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FEATURES OF REPRODUCTION IN LABORATORY-REARED *AEDES FLUVIATILIS* (LUTZ, 1904) (DIPTERA: CULICIDAE)

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Observations were made on 100 female Aedes fluviatilis *(Lutz) maintained individually from the first blood meal onwards and allowed one blood meal during each oviposition cycle, 5% dextrose solution been supplied throughout life. The average length of life was 49.9 ± 17.8 days; the logarithm of the mortality rate increased proportionately to physiological age. The insects took an average of 7.3 ± 3.2 blood meals and produced a mean of 7.9 ± 3.7 clutches of eggs. There was a progressive decrease, proportional to advancing physiological age, in the mean numbers of eggs laid in successive oviposition cycles, in the intervals between blood feeding and oviposition, and in the numbers of larvae that hatched. Delayed oviposition, transient sterility and a total loss of fertility were also recorded.*

Aedes fluviatilis (Lutz, 1904) is a widespread Neotropical mosquito often found breeding in domestic and peridomestic situations (Forattini, 1965; Cônsoli, 1976). The females are strongly anthropophilic, at least in the laboratory (Cônsoli & Williams, 1981). Experimentally, the mosquito is susceptible to infection with yellow fever (YF) virus, *Plasmodium gallinaceum* and *Dirofilaria immitis* (Davis & Shannon, 1931; Tasón de Camargo & Krettli, 1978; Kasai, 1979).

In countries where the maintenance of *Ae. aegypti* (L) colonies is prejudicial to public health interests, *Ae. fluviatilis* can be a suitable substitute as a laboratory animal. We have already reported on aspects of the biology of the mosquito in laboratory conditions (Cônsoli, 1976; Cônsoli & Williams, 1978 and 1981); we now consider changes in egg production, fecundity and fertility in relation to increasing age, measured in number of oviposition cycles, of adult females.

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MATERIAL AND METHODS

The observations were made on females belonging to the colony of *Ae. fluviatilis* maintained at Centro de Pesquisas "René Rachou" (FIOCRUZ). Experiments were carried out in a climatized insectary with a temperature of 26 ± 1°C and a relative humidity of 75 ± 5%. The insects were maintained in a natural light/dark regime. Throughout adult life, the mosquitoes had access to a 5% dextrose solution which was changed every four days.

Until the 4th day after eclosion, female mosquitoes were kept in cages containing males. On the 5th day, the mosquitoes were feed to repletion on a human host. Engorged females were then separated and confined individually in small containers (10 cm in diameter, 8 cm high) with the upper end covered with fine nylon mesh. The insects had continuous access to a 5% dextrose solution and water for oviposition.

After laying a batch of eggs, a mosquito was given the opportunity to take another blood meal; a hand was placed on top of the small container for about 10 minutes. The procedure was repeated daily until the mosquito blood fed, to repletion, or died. Once engorged, no blood sources were offerred until the mosquitoes had deposited their batch of eggs.

The isolated females were examined daily. Records were kept of the intervals between blood feeding and oviposition, and of the numbers of eggs laid in each clutch. Records were also kept of the total number of egg clutches each female produced during adult life and of the chronological age at the time of death.

Each batch of eggs was maintained separately until all larvae had emerged. The numbers of larvae emerging from each clutch were recorded.

RESULTS

The observations relate to 100 female *Ae. fluviatilis* which produced at least three egg batches. Some analyses are restricted to the 30 females which survived long enough to lay a minimum total of 10 clutches.

Longevity

Length of life averaged 49.9 ± 17.8 days. The earliest death occurred after 16 days and one female survived for 96 days after eclosion.

In Fig. 1, the survival curve of the cohort, and the corresponding regression line for the mortality rate of the population, are related to age as expressed by the total number of oviposition cycles completed by individual specimens. The logarithm of the mortality rate increased proportionally to age and conformed to regression equation:

$$\log y = \log 0.17 + 0.79x$$

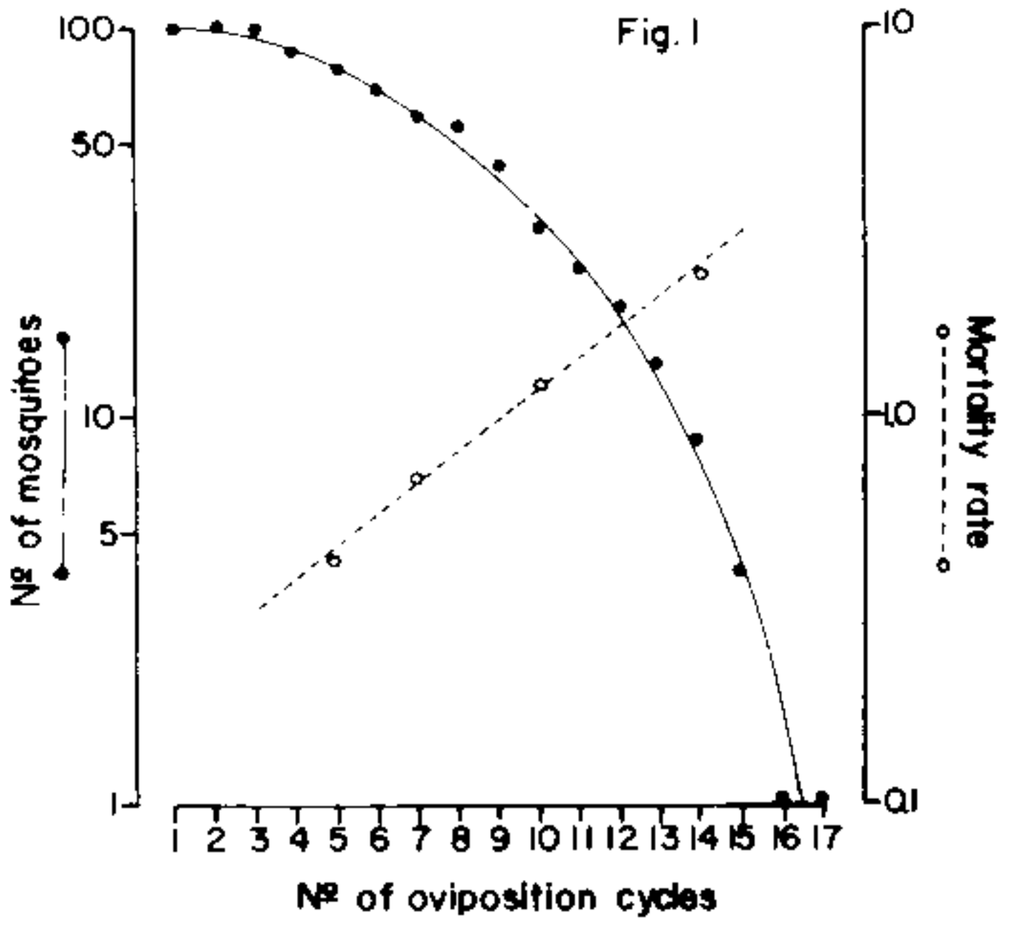
where y is the mortality rate and x is the total number of egg clutches produced.

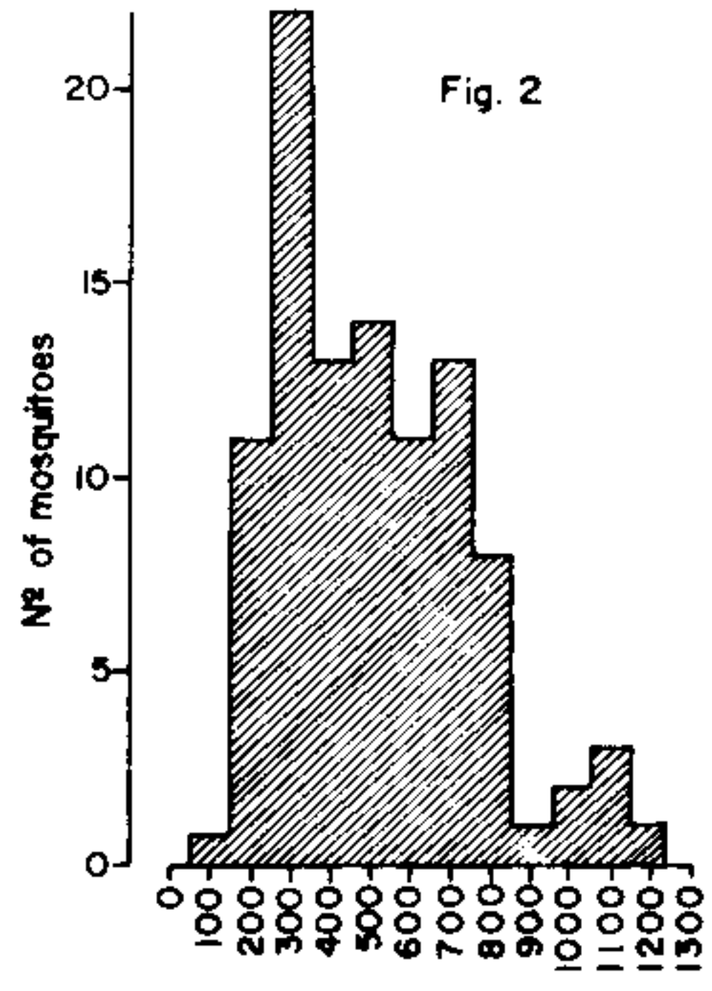
Egg production

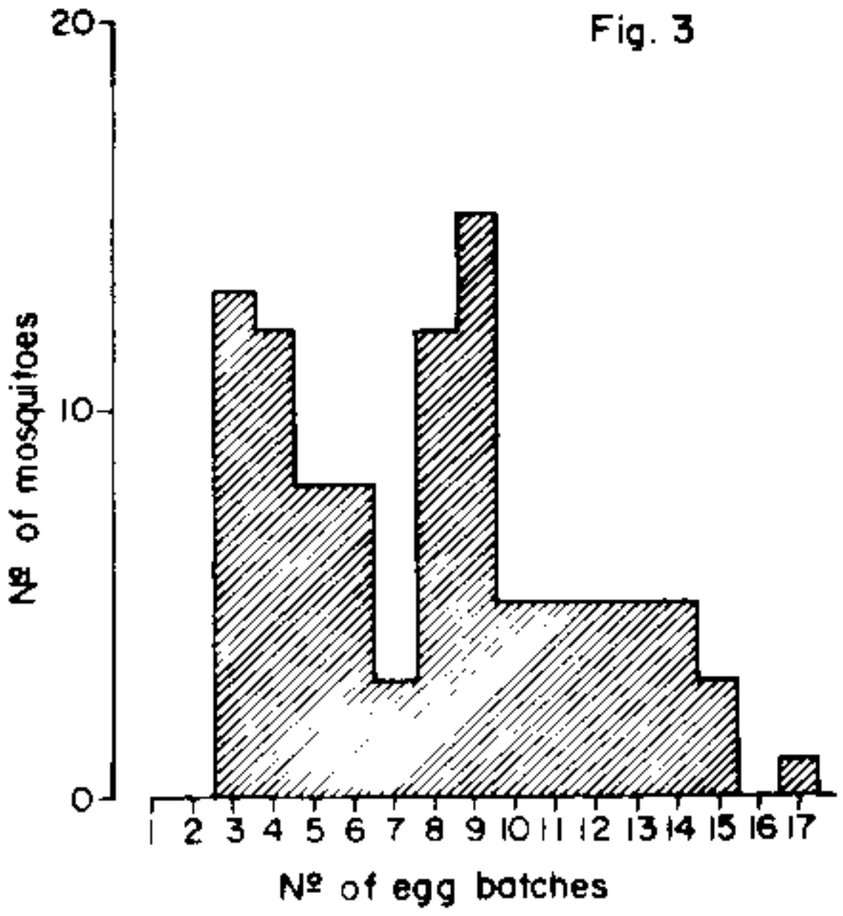
A total of 45,475 eggs were laid during the course of the experiment, the mean being 455 ± 241 eggs/female. The large standard deviation indicates that individuals varied considerably in fecundity though this was obviously influenced by the survival of each

individual. Thus, one female produced only 69 eggs during her life time whereas another laid 1,154 eggs.

Fig. 2 shows the frequency distribution diagram of the total number of eggs produced by each female. The skewness of the diagram reflects the fact that mortality increased logarithmically with increasing age (Fig. 1). Collectively, 82 females produced between 201 and 800 eggs/female, and the mode was 201 – 300 eggs/female.







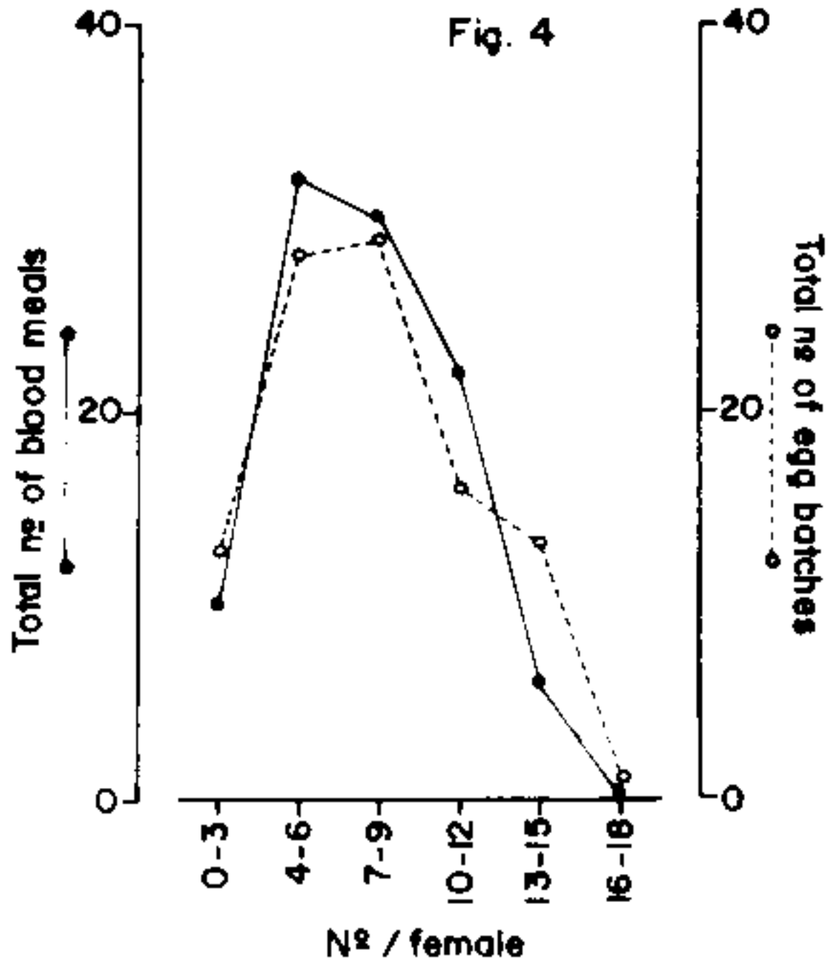


Fig. 1 – Survival curve and mortality rate (No. of females dead per oviposition cycle) in relation to the physiological age of 100 female *Ae. fluviatilis* maintained in laboratory conditions.

Fig. 2 – Frequency distribution diagram for the total number of eggs produced by 100 female *Ae. fluviatilis*.

Fig. 3 – Frequency distribution diagram for the total number of egg clutches laid by 100 female *Ae. fluviatilis*.

Fig. 4 – Comparison between the total number of blood meals taken by 100 female *Ae. fluviatilis* and the total number of egg batches they produced.

Egg production in relation to blood meals

The 100 female mosquitoes took a total of 732 blood meals during the experiment. The range in the number of blood meals ingested by individual females was 1 – 14, with a mean of 7.3 ± 3.2 meals/female. A total of 790 egg batches was produced. The range for individuals was 3 – 17, with a mean of 7.9 ± 3.7 clutches/female.

When these two means are compared by Pearson's coefficient of correlation, r = 0.946, significant at the 1% level ($\alpha = 0.01$).

Although this result indicates congruence between the numbers of blood meals taken and the numbers of egg clutches produced, female *Ae. fluviatilis* are not gonotrophically concordant. The frequency distribution diagram for the number of egg batches/female (Fig. 3) is distinctly bi-modal and unrelated to the survival pattern (Fig. 1) or to total egg production (Fig. 2). Fig. 4 shows that ingestion of blood and production of eggs are closely related but not coincidental.

Egg production in relation to age

When the mean number of eggs/batch and the mean number of total clutches/female are compared by Pearson's coefficient of correlation, r = 0.896, is significant at the 1% level.

Fig. 5 shows the regression line between the decline in egg production in relation to increasing age, as expressed in terms of the number of oviposition cycles. The regression equation conforms with the formula:

$$y = 73.90 - 2.86x$$

where y is the mean number of eggs/female and x is the number of oviposition cycles.

Fig. 5 also shows the percentage decline in egg production between successive oviposition cycles. The decline ranged from 4.3% between the 1st and 2nd cycles to 9.6% between the 14th and 15th. The rate of percentage decrease in egg production tended to increase logarithmically with advancing age and conforms with the regression equation:

$$\log y = \log 3.72 + 0.50x$$

where y is the percentage decrease in egg production between successive oviposition cycles.

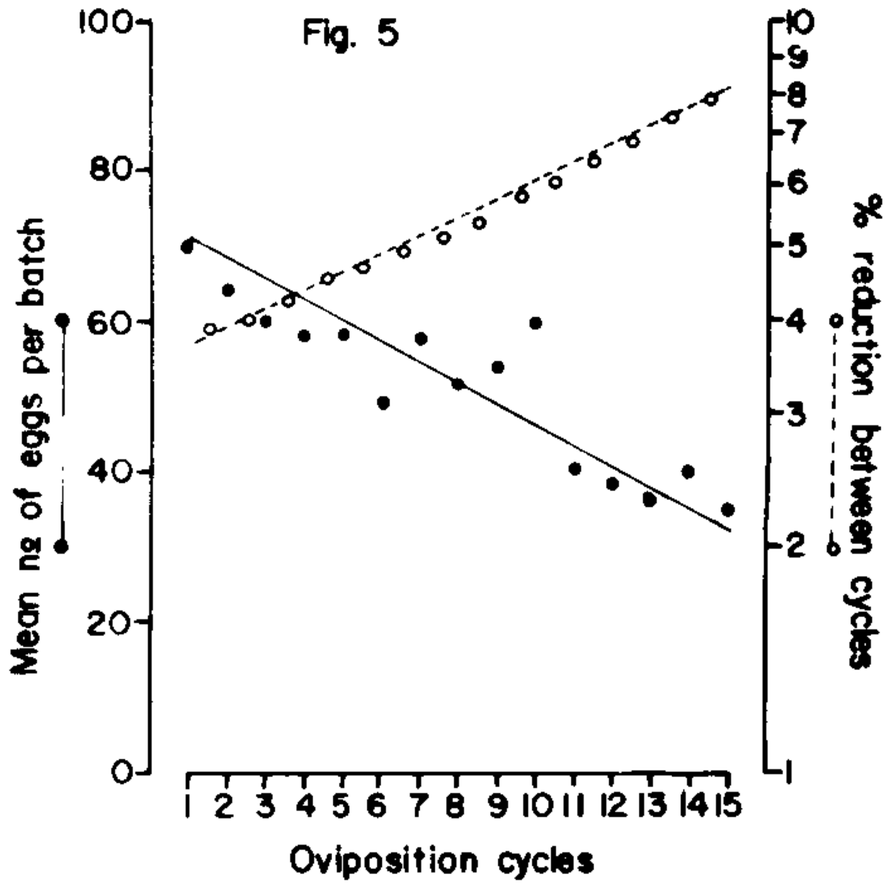
The regression equations given for the logarithmic/arithmetic graphs shown in Figs 5 – 7 are convenient approximations. By inspection of the basic data, given in the Figures, it is clear that the relationship between the respective variables is curvilinear rather than linear.

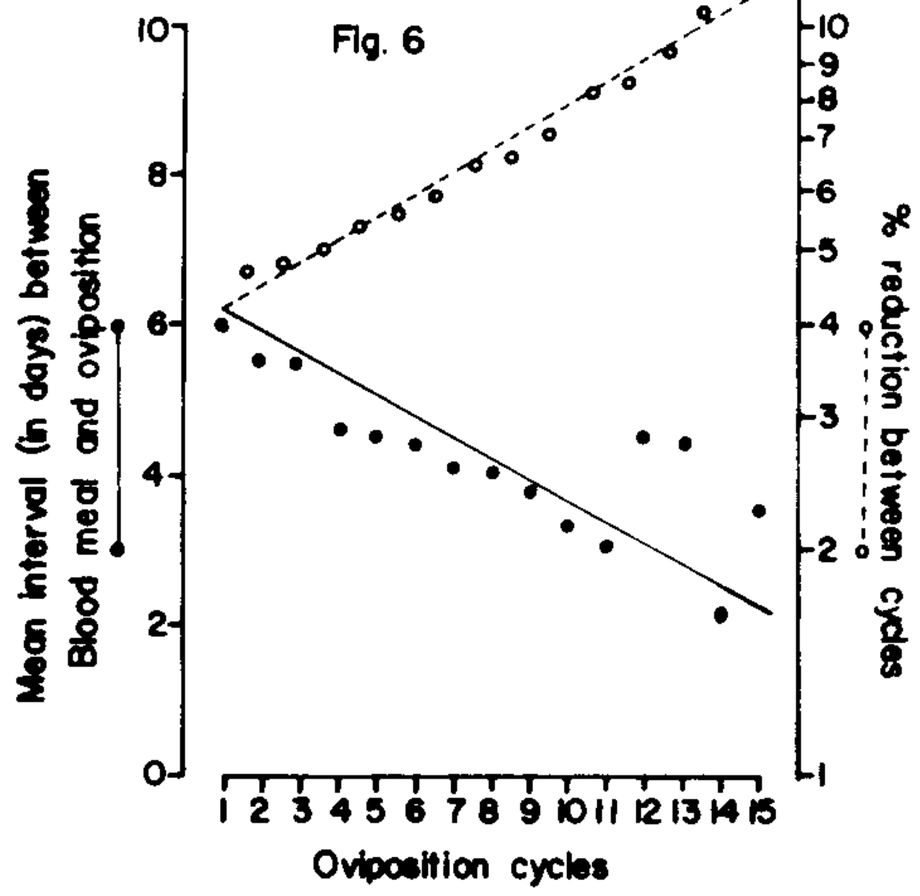
Intervals between blood feeding and oviposition

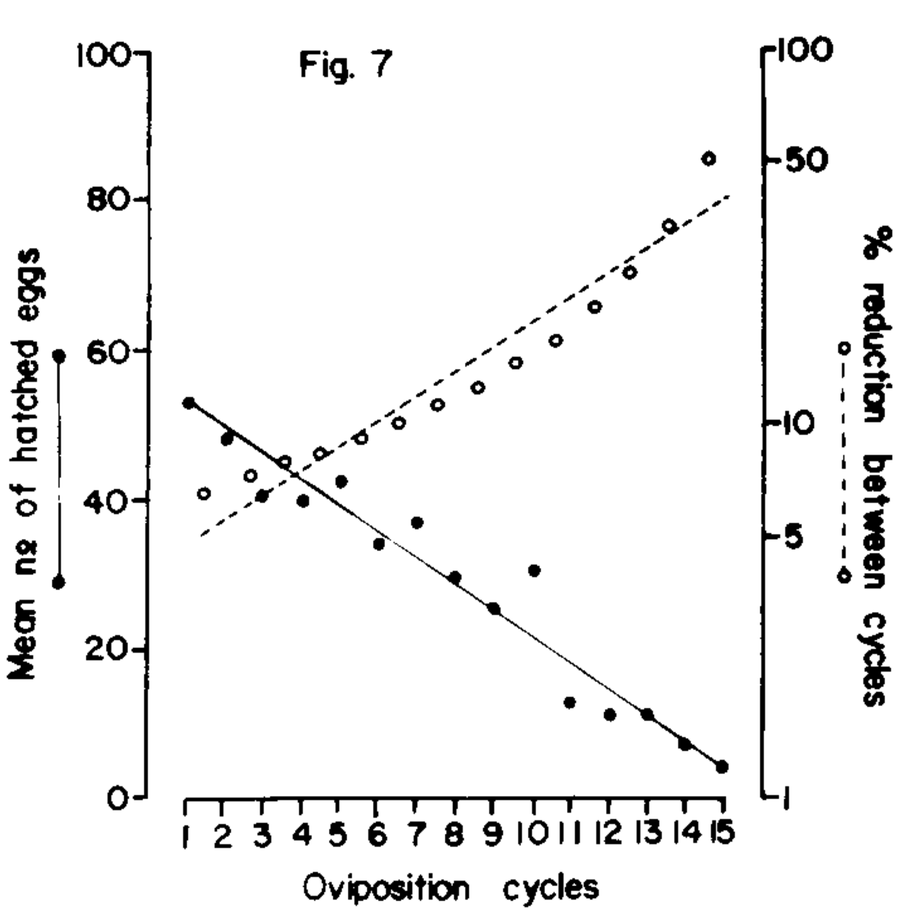
When the time-lapse between blood feeding and subsequent oviposition is compared, by Pearson's coefficient of correlation, to advancing age, r = 0.896, is significant at the 1% level.

The maximum mean value for the interval between blood feeding and oviposition varied from 6.0 ± 2.7 days (1st oviposition cycle) to 2.1 ± 2.0 days (14th cycle). The interval decreased progressively with advancing age (Fig. 6) and the regression equation conforms with the formula:

$$y = 6.48 - 0.29x$$







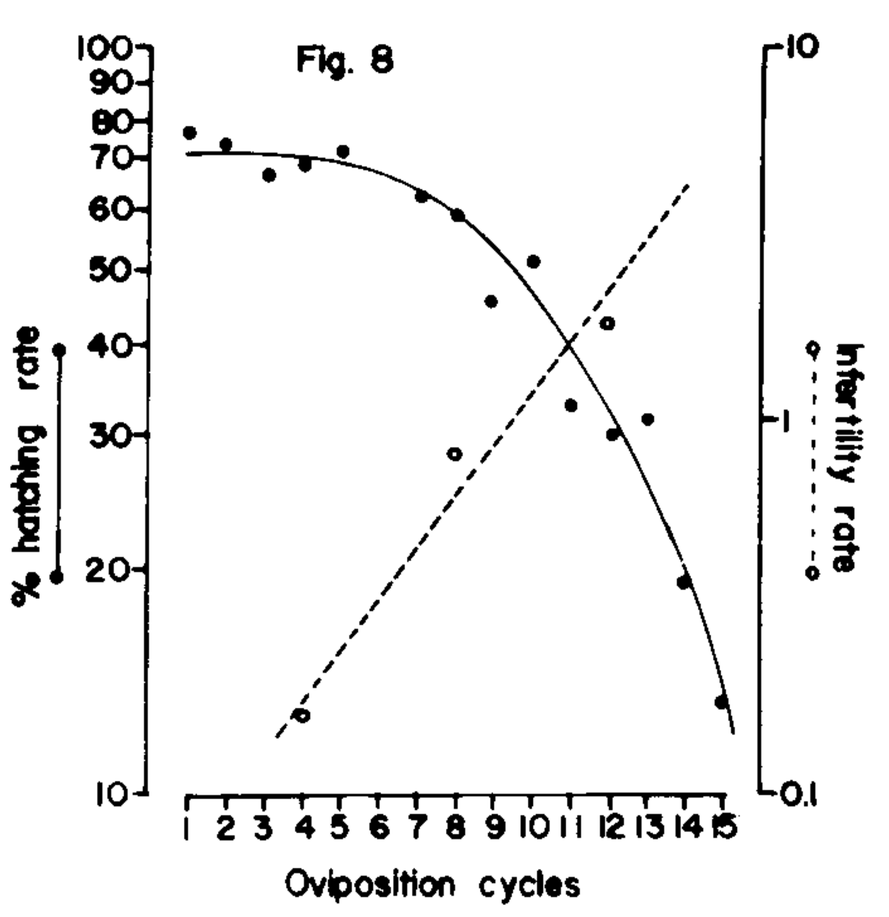


Fig. 5 – Relationship between fecundity and physiological age in female *Ae. fluviatilis* maintained in laboratory conditions.

Fig. 6 – Changes in the interval between blood feeding and oviposition in relation to the physiological age of *Ae. fluviatilis.*

Fig. 7 – Relationship between fertility and physiological age in female *Ae. fluviatilis.*

Fig. 8 – Percentage reduction between successive oviposition cycles in the fertility of eggs produced by 100 female *Ae. fluviatilis,* and the estimated infertility rate in relation to the number of oviposition cycles.

where y is the mean interval, in days, between blood feeding and oviposition and x is the number of oviposition cycles.

Analysis of variance and Duncan's multiple range test (Ducan, 1955) were used to compare egg production in successive oviposition cycles of the 30 females that each

produced a minimum of 10 clutches. The mean number of eggs produced in the 1st oviposition cycle was significantly higher than those for the 4th, 5th, 7th, 8th, 9th and 10th cycles. The mean number of eggs in the 2nd cycle was significantly higher than those of the 7th and 8th cycles.

The percentage decrease, from one oviposition cycle to the next, between blood feeding and oviposition is also shown in Fig. 6. The decrease varied from 4.68% between the 1st and 2nd oviposition cycles to 11.16% between the 14th and 15th. THe regression equation between the two variables conforms to the formula:

$$\log y = \log 3.89 + 0.93x$$

where y is the rate of decrease and x is the corresponding oviposition cycle.

Delayed oviposition

In several instances, oviposition by individual females was delayed well beyond the mean value for the appropriate oviposition cycle. Although only three examples are cited here, others could be reported.

The 7th clutch of female No. 13 was laid 16 days after the preceeding blood meal. Female No. 42 produced her 12th batch of eggs 23 days after blood feeding. The 2nd and 4th clutches of female No. 74 were laid 14 and 17 days, respectively, after blood meals.

Delayed oviposition was not related to age and did not affect longevity. Thus, female No. 13 survived for 81 days and passed through nine oviposition cycles; female No. 74 died 74 days after the first blood meal and produced nine clutches of eggs. In the case of female No. 42, however, delayed oviposition occurred in the last (12th) oviposition cycle.

Hatching rates

Comparing the mean numbers of larvae emerging from eggs produced in each oviposition cycle with the corresponding age of the female parent, $r = 0.972$, is significant at the 1% level.

Fig. 7 shows the regression line between the mean hatching rate of eggs in relation to parental age. The mean number of viable eggs decreased progressively in successive oviposition cycles and conforms with the regression equation:

$$y - 57.17 - 3.57x$$

where y is the mean number of eggs that hatched and x is the corresponding number of oviposition cycles.

Analysis of variance and Duncan's test were applied to data for the 30 females that completed a minimum of 10 oviposition cycles. Statistically significant differences exist between the mean number of viable eggs from the 10th cycle when compared to the means for the 1st, 2nd, 3rd and 5th cycles, between the mean for the 9th cycle and those of the 1st – 5th cycles, and between that of the 8th cycle and those of the 1st, 2nd and 5th cycles.

Fig. 7 also shows the percentage reduction in hatching rates between sucessive oviposition cycles. The decrease varied from 6.68% between the 1st and 2nd cycles to 50.64% between the 14th and 15th. The regressions equation conforms to the formula:

$$\log y = \log 4.07 + 0.65x$$

where y is the percentage decrease in hatching rates of successive oviposition cycles and x is the number of cycles.

In Fig. 8, the percentage of eggs that hatched is plotted against the corresponding oviposition cycle. The proportion of hatching eggs declined from 75.0% in the 1st oviposition cycle to 13.2% in the 15th. One female lived long enough to produce a total of 17 egg clutches, but all eggs laid in the 16th (eight eggs) and 17th (30 eggs) oviposition cycles did not hatch.

The regression line for the infertility rate shown in Fig. 8 was constructed from tangents drawn along the curve for declining hatching rates. The logarithm of the infertility rate increases proportionally to advancing number of oviposition cycles and conforms to the regression equation:

$$\log y = \log. 0.05 + 1.38x$$

where y is the infertility rate and x is parental age.

Some females produced fertile eggs only during their earlier oviposition cycles. Female No. 1 produced a total of 11 egg clutches but, after the 8th oviposition cycle, all eggs were sterile. Similarly, female No. 15 laid eggs on 15 occasions but none were fertile after the 8th oviposition cycle. In total, 10 females did not produce fertile eggs in at least the last two oviposition cycles of their lives. In contrast, some females continued to produce some fertile eggs up to the time they died. Female No. 33 passed through a total of 10 oviposition cycles in 44 days and the fertility rate of each clutch was in the range of 82 – 100%.

Transient infertility

Several females passed through a phase of sterility or low fertility that was not related to advancing age. The first two clutches of female No. 19 were fertile, with hatching rates of 92.6% and 65.5% respectively; all eggs of the 3rd oviposition cycle were sterile; in the next three cycles, fertility rates increased progressively, being (respectively) 17.2%, 29.0% and 87.5%. Female No. 23 produced 100% fertile eggs in the 5th and 7th oviposition cycles but the eggs of the 6th cycle were 100% sterile; thereafter, high fertility rates were recorded until the 11th cycle, after which hatching rates diminished and only 25% of the eggs produced in the last (14th) cycle were fertile. The first clutch of eggs produced by female No. 25 was 100% sterile; in the next four oviposition cycles, hatching rates were in the range of 19.3 – 77.8%. In contrast, 100% fertility was recorded in the 1st oviposition cycle of female No. 70; eggs of the 2nd and 3rd cycles were 100% sterile but fertile eggs were produced in the 4th – 8th cycles.

In some cases, infertility was related to delayed oviposition. Eggs of the 12th oviposition cycle of female No. 42 and those of the 3rd cycle of female No. 43 were 100% sterile. In these instances, the mosquitoes laid eggs 23 and 18 days, respectively, after the preceeding blood meal. On the other hand, oviposition in the 2nd cycle of female No. 41 was delayed for 14 days but the eggs were 100% fertile. Similarly, a hatching rate of 85.5% was recorded for the eggs produced in the 2nd oviposition cycle of female No. 89 but the eggs were laid 17 days after blood feeding.

DISCUSSION

Detinova (1962) defined physiological age of a haematophagous insect as the number of gonotrophic cycles completed. This criterion was questioned by Klowden & Lea (1980) who proposed that gonotrophic age (denoted by irreversible alterations in the

female reproductive system) should be distinguished from physiological age (related to changes associated with senescence). It would be misleading to use the expression gonotrophic age for *Ae. fluviatilis* because it is clear from Figs. 3 and 4 that the species lacks gonotrophic concordance as conceived by Swellegrebel (1929). Instead, the phrase "number of oviposition cycles" has been used as a measure of increasing age. The results of the present studies on *Ae. fluviatilis* revealed that the various parameters examined are all age-specific and, indirectly, reflect senescent changes in the adult female. In such a situation as this, the number of oviposition cycles accords with the definitions of physiological age given by both Detinova (1962) and Klowden & Lea (1980).

The survival curve (Fig. 1) reflects the accumulated total of senescent changes. The survivorship curve is closest to type I of Deevey (1947) and Slobodkin (1962) and indicates that mortality is higher among older individuals. The patterns of survival and mortality for *Ae. fluviatilis* in laboratory conditions correspond to those determined by Kershaw, Chalmers & Lavoipierre (1954) for *Ae. aegypti* and certain other species of mosquitoes. However, Kershaw et al (1954) related the two parameters to chronological age. For *Ae. fluviatilis,* the logarithm of mortality rate progresses proportionately to physiological age.

In the studies of Kershaw et al (1954), female *Ae. aegypti* were maintained, together with an initially equal number of males, in relatively large cages. In the present studies, female *Ae. fluviatilis* were kept individually in small containers. In these confined conditions, one female survived from 96 days after the initial blood meal. This result can best be compared with studies on six Louisiana species of *Aedes* which had a maximum survival of 28 days (Chapman & Woodard, 1965) and on four Alaskan species of *Aedes* with a maximum survival of 131 days (Sommerman, 1969).

Egg production of *Ae. fluviatilis* (454.7 ± 241.4 eggs/female in 7.9 ± 3.7 oviposition cycles) can be compared with that of *Ae. vexans* (Meigen) and *Culex salinarius* Coquillett. Breland & Pickard (1964) determined that *Ae. vexans* produced 162.0 ± 5.7 eggs/female in 3.0 ± 0.3 gonotrophic cycles and Andreadis & Hall (1980) found that *Cx. salinarius* laid 327.0 ± 113.0 eggs/female in 3.2 ± 0.2 cycles. Taking into account the differences in the life spans of the three species, the fecundity of *Ae. fluviatilis* was 43.6% less than that of *Cx. salinarius* whereas the fecundity of *Ae. vexans* was more or less (a difference of only 4.3%) equal to that *Ae. fluviatilis.* The higher fecundity of *Cx. salinarius* probably reflects that the two species of *Aedes,* like other members of this genus (Clements, 1963), have fewer ovarioles in the ovaries. Additionaly, the host species used, as well as the amount of blood ingested could also have influenced these differences.

In considering the fecundity of *Ae. fluviatilis,* reference must be made to the large standard deviations about the means. If fecundity is controlled genetically, the high standard deviations probably reflect the heterogenecity of the colony of *Ae. fluviatilis.* Other possible genetical differences have been observed in the hatching mechanisms of eggs (Cônsoli, 1976; Cônsoli & Williams, 1978) and variations in susceptibility to infection by both *P. gallinaceum* (Tasón de Camargo, 1977) and *D. immitis* (Kasai, 1979). With the accumulative evidence of genetic diversity, it now seems advisable to cull selected lines from the existing stock so that experimental cohorts are more uniform in performance.

Progressive reduction in fecundity in consecutive oviposition cycles has been recorded in several species of *Anopheles* in widely separated parts of the world (Detinova, 1962). Studies on wild-caught *An. maculipennis messae* Falleroni in U.S.S.R. confirmed that this decrease in fecundity is not a laboratory artifact but also occurs in natural populations (Detinova, 1962). Clements (1963) commented that diminishing fecundity in relation to age occurs in all mosquitoes that have been studied. This has been confirmed by the more recent studies of Sommerman (1969) and *Ae. cinereus* Meigen, *Ae. communis* (De Geer), *Ae. impiger* (Walker) and *Ae. punctor* (Kirby) and those of Andreadis & Hall

(1980) on *Cx. salinarius*. Putnam & Shannon (1934) recorded a 15% decrease in fecundity between successive oviposition cycles of *Ae. aegypti*. The rate of decrease in fecundity for *Ae. fluviatilis* is not uniform but increases logarithmically in proportion to advancing age (Fig. 5). In contrast to the observations on *Ae. aegypti*, the percentage decline in fecundity did not attain 10%.

The decline in fecundity between successive ovipositions in mosquitoes is due to a decrease in the numbers of ovarioles that develop to maturity (Detinova, 1962; Clements, 1963). In *Ae. fluviatilis*, declining fecundity is accompanied by a diminution of the time interval between blood feeding and oviposition (Fig. 6). Allowing for scale differences in the graphs, the regression equations for the two processes are almost the same. A similar phenomenon occurs in *An. maculipennis* and Detinova (1962) suggested that it is due to a gradual intensification in the functioning of digestive glands. As an alternative it can be suggested that the ovaries undergo senescence (as indicated by the reduction in the number of follicles that mature) whereas the physiological processes governing blood digestion and oögenesis are usually not subject to senescent changes. By this supposition, the decreasing interval between blood feeding and oviposition is related solely to the diminishing number of oocystes that develop, considering that only one oocyte mature each time in each ovariole.

Clements (1963, p. 299) stated that "a single mating will provide sufficient sperm for the requirements of most females" and referred to observations made by Shute (1936) on *An. labranchiae atroparvus* van Thiel and to two studies on *Ae. aegypti*. Goeldi (1905) recorded that *Ae. aegypti* can produce fertile eggs for 102 days after mating; Bacot (1916) found that one female continued producing fertile eggs for 62 days after mating, laying a total of 711 fertile eggs in 15 oviposition cycles.

In the present study, female *Ae. fluviatilis* were maintained in the presence of males only until the 5th day of adult life. Since all females subsequently produced fertile eggs, all had been inseminated at least once. From the data summarized in Figs. 7 and 8, it is clear that the numbers of spermatozoa stored in the spermathecae were insufficient to meet all the requirements of the females. Even in the 1st oviposition cycle, only 75% of the eggs proved to be fertile (Fig. 8).

According to De Wilde (1964, p. 33): "Senescence in many insects is characterized by decreased fertility". By comparing Figs. 5 and 7, it is clear that decreased fertility in *Ae. fluviatilis* is not due solely to senescent changes in the ovaries, which are expressed as the decline in egg production (Fig. 5). One or more factors affecting the sperms stored in the spermathecae must be operative because the decline in fertility is more rapid than the decrease in fecundity. Senescent processes could influence the vitality and activity of the spermatozoa and so reduce their chances of gaining access to and passing through the micropyle. Senescent changes could also diminish the efficiency of the mechanisms involved in the release of spermatozoa from spermathecae.

The infertility rate (Fig. 8) can be considered to combine the effects of senescent processes on the functioning of the ovaries with the presumed factors involving the survival, wastage, vitality, activity and reproductive capacities of the spermatozoa stored in the spermathecae.

From the foregoing comments, the terminal sterility that occurred in 10% of female *Ae. fluviatilis* is explicable. Transient sterility and periods of low fertility cannot be explained on the same basis. Examination of the available literature has not revealed published reports recording this phenomenon in other species of mosquitoes, though it probably does occur in species other than *Ae. fluviatilis*. It is difficult to envisage how or why it occurs. Fertilization takes place immediately before an egg is laid. Describing the process, Chapman (1971, p. 346) stated: "... as each egg passes down the oviduct a few sperm are released from the spermatheca" but added that "It is not clear how this is

brought about . . ." It can only be assumed that, at times, the passage of eggs of *Ae. fluviatilis* through the oviduct does not stimulate sperm release or that the mechanism controlling the discharge of sperm does not respond to the stimulus.

RESUMO

De 100 fêmeas de *Aedes fluviatilis* (Lutz, 1904) individualmente observadas foram obtidos 45.475 ovos em um total de 790 posturas. As médias de desovas produzidas e de repastos sangüíneos ingeridos por fêmea, foram respectivamente de 7,9 ±3,7 e 7,3 ±3,2 e a longevidade média foi de 49,9 ±17,8 dias. Em análise de regressão linear, foi observado um progressivo decréscimo das médias de ovos por postura, larvas eclodidas e número de dias entre repasto sangüíneo e postura, proporcional ao aumento da idade fisiológica. Foram ainda observadas ocorrências de ingestão de sangue durante o desenvolvimento dos ovos, recusa de alimentação sangüínea entre desovas sucessivas, retenção espontânea de ovos por períodos apreciáveis, esterilidade transitória e esgotamento de fecundidade.

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