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INFLUENCE OF LARVAL AND PUPAL PRODUCTS ON THE OVIPOSITION BEHAVIOUR OF *AEDES FLUVIATILIS* (LUTZ) (DIPTERA: CULICIDAE)

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Several larval and pupal products of Aedes fluviatilis *(Lutz) were tested for their influence on the oviposition behaviour of females of the same species. Significant ($\alpha = 0,05$) attractiveness was shown by: larval water, previously containing 5 to 15 larvae/1,5 ml; larval water, preserved up to 38 days; evaporate and reconstructed larval water extracts up to 2 years after production and water filtered through fresh or dried ground larvae. Hexanic larval water extracts and water filtered through fresh or dried ground pupae did not influence oviposition.*

Key words: *Aedes fluviatilis* – oviposition behaviour – larvae and pupal products

Oviposition site selection is considered by most authors the main factor responsible for the distribution of mosquito breeding places, being most relevant to the distribution of species in nature (Ikeshoji & Mulla, 1970; Ikeshoji et al., 1975; Steffan & Everhuis, 1981). Several studies have shown that ovitraps are very effective devices when employed in surveillance work, for evaluation of the efficacy of insecticides or for the estimation of seasonal population fluctuations, sometimes being superior to larval surveys or light traps (Browne & Bennett, 1981; Leiser & Beier, 1982; Clark et al., 1982; Kloter et al., 1983).

The investigation of factor affecting the oviposition behaviour of female mosquitoes seems relevant, both considering the understanding of the ecology of species and the eventual identification of attracting substances that could provide novel means for assessment and control of mosquito populations (Ikeshoji & Mulla, 1970; Leite, 1980).

Aedes (Ochlerotatus) fluviatilis (Lutz, 1904) is widely distributed in the neotropical region and has been recorded from Nicaragua to Argentina, and in the present work several larval and pupal products of *Ae. fluviatilis* were tested to assess their influence on the oviposition behaviour of females of the same species.

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MATERIAL AND METHODS

Mosquitoes were obtained from a colony maintained since 1974 at Centro de Pesquisas "René Rachou" (FIOCRUZ – MS), Belo Horizonte, Minas Gerais, Brazil. Routine maintenance techniques are described in Consoli & Williams (1978; 1981).

Experiments – Thirteen experiments, each repeated three times, were carried out. For each experiment, 200 males and 200 females, aged between 4 and 6 days, were put into a cage build of "Eucatex" and nylon netting (40 x 40 x 40 cm). A supply of 5% honey solution was provided. Five days after females had taken a blood meal on anaesthetized mice *(Mus musculus)* experimental and control dishes were put into the cages for 24 hours, and the number of egg laid in each dish recorded afterwards. In each experimental replicate, the position of the dishes inside the cages was changed. Only young 4th stage larvae and one day old pupae were used, to avoid, at possibility, stage changes during the experiments. In all tests, sterile transparent glass dishes (9 cm diameter, 150 ml) were used.

Experimental media

1. Larval water, at two concentrations – Batches of 500 and 1500 larvae were rinsed 3 times in distilled water and placed, separately in dishes containing 150 ml distilled water. After 24 hours, larvae were removed by filtration, and the two resulting liquids were offered, to females for oviposition. A similar dish, containing distilled water only, was used as control.

2. Larval water, preserved at 4°C – Experimental and control media were obtained as described above, but before use, were maintained at 4°C for 38 days.

3. Evaporated larval water extract – Larval water, obtained as described in 1, with a batch of 500 larvae, was evaporated in a rotary evaporator at 70°C. The solid residuum was dissolved again in 150 ml distilled water. The same procedure was followed with 150 ml of pure distilled water, used as control.

4. Lyophylized larval water extract – The same procedure, as described in 3, was followed, only changing evaporation for lyophilization.

5. Evaporated larval water extract, used after 2 months and 2 years – One litre of larval water (5 larvae/1.5 ml), was obtained and evaporated as in 3. The residuum was preserved for intervals of 2 months and 2 years at 4°C. Solutions in distilled water, at 100, 10 and 1 ppm were prepared and offered to females together with a control of distilled water that passed the same procedure.

6. Hexanic larval water extracts: solid and liquid fractions – When larval water, obtained as in 3 was extracted with hexane, a solid and a liquid fraction were obtained. Each of these, were reconstituted with 150 ml of distilled water and used with a control dish containing only distilled water.

7. Double evaporated larval water extract – The solid evaporation residuum, obtained as in 3, was washed with hexane and evaporated again. The second residuum was reconstituted with 150 ml of distilled water. As controls, two dishes were used: the first one containing distilled water which passed the same procedure (control 1) and the second one containing simply distilled water (control 2).

8. Fresh and dried ground larvae extracts – Fifty larvae, after being washed 3 times in distilled water, were ground in the middle of a filter paper sheet (15 x 15 cm); 150 ml of distilled water were passed through this paper and then offered to females along with a control of distilled water only. In a second experiment, the paper containing the ground larvae was dried at room temperature (24-25°C) for 24 hours. Afterwards, an experimental media was prepared as before and offered to the females together with a control of distilled water.

9. Fresh and dried ground pupal extracts – The same procedure was used as in 8, with pupal instead of larval.

Statistical evaluation – Student's "T" test and Duncan's test (Levin, 1978) were used to evaluate differences between means. A significance level of $\alpha = 0{,}05$ (5%) was adopted.

RESULTS

Larval water, at two concentrations – In the lower concentration 1660 ± 327,5 eggs were laid against 257,3 ± 71,1 in control; 478,2 ± 95,6 in the stronger concentration and 107,0 ± 21,0 in control. In both cases experimental means were statistically larger than control. The total of eggs counted in these experiments was 7501 (Fig. 1D).

Larval water, preserved at 4°C – Figure 1E shows means and standard deviation of eggs referring to this experiment: 1712,7 ± 728,0 in experimental dishes and 283,7 ± 86,7 in control. Means differed significantly and the total of eggs observed was 5979.

Evaporated and lyophilized larval water extract – In the evaporated extract solution 807,0 ± 76,2 eggs were counted and 245,3 ± 30,6 in the control; 714,7 ± 141,1 in the lyophilized extract solution and 389,3 ± 64,9 in control. Both experimental means were statistically larger then controls. The total of eggs counted was 6469 (Fig. 1B).

Evaporated larval water extract, used after 2 months and 2 years – When the extract was employed after 2 months females laid 3533,3 ± 2042,4 eggs in the 100 ppm solution, 1848 ± 871,4 in 10 ppm and 591,7 ± 311,2 in 1 ppm, against 823,7 ± 542,9 in control; 20.390 was the total of eggs counted in this experiment (Fig. 2B). After 2 years storage, 2607,0 ± 966,2 eggs were laid in a 100 ppm solution, 1711,0 ± 520,0 in 10 ppm and 1077,3 ± 550,8 in 1 ppm, against 614,0 ± 168,4 in the control; the total of eggs was 18.028 (Fig. 2A). In both cases the egg means in 100 ppm solutions were statistically larger than those found in 1 ppm and controls.

Hexanic larval water extracts: solid and liquid fractions – No significant differences

between egg means occurred in this experiment: 2654,0 ± 1822,1 were laid in the solid fraction solution, 1359,7 ± 1198,2 in the liquid fraction solution and 1624,3 ± 2026,6 in control (Fig. 1C).

Double evaporated larval water extract – Again no statistically differences between means occurred: 828,0 ± 969,7 eggs were counted in the experimental dishes, 1585,7 ± 1744,9 in control 1 and 576,0 ± 415,3 in control 2. The total of eggs was 8969 (Fig. 1A).

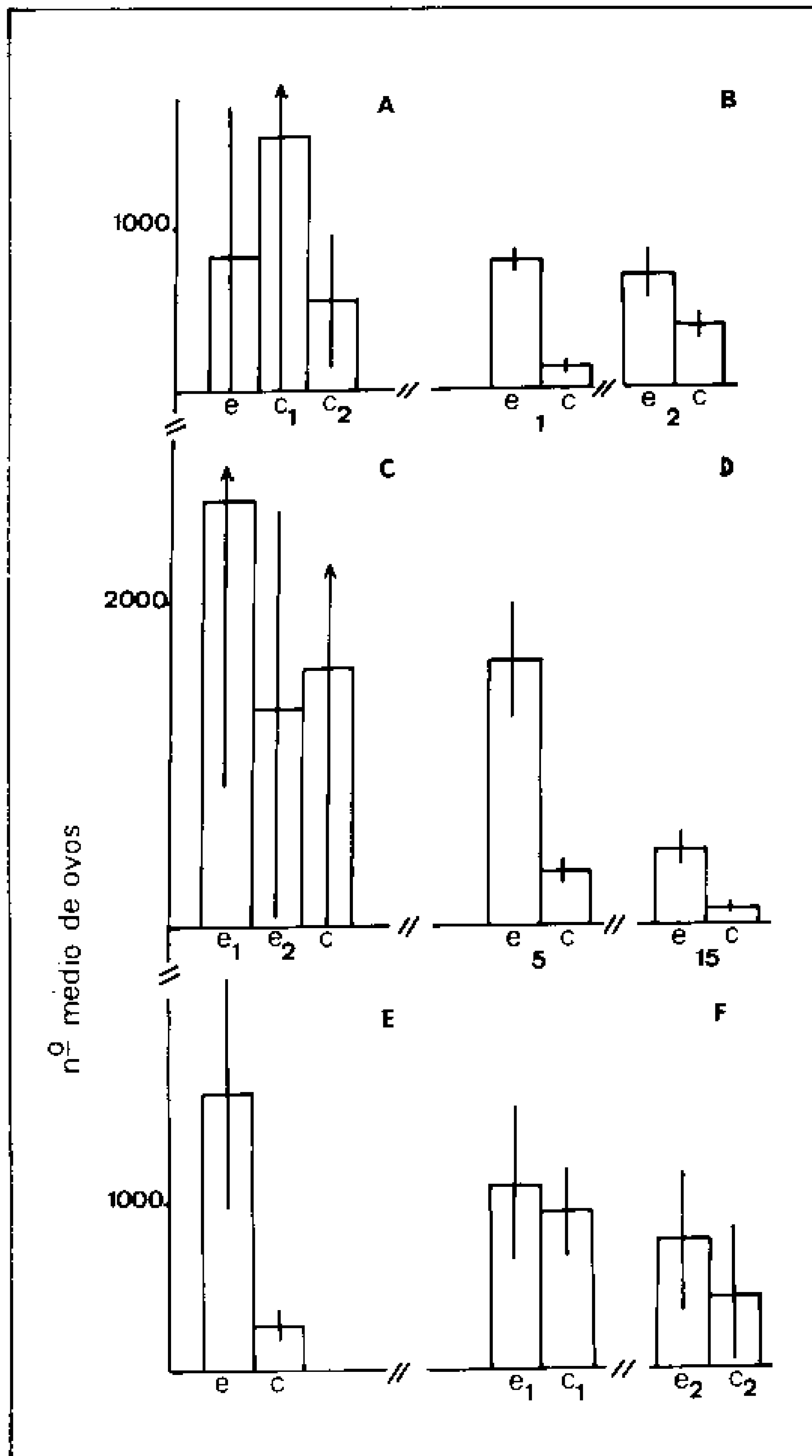

Fig. 1: Means and standard deviations of *Aedes fluviatilis* eggs laid in: A: double evaporated larval water extract (e) and controls (c_1/c_2); B: evaporated larval water extract (e-1) lyophilized larval water extract (e-2) and controls (c-1/c-2); C: hexanic larval water extracts – solid fraction (e_1), liquid fraction (e_2) and control (c); D: larval water at 5 and 15 larval/1.5 ml concentrations (e) and controls (c); E: larval water after maintenance at 4°C (e) and control (c); F: water filtered through fresh (e_1) and dried (e_2) ground pupae and controls (c_1/c_2).

Fresh and dried ground larvae extracts – In dishes holding the fresh extract solution, 2316,6 ± 1590,5 eggs were observed against 308,3 ± 125,9 in the control; 678,7 ± 87,5 were laid in the dried extract solution and 323,0 ± 139,2 in its control. In both instances, experimental means were statistically larger then control. Total of eggs observed was 9874 (Fig. 2C).

Fresh and dried ground pupal extracts – In the solutions prepared with freshly ground pupae 1156,7 ± 467,2 eggs were laid and 981,7 ± 277,6 in the control; in the dried extract solution 797,7 ± 423,7 eggs were observed against 464,3 ± 413,5 in control. No significant differences occurred between means and the total of eggs was 10.201 (Fig. 1F).

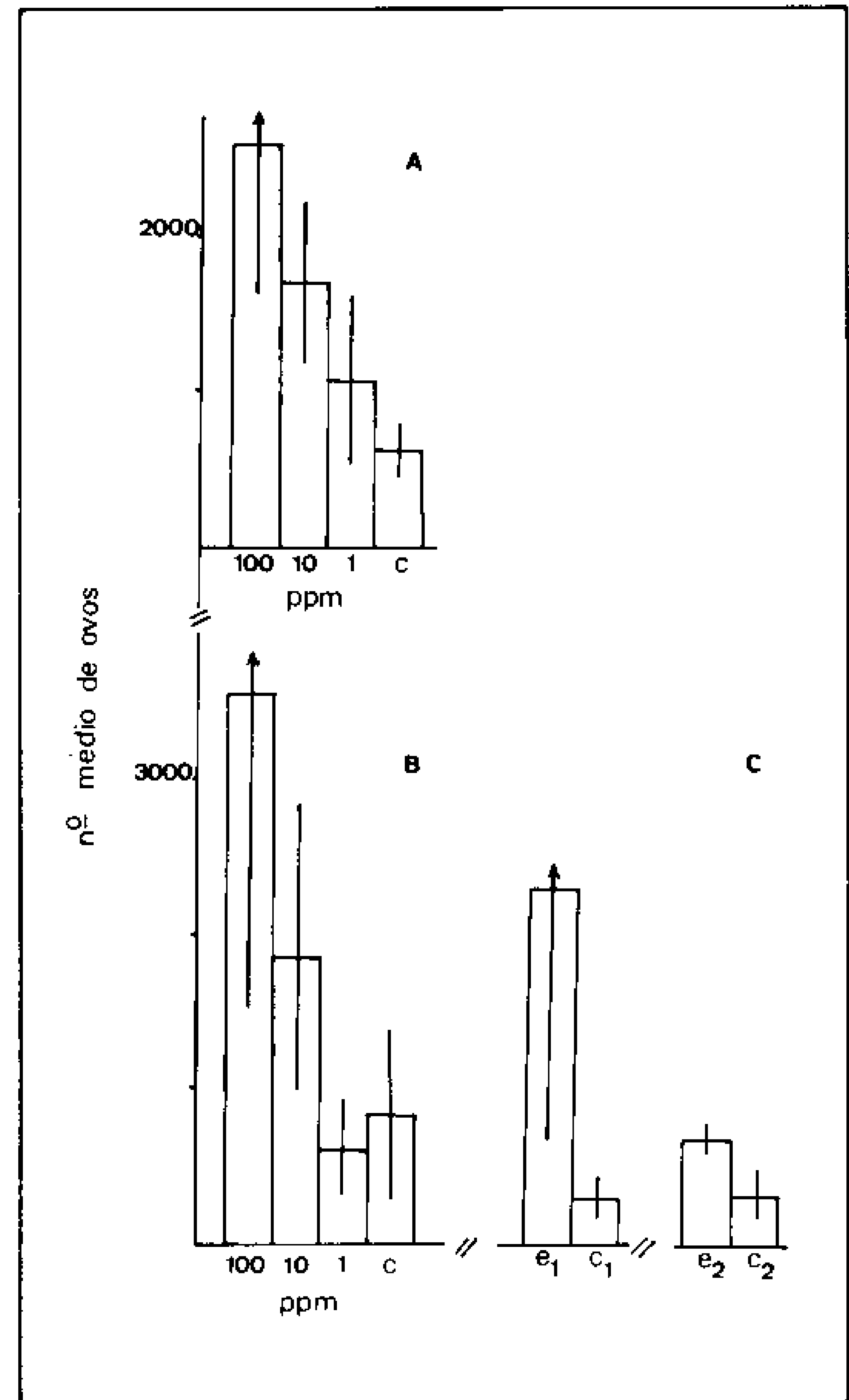

Fig. 2: Means and standard deviations of *Aedes fluviatilis* eggs laid in: A: evaporated larval water extract, dissolved at 100, 10, 1 ppm and control (c) after 2 years preservation; B: evaporated larval water extract, dissolved at 100, 10, 1 ppm and control (c) after 2 months preservation; C: water filtered through fresh (e_1) and dried (e_2) ground larvae and controls (c_1/c_2).

DISCUSSION

As apparent from results, larval water of *Ae. fluviatilis* proved to be attractive to its females, when concentrations of 5 or 15 larvae/ 1,5 ml were employed. Leite (1980) demonstrated the attraction of these females to larval water obtained at 1 larval/ml concentration. Hwang & Mulla (1980) noted that crowded *Culex quinquefasciatus* can produce carbohydrates and fatty acids that inhibit or retard the development of younger larvae, while Dadd & Keinjan (1974) found that distinct and independent autophagostimulants, growth-retardants and oviposition-stimulants can be produced by crowded *Culex pipiens* and *Ae. aegypti* larvae. We have no data concerning the occurrence of autophagostimulants or growth retardants in crowded *Ae. fluviatilis,* but in our experiments, crowding at the mentioned level does not seem to affect oviposition stimulants. The oviposition stimulants in *Ae. fluviatilis* seems to be stable ones, since they retain their activity for 38 days storage in liquid form and up to 2 years in solid form. Stable oviposition attractants seem to occur in other species: Kalpage & Brust (1973) found that larval derivatives in *Aedes atropalpus* maintained their activity for several weeks, even after evaporating and reconstituting the experimental media; Bentley et al. (1976) found that an oviposition attractant related to 4th stage *Aedes triseriatus* larvae could resist distillation at 70°C and could be stored for several weeks; Andreadis (1977) refers to the existence of at least one non-volatile attracting factor, occurring in emergence water of *Culex tarsalis* and Osgood (1971) considers the breeding place water of the same species attractive to females for several weeks. On the other hand, Laurence & Pickett (1982) described a volatile oviposition stimulant related to the apical droplet of *Cx. quinquefasciatus* eggs.

The easy obtainment of such a stable attractive factor in *Ae. fluviatilis* seems to justify field investigations to assess its efficacy when confronted with the multiple physical, chemical and biological factors that influence mosquito oviposition in nature (Wallis, 1954; Belton, 1967; Furlow & Hays, 1972; Ikeshoji et al., 1975; Kramer & Mulla, 1979; Hwang & Mulla, 1980).

In laboratory conditions, larval and pupal water of *Ae. fluviatilis* is attractive to females of this species but not to *Cx. quinquefasciatus* (Consoli & Williams, 1978; Leite, 1980). Ikeshoji & Mulla (1970) described intra and extraspecific oviposition stimulants extracted from natural breeding waters of *Culex peus, Cx. tarsalis, Aedes nicromaculis* and *Aedes taeniorhynchus* and concluded that "the intra and extraspecific activities of these attractants undoubtedly play a role in the selection of oviposition sites by various species of mosquitoes" and that "the discovery, characterization and assessment of oviposition attractants operating in mosquito-breeding sites would provide a basis for an understanding of distribution and abundance of various species of mosquitoes".

Several times organic solvents have been successfully employed as oviposition attractants: McDaniel et al. (1979) used hexanic and ethanolic larval extracts of *Ae. triseriatus;* Ikeshoji & Mulla (1970) extracted several distillates of breeding water with diethyl ether; Osgood (1971) extracted eggs of *Cx. tarsalis* with ether, and Bentley et al. (1976) did the same with eggs of *Ae. triseriatus* and *Ae. atropalpus* using acetone. The hexanic extracts we prepared from *Ae. fluviatilis* larvae did not attract the oviposition of its females: perhaps other solvents and/or other techniques would be more successfull.

Oviposition media prepared with ground larvae, both fresh and dried, were found attractive to female *Ae fluviatilis.* The persistence of attractiveness in the dried extracts is a further fact pointing to the stability of the attracting factors in this species. Soman & Reuben (1970) attracted *Ae. aegypti* females using dead larvae of the same species and concluded that the stimulus involved was not visual. McDaniel et al. (1979), using Kaolin-feed *Ae. triseriatus* larvae, concluded that their attractiveness for females was not related to their intestinal contents. Trimble & Wellington (1980), using Kaolin treated *Aedes togoi* larvae in sterilized water and dishes, showed the attractiveness of this water for females. In our experiments, the possible interference of larval metabolites or bacteria on the attractiveness observed cannot be excluded but the presence of live larvae seems to be unnecessary to stimulate oviposition. The same did not occur with pupae: both fresh and dried extracts did not stimulate *Ae. fluviatilis* females, whereas live pupae present in oviposition water are able to do so (Consoli

& Williams, 1978; Leite, 1980). Nevertheless, Leite (1980) showed that pupal water of *Ae. fluviatilis* is not attractive to females of this species and Trimble & Wellington (1980) observed the same when using pupal water of *Ae. togoi*, no matter if it was sterilized or not. On the other hand, Andreadis (1977) found that *Culex salinarius* females preferred to lay eggs in the pupal water of this species rather then on water containing larval food. Also Consoli & Espinola (1973) and Leite (1980) demonstrated the attractive properties of *Cx. quinquefasciatus* pupal water to the females of this specie. The possibility that some oviposition stimulants, related to *Culex* pupae may be lacking in *Aedes* pupae deserves investigation.

RESUMO

Influência de derivados de larvas e pupas sobre o comportamento de oviposição de *Aedes fluviatilis* (Lutz) (Diptera: Culicidae) – Estudou-se a influência sobre o comportamento de oviposição das fêmeas de *Aedes fluviatilis* (Lutz) de produtos derivados das formas imaturas da mesma espécie. As fêmeas foram atraídas significativamente ($\alpha = 0,05$) por ocasião da oviposição por: água destilada que contivera 5 ou 15 larvas/1,5 ml; a mesma água (5 larvas/1,5 ml) após sua preservação por 38 dias; extratos evaporados e reconstituídos de água que conteve larvas, por até dois anos a sua produção, e filtrados de macerados frescos e secos de larvas. Extratos hexânicos de água que conteve larvas e filtrados de macerados frescos e secos de pupas não atraíram a oviposição das fêmeas.

Palavras-chaves: *Aedes fluviatilis* – comportamento de oviposição – derivados de larvas e pupas

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REFERENCES

ANDREADIS, T. G., 1977. An oviposition attractant of pupal origin in *Culex salinarius*. *Mosquito News, 37:* 53-56.

BELTON, P., 1967. The effect of illumination and pool brightness on oviposition by *Culex restuans* (Theob.) in the field. *Mosquito News, 27:* 66-68.

BENTLEY, M. D.; McDANIEL, J. N.; LEE, H. P.; STIEHL, B. & YATAGAI, M., 1976. Studies of *Aedes triseriatus* oviposition attractants produced by larvae of *Aedes triseriatus* and *Aedes atropalpus* (Diptera: Culicidae). *J. Med. Entomol., 13:* 112-115.

BROWNE, S. M. & BENNETT, G. F., 1981. Responses of mosquitoes (Diptera: Culicidae) to visual stimuli. *J. Med. Entomol., 18:* 505-521.

CLARK, G. G.; ROHRER, W. H.; ROBBINS, D. N.; PRETULA, H. L. & HARROFF, R. N., 1982. Lacrosse virus activity in Illinois detected by ovitraps. *Mosquito News, 42:* 551-557.

CONSOLI, R. A. G. B., 1987. *Influência de diversos fatores físicos e químicos sobre o comportamento de oviposição das fêmeas de* Aedes (Ochlerotatus) fluviatilis *(Lutz, 1904) e suscetibilidade das larvas da mesma espécie a diversos extratos vegetais.* Thesis, ICB/UFMG, Belo Horizonte, 187 p.

CONSOLI, R. A. G. B. & ESPINOLA, H. N., 1973. Possíveis fatores químicos na água que influenciam as fêmeas de *Culex pipiens fatigans* para a oviposição. *Rev. Pat. Trop., 1:* 49-54.

CONSOLI, R. A. G. B. & WILLIAMS, P., 1978. Laboratory observations on the bionomics of *Aedes fluviatilis* (Lutz, 1904). *Bull. Ent. Res., 68:* 123-136.

CONSOLI, R. A. G. B. & WILLIAMS, P., 1981. Aspects of the biology of laboratory-reared female *Aedes fluviatilis* (Lutz) (Diptera: Culicidae). *Mosquito News, 41:* 40-46.

DADD, R. H. & KLEINJAN, J. E., 1974. Autophastimulant from *Culex pipiens* larvae: distinction from other mosquito larval factors. *Environ. Entomol., 3:* 21-28.

FURLOW, B. M. & HAYS, K. L., 1972. Some influences of aquatic vegetation on the species and number of Culicidae (Diptera) in small pools of water. *Mosquito News, 32:* 595-599.

HWANG, Y. S. & MULLA, M. S., 1980. Chemicals and the future. *Californ. Agriculture, 34:* 32-33.

IKESHOJI, T. & MULLA, M. S., 1970. Oviposition attractants for four species of mosquitoes in natural breeding waters. *Ann. Entomol. Soc. America, 63:* 1322-1327.

IKESHOJI, T.; SAITO, K. & YANO, A., 1975. Bacterial production of the ovipositional attractants for mosquitoes on fatty acid substrates. *Appl. Entomol. Zool., 10:* 239-242.

KALPAGE, K. S. P. & BRUST, R. A., 1973. Oviposition attractant produced by immature *Aedes atropalpus. Environ. Entomol., 2:* 729-730.

KLOTER, K. O.; BOWMAN, D. P. & CARROLL, M. K., 1983. Evaluation of some ovitrap materials used for *Aedes aegypti* surveillance. *Mosquito News, 43:* 438-441.

KRAMER, W. L. & MULLA, M. S., 1979. Oviposition attractants and repellents of mosquitoes: oviposition responses of *Culex* mosquitoes to organic infusions. *Environ. Entomol., 8:* 1111-1117.

LAURENCE, B. R. & PICKETT, J. A., 1982. Erithro-6-acetoxy-5-hexadecanolide, the major component of a mosquito oviposition attractant. *J. Chem. Soc. Chem. Commun., 1:* 59-60.

LEISER, L. B. & BEIER, J. C., 1982. A comparison of oviposition traps and New Jersey light traps for *Culex* population surveillance. *Mosquito News, 42:* 391-395.

LEITE, M. A. B., 1980. *Comportamento de oviposição e interação das formas imaturas de* Aedes fluviatilis *(Lutz, 1904) e* Culex pipiens fatigans *Wiedemann,*

1828 (Diptera: Culicidae) em laboratório. Thesis, ICB/UFMG, Belo Horizonte, 103 p.

LEVIN, J., 1978. *Estatística aplicada à Ciências Humanas.* Editora Harper & Row do Brasil Ltda, SP, 310 p.

McDANIEL, J. N.; BENTLEY, M. D.; LEE, H. P. & YATAGAI, M., 1979. Studies of *Aedes fluviatilis* (Diptera: Culicidae) oviposition attractants. Evidence for attractant production by Kaolin-treated larvae. *Can. Ent., 111:* 143-147.

OSGOOD, C. E., 1971. An oviposition pheromone associated with the egg rafts of *Culex tarsalis. J. Econ. Entomol., 64:* 1038-1041.

SOMAN, R. S. & REUBEN, R., 1970. Studies on the preference shown by ovipositing females of *Aedes aegypti* for water containing immature stages of the same species. *J. Med. Ent., 7:* 485-489.

STEFFAN, W. A. & EVENHUIS, N. L., 1981. Biology of Toxorhynchites. *Ann. Rev. Entomol., 26:* 159-181.

TRIMBLE, R. M. & WELLINGTON, W. G., 1980. Oviposition stimulant associated with fourth-instar larvae of *Aedes togoi* (D. C.). *J. Med. Entomol., 17:* 508-514.

WALLIS, R. C., 1954. A study of oviposition activity of mosquitoes. *Am. J. Hyg., 60:* 135-168.