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BEHAVIOR OF TRIATOMINES (HEMIPTERA: REDUVIIDAE) VECTORS OF CHAGAS' DISEASE. IV. FECUNDITY, FERTILITY AND LONGEVITY OF *PANSTRONGYLUS MEGISTUS* (BURM., 1835) PAIRS AND VIRGIN FEMALES STARVED UNDER LABORATORY CONDITIONS

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A laboratory study was conducted on the fecundity, fertility and life span of Panstrongylus megistus *pairs and on the fecundity and life span of* P. megistus *virgin females submitted to starvation after the last moulting. Of the mated females, 22.2% laid eggs, 4.4% of which were fertile. Females resisted starvation more than males. Of the starved virgin females, only 10% laid eggs, with a low egg-laying rate (0.47) per female. Resistance to starvation was lower in virgin than in mated females.*

Key words: Chagas' disease – reproduction – fecundity – longevity – triatomines – ***Panstrongylus megistus***

The resistance of triatomines to starvation is a highly important characteristic in the ecology and epidemiology of Chagas' disease (Gajardo-Tobar, 1952; Zeledón et al., 1970; Perondini, 1973). Because of this resistance they remain alive spontaneously moving from a region to another (Costa & Perondini, 1973). This factor is also very important in terms of insecticide application. According to Perlowagora-Szumlewicz (1969), these triatomines may continue to stay in their hiding places even after a dwelling has been treated with insecticides, so that part of population may survive and repopulate the area after the deleterious effect of the toxic agent has passed.

The objective of the present study was to determine the effect of feeding on the fecundity, fertility and longevity of *Panstrongylus megistus* as part of a continuing series of investigations on the reproduction of this species (Lima et. al., 1986 a,b; Lima et. al., 1987).

MATERIAL AND METHODS

Fifth-instar nymphs obtained from the Entomology Departament of the "Instituto Oswaldo Cruz" were separated by sex (Espinola, 1966) and allowed to feed weekly on pigeons up to the imaginal moulting. The adults were marked individually (Mac Cord et. al., 1983).

The tests were divided into two phases:

Phase I – Thirty pairs were separated on the day of the last moulting into 7.5 x 5.5 cm glass bottles and received no food. The bottles were observed daily and egg-laying, fertility and possible death of each insect were recorded. Dead insects were not replaced. The observations covered the period from April to May 1984. Mean temperature was 29.3 ± 1.8°C and relative atmospheric humidity was 79.3 ± 5.5%.

Phase II – Assuming that the nutrient supply provided by the last nymphal instar may affect egg-laying, egg fertility and duration of the imaginal period in the absence of food, the tests were repeated after the death of all insects used in Phase I. Thirty males and 30 females forming pairs and 30 virgin females were weighed individually on an analitical precision scale on the day of the last moulting. Observations were made from October to December 1984. Mean temperature and humidity were 28.3 ± 1.9°C and 80.9 ± 1.9%.

RESULTS

Phase I – Thirty pairs were separated on the day of the last moulting but only 15 which survived more than 8 days were analized, since the others would have no time to start egg-laying. The data are given in Table I. Five females (33.3%) laid eggs, and 2 of these (40%) laid fertile eggs, with a high fertility rate (90%). The last egg-laying ocurred at a minimum of 1 day and a maximum of 10 days before the death of females ($\bar{X}$ = 4.8 ± 4.0 days).

Females were more resistant to food deprivation than males (T = 24.5; $P < 0.05$), surviving a miminum of 10 and a maximum of 54 days ($\bar{X}$ = 20.4 ± 10.6 days) (Fig. 1).

Phase II – Among the 30 pairs under observations, 5 females laid eggs, but none of them was fertile. Five females expelled spermatophores, indicating that copulation had occurred.

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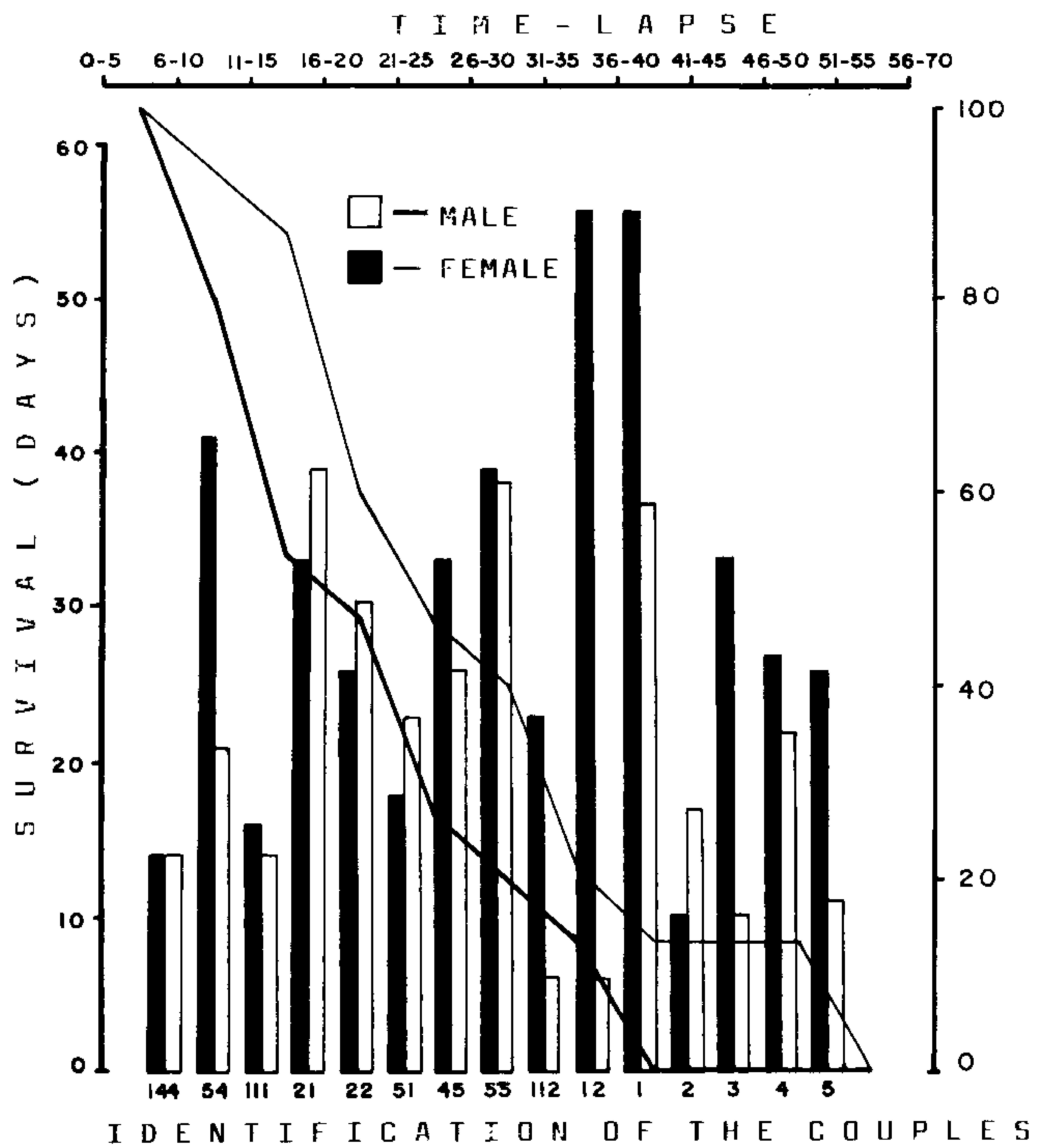

Fig. 1: Survival of males and females of *Panstrongylus megistus* (Phase I) submitted to starvation after the last moulting (n=15).

The number varied from 1 to 2 ($\bar{X}$ = 1.2 ± 0.4 spermatophores/females). Two females expelled spermatophore but laid no eggs, and 3 laid eggs but did not eliminate any spermatophores. The number of egg-laying varied from 2 to 4, with a total of 21 eggs (a minimum of 3 and a maximum of 8 eggs per female, $\bar{X}$ = 0.70). The last egg-laying occurred 1 to 12 days before death of females ($\bar{X}$ = 5.8 ± 4.5 days) (Table II).

Table III shows the relationship between initial insect weight, duration of the imaginal period and weight after death. The highest weight was not always related to egg-laying. Female no. 1, which laid eggs, weight less than several other females, such as, for example, nos. 3, 31 and 32 whose weights exceeded 500 mg and which did not lay eggs. Male no. 111, whose weight was also one of the lowest, copulated.

Analysis of Tables II and III showed no significant correlation between weight at last nymph instar and the factors tested (r = 0.23; > 0.05).

Again, as was also the case for Phase I, males proved to be less resistant to starvation than females (Fig. 2).

Of the 30 virgin females, only 3 (10%) laid eggs with a very low rate of eggs laid per female (0.47).

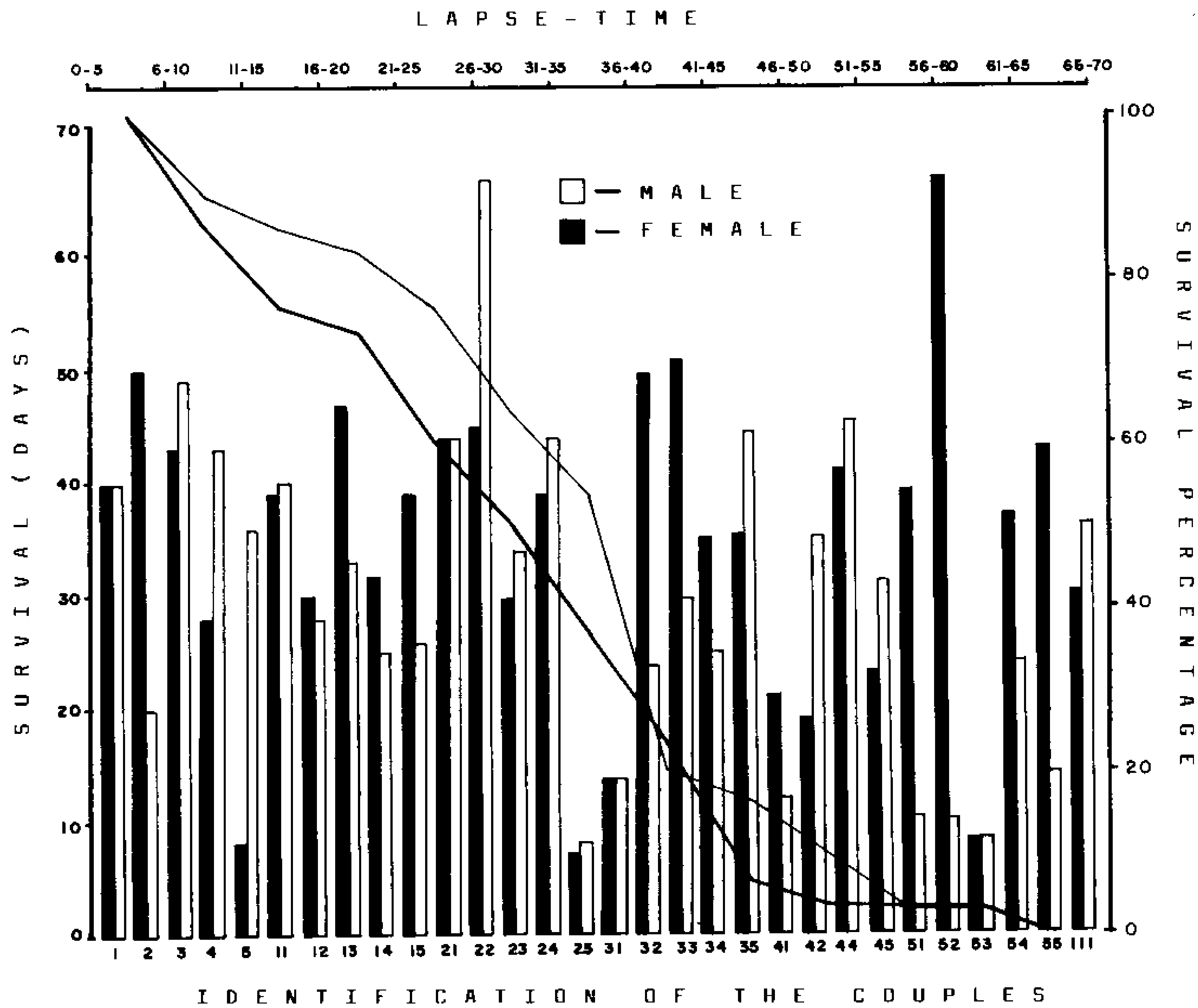

Fig. 2: Survival of males and females of *Panstrongylus megistus* (Phase II) submitted to starvation after last moulting (n=30).

Table IV shows the results of the observations. The last egg-laying occurred 1 to 15 days ($\bar{X}$ = 3.0 ± 1.6) before death of the females, and the mean weight of the 3 females that laid eggs was 423.5 ± 65.4 mg.

Table V shows the initial weights of the 30 virgin females, the duration of their imaginal period and their weights after death. Female no. 21, which laid eggs, weighed less than several other females that did not lay eggs, probably owing to a smaller nutrient originating from the 5th nymph instar. On the other hand, female no. 31, which was the heaviest among the 30 females studied, laid more eggs (11), with a larger number of egg-laying (5) and with the maximum number of eggs laid per day (4).

The duration of the imaginal period was much shorter than for the mated females, reaching approximately the same value as for the mated males which did not feed (Fig. 3). The Wilcoxon test showed a significant difference ($Z = -3.2$; $p < 0.05$) in relation to the 30 mated females (Phase II) observed simultaneously (Fig. 3).

DISCUSSION

As observed by Zeledón et al. (1970) in *Triatoma dimidiata*, we noted that *P. megistus* is a species with high reproductive potential. It was actually possible to obtain offspring even from pairs that did not feed after the last moulting.

In *Rhodnius prolixus*, Buxton (1930) observed that egg-laying depends on feeding and mating and that virgin females lay smaller amounts of eggs than mated females and start egg-laying later. In the present study, the virgin females that did not feed after the imaginal moulting laid very small numbers of eggs, and only 10% of them laid eggs, with a mean number

TABLE I

Oviposition, fertility and life span of *Panstrongulus megistus* submitted to starvation after the last moulting (Phase I)

Identification of the couples	Preoviposition period (days)	Total number of eggs laid	Fertile eggs	Life span (days)	
				females	males
144	14	5	–	14	14
54	–	–	–	38	21
111	–	–	–	16	14
21	–	–	–	31	36
22	14	20	18	25	28
51	–	–	–	17	23
45	15	4	–	31	25
55	15	23	20	36	35
112	–	–	–	23	6
12	26	27	–	54	6
1	–	–	–	54	38
2	–	–	–	10	17
3	–	–	–	31	10
4	–	–	–	26	22
5	–	–	–	25	11
Total	–	79	38	–	–
$\bar{X} \pm SD$*	16.4 ± 5.2	15.8 ± 10.8	19.0 ± 1.9	28.7 ± 13.0	20.0 ± 10.6

* Mean and standard deviation.

TABLE II

Oviposition, and spermatophores of *Panstrongylus megistus* submitted to starvation after the last moulting (Phase II)

Identification of the couples	Preoviposition period (days)	Total number of eggs laid	Fertile eggs	Maximum eggs female/day	Spermatophore/ female
1	20	3	–	2	–
3	29	3	–	3	2
11	–	–	–	–	1
33	34	4	–	2	–
35	22	3	–	2	1
44	–	–	–	–	1
51	31	8	–	3	–
55	–	–	–	–	1
X ± SD*	15.2 ± 14.0	4.2 ± 1.9	–	2.0 ± 0.6	1.2 ± 0.4

* Mean and standard deviation.

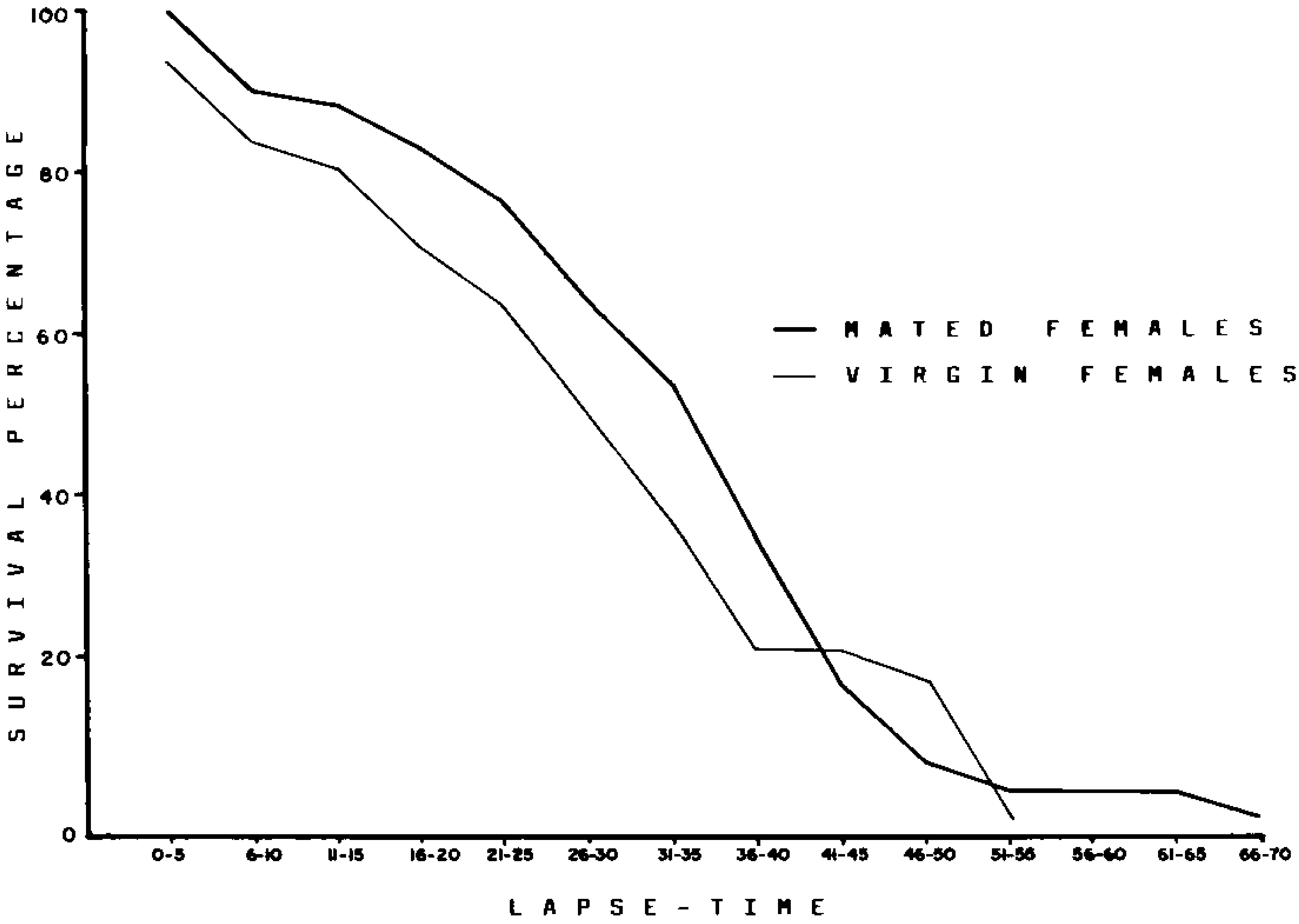

Fig. 3: Survival curve of mated females (Phase II) and virgin females of *Panstrogylus megistus* submitted to starvation after the last moulting (n=30, for each stituation).

of eggs per female of 0.47 ± 2.0. According to Wigglesworth (1936), starved *R. prolixus* females partially reabsorb their eggs with consequent contraction and inactivity of the "corpus allatum".

Furtado & Queiroz (1978) obtained a mean of 0.1 egg per female of *P. megistus* allowed to mate but deprived of food. Fertile egg-laying in the absence of food has been observed in other species of triatomines, such as *R. prolixus* (Buxton, 1930; Galliard, 1936), *Triatoma protracta protracta* (Ryckman, 1962), and *Triatoma dimidiata* (Zeledón et al., 1970). In the present study, starved females forming pairs with starved males, in addition to laying fertile eggs in some cases, had a higher egg-laying rate than the 30 starved virgin females, with 22% of them laying eggs (considering both Phases I and II, n = 45), even though copulation was not always observed. According to Pratt & Davey (1972), mated females may produce more than two oocytes per ovariole, whereas virgin females produced little more than one. According to these authors (op. cit.), the first cycle of oogenesis occurs identically in the two situations, i.e. both in virgin and mated females, but the second cycle is inhibited in virgin insects. In *R. prolixus*, egg production depends on feeding and mating owing to endocrine factors. According to Davey (1974), feeding stimulates juvenile hormone secretion by the "corpus allatum", which acts on the fat body producing vitellogenin, and on the ovaries promoting the transfer vitellogenin from the hemolymph to the egg cell. Buxton (1930) and Davey (1967) have suggested that the increased egg-laying of *R. prolixus* may be mediated by the myotropic factor released by the neurosecretory cells of the "pars intercerebralis", which induces ovipositing by acting on the ovarin muscles (Kriger & Davey, 1984). In the absence of food, the female lives on her nutrient reserves including ovarian proteins which stimulate the endocrine system, with consequent egg production.

In *P. megistus* we could not find a relationship between the egg-laying of starved females with the nutrient reserve supplied by the last nymph instar. In a study on *Triatoma brasiliensis*, Costa & Perondini (1973) and Costa et al. (1975) concluded that egg production and resistance to fasting depend on body weight, which derives from the last nymph instar. Since egg production depends on the stimulation which is not only induced by mating but also by feed-

TABLE III

Initial insect weight, life span and weight after death of *Panstrongylus megistus* submitted to starvation after the last moulting (Phase II)

Ident. of couples	Weight after moulting (mg)		Weight after death (mg)		Life span (days)	
	male	female	male	female	male	female
1	178.2	335.5	103.0	185.0	40	40
2	293.5	309.3	113.6	191.1	18	50
3	387.7	500.3	245.3	247.2	49	43
4	290.9	314.8	137.5	148.6	43	28
5	250.3	329.4	108.4	266.6	36	8
11	314.8	326.4	137.5	138.5	40	39
12	247.6	257.5	120.6	128.8	28	30
13	204.1	475.9	113.0	195.7	33	47
14	267.7	354.9	108.3	124.2	25	32
15	231.3	380.6	135.8	156.2	26	39
21	279.3	415.4	161.0	181.9	44	44
22	448.1	377.6	175.5	161.8	65	45
23	284.9	260.4	129.2	120.8	34	30
24	310.4	403.1	161.1	200.8	44	39
25	251.8	284.6	191.6	189.6	8	7
31	247.6	570.5	200.5	389.1	13	14
32	194.5	547.9	97.1	182.6	24	50
33	255.3	599.6	161.0	240.6	30	51
34	180.9	364.6	94.3	158.8	25	35
35	306.8	528.1	118.7	250.7	44	35
41	338.7	420.2	271.6	192.8	11	21
42	246.6	170.5	103.3	126.0	35	19
43	328.9	460.3	127.7	145.1	45	41
44	159.5	350.4	80.0	142.0	31	23
45	192.4	377.8	125.8	155.5	10	39
51	315.1	545.0	213.4	151.9	10	65
52	275.0	218.9	169.3	165.2	8	8
53	321.8	458.4	158.8	203.1	24	37
54	386.7	423.7	229.2	215.5	14	43
55	234.4	233.9	114.7	119.7	36	30
$\bar{X} \pm SD$*	274.2±65.3	384.1±108.9	135.9±41.4	182.5±55.1	28.9±1.4	34.4±1.6

*Mean and standard deviation.

TABLE IV

Oviposition of virgin females of *Panstrongylus megistus* submitted to starvation after last moulting

Identification of the female	Preoviposition period (days)	Total number of eggs laid	Maximum eggs female/day	Number of ovipositions
12	27	1	1	1
21	23	2	1	2
31	26	11	4	5
$\bar{X} \pm SD$*	25.3±1.7	4.3±4.7	2.0±1.4	2.7±1.7

*Mean and standard deviation.

TABLE V

Initial weight, weight after death and life span of virgin female of *Panstrongylus megistus* submitted to starvation after the last moulting

Identification of the females	Weight after the moulting (mg)	Weight after the death (mg)	Life span (days)
1	337.5	182.7	30
2	283.0	211.6	8
3	291.2	115.2	40
4	421.6	326.0	8
5	286.8	207.9	10
11	476.7	225.5	28
12	431.1	186.7	28
13	293.2	116.0	24
14	385.1	155.0	40
15	350.2	155.9	39
21	339.8	150.1	31
22	236.2	114.8	17
23	313.5	128.0	19
24	212.1	104.8	10
25	283.9	135.4	31
31	499.5	195.0	47
32	301.8	159.4	31
33	138.8	121.4	3
34	352.0	179.4	15
35	421.7	187.9	30
41	415.1	143.2	29
42	241.4	103.8	46
43	359.0	141.8	31
44	358.2	155.7	40
45	218.3	99.0	27
51	394.8	182.0	48
52	321.9	133.4	38
53	324.3	194.4	16
54	297.2	225.0	5
55	366.3	161.7	21
$\bar{X} \pm SD$*	331.0 ± 80.1	165.3 ± 51.1	26.3 ± 13.0

* Mean and standard deviation.

ing, egg-laying under starving conditions becomes possible for females having sufficient food stores in their digestive tract to activate the "corpus allatum" for egg production (Garcia & Azambuja, 1985). The number of eggs is small because food is not replaced. In the present study, in some cases we obtained results similar to those cited above when relating body weight to ovipositing by starved virgin and mated females. No correlation between female weight and number of eggs laid was obtained by the Pearson's correlation test ($r = 0.23$; $p > 0.05$).

This lack of relationship could be due to the relatively small size of the sample. This point could be clarified with a larger sample of females laying eggs. According to Collier et al. (1977) the fraction of energy used in metabolism of *Triatoma phyllosoma*, did not change appreciably until instar V, when it increased. About 12.713 joules of energy were contained in eggs produced by a female of average longevity. The authors deduce that an adult female of *T. phyllosoma* requires 12.3 feedings during adulthood to supply the energy needed for this level of reproductive activity.

As to the duration of the imaginal period, females resisted starvation more than males in both groups. This fact had also been observed in *Triatoma infestans* (Gajardo-Tobar, 1952; Perlowagora-Szumlewicz, 1976), *Triatoma spi-*

nolai (Gajardo-Tobar, 1952), *Rhodnius neglectus* (Costa et al., 1967) and *T. brasiliensis* (Costa & Perondini, 1973). In *T. dimidiata*, Zeledón et al. (1970) observed that females tended to be more resistant than males, though this is not a general rule. The results of this study contradict those reported by Perlowagora-Szumlewicz (1976), who found *P. megistus* males to be more resistant to starvation. Two explanations could be proposed for these differences: 1) Perlowagora-Szumlewicz worked with insects collected in the field and with the F1 obtained in the laboratory; and 2) her results referred to a sample of 10 individuals, whereas the *P. megistus* studied here originated from a countless generations reared in the laboratory and the sample consisted of 45 individuals of each sex observed in two replications.

Neiva (1910) noted that *P. megistus* males suck with the same intensity as females but are less resistant to adverse weather conditions and, even under favorable conditions, feed and live less than females. More recently Heitzmann-Fontenelle (1976) observed that, under favorable laboratory conditions, *P. megistus* males have a much shorter life span than females. In the present experiments, the mated females in the Phase II (n = 30) lived longer than virgin females (n = 30), whose survival was close to that of starved males in Phase II (which were observed simultaneously). Davey (1965) and Brasileiro (1982), respectively studying *R. prolixus* and *T. brasiliensis* fed at regular intervals, found that virgin females were more resistant than mated females, living for a longer period of time.

RESUMO

Comportamento de triatomíneos Hemiptera: Reduviidae) vetores da doença de Chagas. IV. Fecundidade, fertilidade e longevidade de casais e fêmeas adultas de *Panstrongylus megistus* Burm., 1835), privados de alimento, em condições de laboratório – Efetuou-se, em laboratório, um estudo sobre a fecundidade, fertilidade e duração de vida de casais de *Panstrongylus megistus* e da fecundidade e duração de vida de fêmeas virgens dessa espécie, submetidos a jejum, após a última ecdise. Das fêmeas acasaladas, 22,2% puseram ovos, dos quais 4,4% estavam férteis. As fêmeas tiveram maior resistência ao jejum que os machos. Das fêmeas virgens em jejum, apenas 10% puseram ovos, com baixa de ovipostura por fêmeas (0,47). A resistência ao jejum foi mais baixa nas fêmeas virgens do que naquelas que permaneceram sempre com os machos.

Palavras-chave: doença de Chagas – reprodução – fecundidade – longevidade – triatomíneos – *Panstrongylus megistus*

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