), where they were kept in captivity under the natural variations of photoperiod, temperature, and humidity registered in that town, and protected from wind and rain. Climatologically, La Para and Villa del Totoral are similar, belonging to the same thermal region, and semidry flat-land biome (Capitanelli 1979).

*Number and length of gonotrophic cycles during different seasons:* The collected females were individually placed in 70-ml plastic flasks, each containing a piece of wet cotton at the bottom and filter paper covering it and the walls of the flask to provide humidity and as a substrate for oviposition. Each flask was covered with netting. Females were fed on a human host by having one of the operators put his hand over the netting long enough for each mosquito to feed. A piece of cotton soaked in 10% sugar solution also was placed on top of the netting and was changed twice a week. To count the number of days from the 1st bloodfeeding until oviposition, females were checked every 48 h in the autumn and winter, because their metabolism is slower at this time of the year (Clements 1992), and every 24 h in the spring and summer.

Females taken in each collection were divided into 2 groups and each group was exposed to a different regimen of bloodfeeding until oviposition occurred. One group of females received only one blood meal (treatment A) and then only sugar solution, whereas the other group received several blood meals until oviposition took place (treatment B). In this case, only 2 or 3 bloodfeedings occurred per gonotrophic cycle. This was repeated with each female for both treatments as many times as pos-

sible to obtain the highest number of gonotrophic cycles. A minimum of 20 females per collection, when possible, was used for each treatment.

The number of females that ingested blood, the number and dates of feedings, and the dates of oviposition were recorded, which allowed determination of the number and length of gonotrophic cycles during different seasons. To determine the number of gonotrophic cycles and to discriminate which cycle the females had attained, they were dissected, after each died, to count the number of pedicel dilatations. When females died, they were preserved in AGA (ethyl alcohol 96%, distilled water, glycerin, and glacial acetic acid in the proportions 9:5:1:1, respectively). Dissections were carried out in normal saline solution under a stereoscopic microscope, and the dilatations were counted under a compound microscope at 400× magnification (Almirón et al. 2000). The daily maximum and minimum temperatures in Villa del Totoral were recorded to correlate them with the number and length of gonotrophic cycles at different times of the year. To do that, the mean temperature between the days when the gonotrophic cycle for each female developed was calculated. The length of the gonotrophic cycles of females with 1, 2, or 3 bloodfeedings was compared by means of analysis of variance (ANOVA). The same tests were used to analyze the length of the gonotrophic cycles during the different seasons of the year.

*Egg production according to the number of blood meals, per gonotrophic cycle, and in different seasons:* For females of treatments A and B, the total number of eggs laid per individual in each gonotrophic cycle throughout the year, and the number of eggs according to the number of bloodfeedings was recorded. To detect differences in fecundity in females collected in different seasons, the number of eggs laid per gonotrophic cycle was analyzed by ANOVA, considering the different seasons of the year. A similar analysis was done by comparing the number of eggs laid in different gonotrophic cycles for all the females captured on different dates and according to the number of blood meals. The differences between the number of eggs laid per female with a full blood meal (treatment A) and with several blood meals (treatment B) in a gonotrophic cycle were detected by a *t*-test.

## RESULTS

### Number of gonotrophic cycles during different seasons

A total of 658 female *Oc. albifasciatus* were captured between February and November 2000. No females were collected on July 17 because of high winds and very low densities that were not captured by this method. The number of completed gonotrophic cycles could be determined for 498 individuals by using ovariole dissections (Table 1). Most

Table 1. Female *Ochlerotatus albifasciatus* collected between February and November 2000 along the southern coast of Mar Chiquita Lake, and their proportional distribution according to the gonotrophic cycle that began in captivity. The total number of gonotrophic cycles completed by each female was determined through ovariole dissection after the mosquito died.

Date	Sam- ple	Gonotrophic cycle					n <sup>1</sup>
		1	2	3	4	5	
Feb. 1	1	0.33	0.49	0.18	—	—	43
Feb. 10	2	0.45	0.37	0.15	0.03	—	47
Feb. 26	3	0.33	0.34	0.30	0.03	—	36
March 25	4	0.30	0.41	0.19	0.10	—	35
April 8	5	0.45	0.38	0.17	—	—	29
April 23	6	0.50	0.42	0.08	—	—	36
May 25	7	0.03	0.37	0.03	0.03	—	19
June 8	8	0.04	0.14	0.45	0.32	0.04	21
June 29	9	—	—	0.35	0.40	0.25	17
July 17 <sup>2</sup>	10	—	—	—	—	—	0
July 29	11	—	0.07	0.50	0.22	0.21	25
Aug. 19	12	—	0.05	0.30	0.55	0.10	15
Sept. 21	13	0.06	0.10	0.31	0.37	0.16	31
Oct. 21	14	0.16	0.45	0.33	0.03	0.03	36
Nov. 9	15	0.47	0.30	0.19	0.04	—	51
Nov. 26	16	0.50	0.25	0.23	0.02	—	57

<sup>1</sup> Number of dissected females.

<sup>2</sup> No females were collected on July 17.

of the females collected during the spring and summer and at the beginning of the autumn began captivity in gonotrophic cycle 1 or 2, whereas those captured during the winter began captivity in gonotrophic cycle 3, 4, or 5. A maximum of 5 gonotrophic cycles with an average of  $2.24 \pm 1.11$  was determined by ovariole dissection as each mosquito died (Table 1).

Forty-two percent of the dissected females ( $n =$

208) had fed on blood 2 or 3 times per gonotrophic cycle, laying 90 eggs on average per oviposition; the mean time between a blood meal and oviposition was 11.23 (SD = 4.06) days. The mean number of eggs per oviposition for females that completed a gonotrophic cycle after only 1 blood meal was 80, after 9.02 (SD = 5) days (the mean time between bloodfeeding and oviposition).

**Length of gonotrophic cycles during different seasons in relation to the number of bloodfeedings**

One hundred forty-nine females that had completed their 1st gonotrophic cycle were recorded after being captured from February through April, and from September through November. Recently emerged females were not found during the cold season (May–August), except for 1 female captured on June 8 that belonged to treatment A. An increase in the number of bloodfeedings produced an increase in the length of the 1st gonotrophic cycle (Table 2), with significant differences between 1 bloodfeeding, and to 2 or 3 blood meals ( $F = 22.43$ ;  $df = 2,146$ ;  $P < 0.01$ ). The length of the 1st gonotrophic cycle for treatment A females was significantly longer in summer and spring compared to autumn ( $F = 5.15$ ;  $df = 2,78$ ;  $P < 0.01$ ). No female that had completed its 1st gonotrophic cycle during winter was found. For treatment B females, the length of their 1st cycle was shorter in spring compared to summer and autumn, but no significant differences were found (Table 3).

A 2nd gonotrophic cycle was found in 79 females belonging to treatment A and 75 of treatment B females captured on all of the sampling dates, except for June 29 (21 treatment B females that had fed on blood twice during the 1st cycle but that had

Table 2. Length (in days) and standard deviation for 5 gonotrophic cycles of female *Ochlerotatus albifasciatus* belonging to treatment A (1 bloodfeeding/gonotrophic cycle) and treatment B (2 or 3 bloodfeedings/gonotrophic cycle).<sup>1</sup>

Cycle	Number of bloodfeedings	Treatment A		Treatment B	
		n	Length (days)	n	Length (days)
1	1	81	7.7 ± 3.8 a	—	—
	2	—	—	61	10.7 ± 3.6 b
	3	—	—	7	14 ± 4.6 b
2	1	79	7.8 ± 4.7 a	—	—
	2	—	—	68	10.9 ± 3.7 b
	3	—	—	7	13.8 ± 3.1 b
3	1	81	10.3 ± 5.1 a	—	—
	2	—	—	39	10.9 ± 3.9 a
	3	—	—	2	14 ± 7.1 a
4	1	38	11.7 ± 6.4 a	—	—
	2	—	—	16	12.2 ± 3.9 a
5	1	11	11 ± 3.6 a	—	—
	2	—	—	8	9.5 ± 1.6 a

<sup>1</sup> Different lowercase letters indicate significant differences in the length of the gonotrophic cycles, for each treatment and among treatments, respectively.

Table 3. Length (in days) and standard deviation for 5 gonotrophic cycles of female *Ochlerotatus albifasciatus* belonging to treatment A (1 bloodfeeding/gonotrophic cycle) and treatment B (2 or 3 bloodfeedings/gonotrophic cycle) during different seasons of the year.<sup>1</sup>

Season	Gonotrophic cycle, treatment A					Gonotrophic cycle, treatment B				
	1	2	3	4	5	1	2	3	4	5
Summer	8.3 ± 5.4 b	9 ± 5.4 a	9.9 ± 5.4 a	6 ± 2.8 a	—	11.6 ± 4.9 ab	10.8 ± 3.9 a	10.6 ± 3.5 ab	—	—
Autumn	7.3 ± 2.4 a	6.6 ± 4.8 a	10.5 ± 6.4 a	12.6 ± 7.7 a	13 a	12.8 ± 4.1 b	14.4 ± 3.8 b	14.3 ± 4.8 c	15.4 ± 3.8 b	—
Winter	—	15 ± 3 b	10.8 ± 4.6 a	11.4 ± 4.2 a	11 ± 4.1 a	—	—	12.6 ± 5.1 bc	12.8 ± 3.7 ab	9.3 ± 1.9 a
Spring	8.3 ± 2.5 b	7.1 ± 2.3 a	10.2 ± 4.2 a	12.4 ± 8.1 a	10 ± 2.8 a	10 ± 2.1 a	9.4 ± 1.8 a	8.9 ± 1.5 a	9.2 ± 2.3 a	9.8 ± 1.5 a

<sup>1</sup> Different lowercase letters in each column indicate significant differences in the length of each gonotrophic cycle during the different seasons of the year, and between the 2 treatments of bloodfeeding.

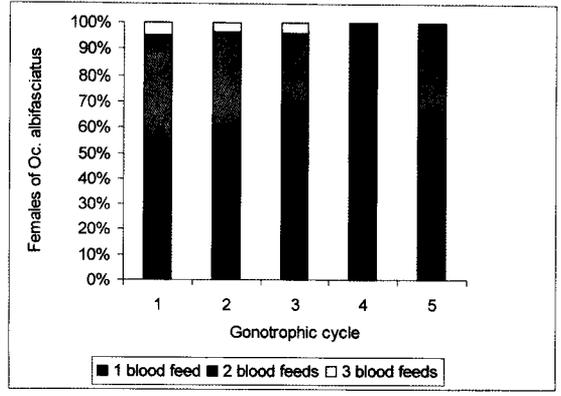


Fig. 1. Percentage of female *Ochlerotatus albifasciatus* that completed 1, 2, and 3 bloodfeedings per gonotrophic cycle.

only 1 blood meal during cycle 2 were not included in this analysis). The length of the 2nd cycle was significantly longer after 2 bloodfeedings ( $F = 13.56$ ;  $df = 2,151$ ;  $P < 0.01$ ; Table 2). The duration of this cycle for treatment A females was markedly longer during the coolest months when compared with the rest of the year ( $F = 4.17$ ;  $df = 3,75$ ;  $P < 0.01$ ; Table 3). Females captured on June 29, and assigned to treatment A, did not complete this cycle. No treatment B females that had completed cycle 2 were recorded during the winter. This may be due to the length of this cycle being longer in the autumn than in the warmer spring and summer months ( $F = 13.74$ ;  $df = 2,72$ ;  $P < 0.01$ ; Table 3).

A 3rd gonotrophic cycle was observed in 81 treatment A females and in 41 treatment B females, 2 of which took 3 blood meals (Table 2), for all sampling dates, except for those taken on April 23, June 29, and August 19. The number of females completing this cycle after being collected was variable throughout the year (Table 1), being more frequent at the end of autumn and during the winter. Excluded from the analysis were 14 females that had fed on blood 2 times to complete both cycle 1 and cycle 2, but that had bloodfed only once to reach cycle 3. The length of this cycle, particularly at the end of summer and the beginning of autumn, did not show the same tendency to increase with the number of bloodfeedings as had happened previously (Table 2). Significant differences were not observed in the length of cycle 3 throughout the year for treatment A females. However, the length of the 3rd cycle was significantly longer for those females in treatment B during the autumn and winter compared to spring ( $F = 5.52$ ;  $df = 3,37$ ;  $P < 0.01$ ; Table 3).

The 4th gonotrophic cycle was determined in 38 treatment A females for almost all sampling dates, except for February 1, April 8, April 23, and October 21; and on February 10, February 26, and November 26, only 1 treatment A female was col-

Table 4. Egg production per female *Ochlerotatus albifasciatus* according to the number of bloodfeedings and per gonotrophic cycle.<sup>1</sup>

Cycle	Number of bloodfeedings	Treatment A		Treatment B	
		<i>n</i>	Number of eggs and standard deviation	<i>n</i>	Number of eggs and standard deviation
1	1	81	84.4 ± 20.3 a	—	—
	2	—	—	61	92.9 ± 31.9 a
	3	—	—	8	126.4 ± 47.7 b
2	1	79	94.1 ± 32.8 a	—	—
	2	—	—	68	102.7 ± 44.2 a
	3	—	—	7	136.1 ± 42.9 b
3	1	81	77.9 ± 29.4 a	—	—
	2	—	—	39	75.5 ± 36.4 a
	3	—	—	2	136 ± 15.6 b
4	1	38	57.5 ± 20.6 a	—	—
	2	—	—	16	58.5 ± 24.6 a
5	1	11	38.1 ± 13.1 a	—	—
	2	—	—	8	27 ± 13 a

<sup>1</sup> Different lowercase letters indicate significant differences in the number of eggs laid in each gonotrophic cycle, for each treatment and among treatments, respectively.

lected that had completed the 4th cycle. For treatment B, cycle 4 was completed in 16 females that took 2 blood meals, and were collected in May–June and August–November. Cycle 4 was not found in females during the rest of the year. Not included in this analysis were 9 females that bloodfed 2 times in each of the previous cycles, but that had only fed once in this cycle. The length of cycle 4 did not show significant differences between 1 and 2 bloodfeedings (Table 2). With regard to the length of cycle 4 at other times of the year, no significant differences were observed for treatment A females, but treatment B females showed a significantly longer cycle 4 in autumn as compared with those collected in winter and spring ( $F = 4.99$ ;  $df = 2,13$ ;  $P < 0.05$ ; Table 3).

Nineteen of the collected females had begun their 5th gonotrophic cycle by the end of autumn, during the winter months, and during the spring (June–October). One female collected on October 21 had completed this cycle having bloodfed only once. Eleven females that had completed cycle 5 belonged to treatment A and 8 belonged to treatment B. The length of this cycle was somewhat longer in females that had bloodfed only once com-

pared to those that had fed twice, but this was not statistically different (Table 2). No significant seasonal differences were found for the length of cycle 5 throughout the year for both treatments (Table 3). In general, the length of the gonotrophic cycles was increased with the number of bloodfeedings (Table 3); however, no correlation was seen between temperature and length of the different cycles.

#### Egg production according to the number of blood meals, and per gonotrophic cycle

More than 50% of the females bloodfed only once per gonotrophic cycle (Fig. 1). A number of females bloodfed 2 or 3 times per gonotrophic cycle, with approximate proportions of 45% for cycle 1, 40% for cycle 2, 30% for cycle 3, 25% for cycle 4, and 42% for cycle 5. For both treatments A and B, a peak in egg production was observed in cycle 2 compared with cycle 1, and a decrease in the number of eggs produced was seen in cycles 3, 4, and 5, independent of the number of bloodfeedings (Table 4).

Highly significant differences were found when analyzing the number of developed eggs in all the

Table 5. Mean egg production and standard deviation for 5 gonotrophic cycles of female *Ochlerotatus albifasciatus* belonging to treatment A (1 bloodfeeding/gonotrophic cycle) and treatment B (2 or 3 bloodfeedings/gonotrophic cycle) during different seasons of the year.<sup>1</sup>

Season	Gonotrophic cycle, treatment A				
	1	2	3	4	5
Summer	84.6 ± 19.5 a	86.7 ± 37.5 a	55.7 ± 32.9 a	33 ± 19.8 a	—
Autumn	79.8 ± 21.2 a	94.2 ± 37.9 a	75.8 ± 26.3 b	48.5 ± 20.8 ab	26 a
Winter	—	103.3 ± 35.2 a	82.5 ± 30.9 b	55.8 ± 14.9 b	39.6 ± 13.9 a
Spring	89.8 ± 18.9 a	101.4 ± 13.1 a	92.7 ± 16.4 b	74.9 ± 17.5 c	38 ± 14.1 a

<sup>1</sup> Different lowercase letters in each column indicate significant differences in egg production for each cycle during different seasons of the year, and between the 2 treatments of bloodfeeding.

gonotrophic cycles ( $F = 31.98$ ;  $df = 4,493$ ;  $p < 0.01$ ). Duncan's test identified 4 homogeneous groups, with cycles 1 and 2 in 1 group, whereas cycles 3, 4, and 5 were separated. In gonotrophic cycles 1, 2, and 4, the mean number of eggs was always higher in treatment B females (Table 4), with highly significant differences between treatments ( $T = -3.31$ ;  $df = 496$ ;  $P < 0.01$ ). In cycle 3 as well as in cycle 5, the mean number of eggs laid was slightly higher after 1 blood meal than after 2 blood meals (Table 4). When analyzing the total number of eggs laid by all females, the highest number was obtained from females that took 3 blood meals ( $F = 17.77$ ;  $df = 2,495$ ;  $P < 0.01$ ).

The number of eggs laid was significantly higher after 3 bloodfeedings compared with 1 or 2 blood meals for cycles 1 ( $F = 8.36$ ;  $df = 2,146$ ;  $P < 0.01$ ), 2 ( $F = 4.15$ ;  $df = 2,151$ ;  $P < 0.05$ ), and 3 ( $F = 3.46$ ;  $df = 2,119$ ;  $P < 0.05$ ; Table 4). Significant differences in the number of eggs laid were not observed between both treatments in cycles 4 and 5 (Table 4).

#### Egg production during different seasons

No significant differences were found in egg production in cycles 1, 2, and 5 for treatment A females throughout the year (Table 5). Treatment A females laid significantly fewer eggs in cycle 3 in summer as they did during the rest of the year ( $F = 7.35$ ;  $df = 3,77$ ;  $P < 0.01$ ; Table 5). Treatment A females that completed cycle 4 were collected throughout the year, with significant seasonal differences ( $F = 5.51$ ;  $df = 3,34$ ;  $P < 0.01$ ).

Treatment B females, in gonotrophic cycle 3, showed a significant seasonal difference in egg production, with the highest number of eggs laid recorded in the spring ( $F = 4.29$ ;  $df = 3,37$ ;  $P < 0.01$ ). No significant differences were found in the number of laid eggs in cycles 1, 2, 4, and 5 (Table 5).

#### DISCUSSION

In Corrientes Province (northeastern Argentina), Hack et al. (1978) observed a predominance of *Oc. albifasciatus* during the autumn and winter months, with an increase in their populations in June. In Córdoba Province, peak abundance of *Oc. albifasciatus* occurs during the spring and summer months, with increasing populations seen in No-

vember and December, and February through March. Immatures and adults also are found in autumn and winter (Almirón and Brewer 1994, Ludueña Almeida and Gorla 1995a, Gleiser and Gorla 1997). According to Fava et al. (2001), female *Oc. albifasciatus* remain active biters during the autumn and winter, an observation supported by this work. In collections made throughout year 2000, females continued to feed during the cold season, but in substantially lower numbers than seen in the warm season. We note that no females were captured on July 17, probably because of high winds and low mosquito density, but in spite of the low temperatures, some biting females were taken on July 29. It was later found that these females had likely emerged beforehand and some of them were quite resistant to low temperatures. Almirón et al. (2000) suggested that female *Oc. albifasciatus* may continue their gonotrophic activity during the autumn and winter in Córdoba Province and they may have more than 1 blood meal per gonotrophic cycle. Fava et al. (2001) found that female *Oc. albifasciatus* that ingested blood and a sugar solution had a greater average survival time than those that ingested only the sugar solution. In this work, all of the females that belonged to treatment B completed 2 or 3 bloodfeedings per gonotrophic cycle, verifying the proposal of Almirón et al. (2000).

Through ovariole dissection, a maximum of 5 gonotrophic cycles was recorded in this study, which is in agreement with the findings of Ludueña Almeida and Gorla (1995a). Females that completed the maximum number of cycles were collected in the cold months after having completed their early gonotrophic cycles in the field (cycles 1 and 2, and in some cases even cycle 3). This might suggest that these females could be the more resistant to low temperatures, and that there had not been a recent emergence of young females. A high proportion of recently emerged females was collected in the warm season and they completed up to 3 gonotrophic cycles in captivity. These were later proved to be cycles 1, 2, and 3, through ovariole dissection, showing that the females were relatively new individuals that had just been incorporated into the population. The fact that no females in cycle 5 were seen during the warm season does not necessarily mean that these individuals were absent, but that they would be proportionally more scarce

Table 5. Extended.

Gonotrophic cycle, treatment B				
1	2	3	4	5
101.9 ± 41.9 a	97.4 ± 49.9 a	47.8 ± 18.5 a	—	—
75.8 ± 36.3 b	123.8 ± 57.7 a	87.4 ± 52 bc	50.6 ± 32.3 a	—
—	—	56.2 ± 39.6 ab	52.4 ± 24.8 a	23.3 ± 4.6 a
99 ± 25.1 a	102.1 ± 22.2 a	93.4 ± 23.8 c	70.2 ± 18.5 a	30.8 ± 18.4 a

because of the increased emergence of new females.

Fava et al. (2001) reported values higher than 10 days for the length of the gonotrophic cycles of *Oc. albifasciatus*, reaching 53 days during autumn and winter, although they do not discriminate the cycles. Ludueña Almeida (1994) had already observed that the mean time between bloodfeeding and oviposition was 3 days. The time intervals observed here for each gonotrophic cycle through the year were variable, depending mainly on the number of bloodfeedings per cycle. This did not happen for cycle 5, the duration of which was longer with just 1 bloodfeeding as compared to 2 feedings (Table 2). In general, the greatest length of these cycles was observed during the cold months, with values up to 34 days in some individuals. These observations tend to agree with those data reported by Fava et al. (2001) concerning the length of the cycles during winter.

No correlation between temperature and the length of cycles was observed, suggesting that temperature is not a limiting factor (Fava et al. 2001) and that cycle length is mainly influenced by the number of bloodfeedings. Fava et al. (2001) estimated the development threshold for the egg of *Oc. albifasciatus* as 2.28°C, and 4.71°C for larvae and pupae. They found that eggs hatched when the minimum temperatures were as low as -0.5°C. The lowest mean temperature registered during the cold season for this work was 7.5°C, higher than the development threshold for this species. According to results of this study, the length of the cycles depends primarily on the number of bloodfeedings. This could mean that the length of the cycles tends to be longer when females have a chance of taking several blood meals, thus increasing egg production as well.

Ludueña Almeida and Gorla (1995a) found a mean number of eggs per gonotrophic cycle of 110 (SD = 39.65). Fava et al. (2001) reported a mean number of eggs laid per cycle to be 62.65 (SD = 38.62) after the 1st bloodfeeding, and 42.42 (SD = 50.96) after the 2nd feeding for females captured in autumn and winter. This differs from 84.4 (SD = 20.3) eggs laid after the 1st feeding for the 1st gonotrophic cycle, and 92.93 (SD = 31.9) after the 2nd bloodfeeding for the same cycle as reported in this work. After 3 bloodfeedings in the 1st cycle, significant differences were observed, with values of 126.4 (SD = 47.7). The reason for this could be that all captured females in this study were considered independent of the time of the year, not only for autumn and winter. The mean number of eggs laid per gonotrophic cycle increased with an increase in the number of blood meals and was significantly higher in cycles 1, 2, and 3 for females that had 3 bloodfeedings (Table 4); for females that completed cycles 4 and 5, the mean number of eggs decreased independent of the number of bloodfeedings. This may prove the importance of the amount

of blood ingested in relation to the fecundity of young females, but on the other hand could suggest that there is a decrease in egg production with the increased physiological age of these females, independent of the number of bloodfeedings.

In all gonotrophic cycles, the greatest variability in the number of eggs per oviposition was observed in treatment B females when compared with those of treatment A, which could be due to the greater amount of blood ingested, meaning that females might develop longer cycles when they have a possibility of increasing the total amount of blood ingested, and therefore increase the number of eggs laid per gonotrophic cycle.

According to this and previous studies, we can state that in Córdoba Province the peak abundance of female *Oc. albifasciatus* occurs in the summer and autumn months, even though they remain active biters throughout the year. They do not appear to enter diapause, but their abundance decreases considerably during cold and dry periods. During the warm season within each gonotrophic cycle, they may bloodfeed several times, thus increasing the length of the cycles and allowing extra time to contact more hosts, thus increasing the possible transmission of pathogens. The low autumn and winter temperatures do not appear to limit the females, because they remain active, ingest blood, and lay eggs during this time, contributing to the adult population during the next favorable breeding season. The length of the gonotrophic cycles is extended in females that emerge at the end of summer and survive until the autumn and winter months, but this may depend more on the number of blood meals than on temperature.

Female survivorship is higher after bloodfeeding; however, those that emerge at the end of summer and at the beginning of autumn will not live until the next warm rainy season (Almirón et al. 2000). Diapausing eggs may provide a mechanism through which *Oc. albifasciatus* survives the autumn and winter months (dry season). Immature stages continue their development even during the cold months in Córdoba Province and some new adults may emerge to be incorporated into the population during this time. According to Fava et al. (2001), the thermal threshold of development (4.71°C) estimated for *Oc. albifasciatus* may allow this species to extend its range to the southernmost areas of Córdoba Province, where temperatures permit.

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