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Eggs Fertility and Biological Aspects of the Life Cycle in Natural Populations of *Simulium* (Diptera, Simuliidae)

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ABSTRACT

Eggs fertility rate and some biological aspects of the immature forms were studied on six Simulium species from ovipositions collected in natural breeding places located in rural area. The fertility was considerably high. For a total of 213 ovipositions containing 74,906 eggs, the average fertility rate was 99.13% with variation from 94 to 100%. The developmental cycle of the immature forms was observed from egg eclosion to adult emergence under temperature from 16.5 to 28°C. In S. orbitale the life cycle duration was 30 to 32 days, for S. perflavum 32 to 35 days, for S. subnigrum 33 to 39 days, for S. inaequale 41 to 47 days, for S. pertinax 42 to 48 days, and finally, the longest duration was observed for S. distinctum to range 48 to 49 days.

Key words: Blackfly, egg fertility, life-cycle, oviposition, Simuliidae, *Simulium*

INTRODUCTION

In many parts of the world, hematophagous blackflies are known to cause a negative impact on the economy and public health by transmission of diseases, affecting tourism and welfare of human and animal populations. In extreme cases, it causes death of livestock by toxaemia, anaphylactic shock and respiratory problems due to the inhalation of flies (Robinson, 1997). Females form into swarms and usually bite intensively during daylight producing distress and affliction to the hosts (Coscarón 1989; Kim and Merritt 1997; Lane and Crosskey 1993). Simuliidae can cause problems both in developing and wealthy countries. A number of hematophagous species have been identified as vectors of pathogenic organisms to humans and also domestic and wild animals. Although some areas are not affected by transmission of parasites, the negative health impact is evident by the presence of severe bites. Pathogenic organisms such as *Leucocytozoon* contain approximately 70 species transmitted to birds by Simuliidae. The filarid *Onchocerca volvulus* is the main parasite of humans in Afrotropical and Neotropical regions (Kettle, 1995). Approximately 85.5 million people live in endemic areas and are under risk of onchocerciasis contamination, a leading cause of cutaneous, lymphadenitis and ocular infections (Etya'ale, 2001; Eezzuduemhoi and Wilson, 2004). In onchodermatitis, the chronic pruritic skin can cause serious dermic lesion. In some endemic areas in Africa, onchodermatitis can affect 80% of local population in which individuals live in poverty and ostracism situation. In pregnant women infected with microfilariae of *Onchocerca volvulus* damage in the pituitary gland on fetus has

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been registered resulting later in a deficiency of growth hormone in children (Norwood, 2004). Onchocerciasis (or river blindness) is the second main cause of infectious blindness in humans. The disease is found in 36 countries in Africa, in one country in Arabian peninsula (Yemen), as well as in Guatemala, southern Mexico, some areas of Venezuela, small areas in Brazil, Colombia and Ecuador. In the Amazon forest in Brazil, some indigenous Yanomami communities have been affected by the onchocerciasis (Coelho et al., 1998; Thylefors, 2001; Okulicz et al., 2002; Shelley, 2002; Carabin et al., 2003).

Although there is a vast literature concerning Simuliidae, studies related to biological cycle are few in number and, consequently, the life cycle of most Simuliidade remain incompletely described (Colbo and Moorhouse, 1974; Pegoraro, 1993; Colbo and Wotton, 1979; Lozovei et al., 1989; Kim and Merritt, 1997). Lack of information can lead to inefficient forms of immature control as for example the indiscriminate use of insecticide. Methods of control are generally applied to aquatic forms and most blackfly species breed in fast-flowing streams where it is difficult to evaluate the efficiency of a product. Therefore, studies concerning the fertility of eggs and aspects of the life cycle of the immature forms are important to choose a proper targeting control application in time and space, thus, avoiding unnecessary environmental impacts. Moreover, several species can share the same breeding site, with each one presenting a different life cycle duration and oviposition preference to a specific substrate.

Recent work carried out in the same location of this study revealed the presence of seven species breeding in the “Riacho dos Padres” river closed to Almirante Tamandaré town (Lozovei et al., 2004). The most expressive was *S.* (*Inaequalium*) *inaequale* Peterson and Shannon, 1927 followed by *S.* (*Chirostilbia*) *pertinax* Kollar, 1932, *S.* (*Tyrsopelma*) *orbitale* Lutz, 1910, *S.* (*Ectemnaspis*) *perflavum* Rouband, 1906, *S.* (*Inaequalium*) *subnigrum* Lutz, 1910, *S.* (*Chirostilbia*) *distinctum* Lutz, 1910 and *S.* (*Psaroniompsa*) *incrustatum* Lutz, 1910. The aim of the present work was to better understand egg fertility and aspects related to the life cycle of immature stages of *Simulium* spp from egg to adult. Besides the presence of natural flowing rivers in the region, there are slopes (spillway) made of concrete attached to reservoirs to ensure the water flowing and that are also breeding places for Simuliidae. Although in 2002, the local population applied a blackfly integrate control (*Bacillus thuringiensis israelensis* "Bti" + mechanical), the timetable was not accurately followed and the control failed. This study could form the basis for the establishment of a blackfly control program involving biological and mechanical management in the region.

MATERIALS AND METHODS

Simuliidae collections were performed in the “Riacho dos Padres” river at the “Chácara Evíssima” cottage in the Almirante Tamandaré (25°18’ S, 48°18’ W), 20km North of Curitiba, capital of Paraná State. It is a touristic natural resort where the site is surrounded by mountain and fast flowing stream rivers which provide the ideal conditions for blackfly breeding. The local climate is subtropical (annual average 18 to 22°C) with hot summer and cold winter with occasional frostings. In this cottage, the river “Riacho dos Padres” contains small artificial dams that block the water flowing to form five successive reservoirs for fish breeding. Connected to these reservoirs, there are artificial spillway to ensure the access of water and that are important breeding places for blackflies.

The collections were carried out from January to December 2004. Artificial polypropylene strips (length 2.0m x width 0.5cm) were installed in natural sides of the river and in the lateral walls of the spillways connected to the reservoirs. Strips of specific colours such as light yellow, white and dark blue colours were used to allow visual observation of the ovipositions. The strips were replaced after every collection, i.e. once a week. Also, collections of aquatic plants that were pending or submerged on the river side were performed. Strips and plants were kept in moisten plastic bags to ensure humidity. Polystyrene boxes with ice cubs inside to keep temperature were used to transport the material from the field to the laboratory.

In the laboratory, small fragments of substrate containing ovipositions were transferred to Petri dishes (diameter 9.0cm x height 1.5 cm) and filled with 30ml of distilled water. The Petri dishes were always kept closed to ensure humidity and protection against predator insects. The laboratory was kept under natural photoperiod and non-

controlled temperature. The ovipositions were constantly monitored until completed the total larvae eclosion before verifying the fertility rates.
The egg fertility rate was studied by the recent-ecloded larvae, empty exocorium and unfeasible eggs verified under stereoscope microscope. The data were computed considering the total number of *Simulium* spp eggs. In order to study the life cycle, 78 well-delineated ovipositions with different shapes were separated from each kind of substrate (strips and plants) and placed into glass flasks (6.0 cm diameter x height 14 cm) with 500 ml of distilled water and fish food TetraMin®. Water was replaced every three days or whenever food fermentation took place. The aeration was constantly kept using electric air pumps. During the experiment egg incubation time and larval and pupal stages duration were observed. Identification of the specimens was based on dicothomic keys and species descriptions from Coscarón and Wygodzinsky (1984), Coscarón (1991) e Strieder et al. (1992).

RESULTS

Recent literature indicated seven species breeding in the same location of the present research (Lozovei et al., 2004). In descending order of prevalence, the species were *S. inaequale* (34.91%), *S. pertinax* (19.74%), *S. orbitale* (18.75%), *S. perflavum* (14.85%), *S. subnigrum* (9.84%), *S. distinctum* (1.68%) and *S. incrustatum* (0.23%). However, egg fertility and aspects of immature life cycle were studied on six out of these seven species. Exception was made to *S. incrustatum* since very few ovipositions were obtained from this species due to its sporadic prevalence in the place.

Substrates and types of ovipositions

Large quantity of eggs were collected for the six species of *Simulium*. The artificial polypropylene strips were very effective to obtain intact ovipositions. Besides the good number and condition of the eggs, it was observed that strips were also efficient for better visualization of eggs and recent-ecloded larvae. Non species-specific preference was observed for strip colours, although more ovipositions were found on light colours such as light yellow and white as well as in dark blue strips. However, this was not conclusive and more experiments would be need to be done. Well defined and intact ovipositions were also found in the aquatic plant collections.
The ovipositions of blackflies from "Riacho dos Padres" river were differentiated into three categories according to the number of eggs and protection against dissecation: **a)** ovipositions with disperse eggs in small amounts and little gelatinous matrix; **b)** ovipositions with eggs superposed in two or three layers clearly bordered and covered by a substantial gelatinous mass; and **c)** ovipositions aggregated in several layers superposed and unclearly bordered with thousands eggs covered by abundant matrix and the ovipositions laid by several females. It was worth noting that the ovipositions of *S. distinctum* and *S. pertinax* did not presented completely covered by matrix, with only a small quantity used to fix them to the substrate like in **a)** description. However, in more than one occasion the ovipositions of both species were found bonded together in the same substrate. On many occasions, the phenomenon of aggregated oviposition, as described in **c)** category, was noticed in some mornings at one specific place. The site was inside a closed spillway used for draining water from the reservoir on which the lateral walls were covered with moss. Isolated females were spotted laying eggs on pre-existing ovipositions. This category of ovipositions was also found on the strips substrates.

Number of eggs per oviposition and egg fertility

Despite the three categories used to describe ovipositions, only **a)** and **b)** were object for the egg study. A total of 74,906 eggs were computed from 213 ovipositions analysed. As for the number of eggs per oviposition, the majority (206) presented from 104 to 850 eggs. In four, it was less than 100 eggs (23, 27, 50 and 98 eggs) and in only three more than 1,000 eggs. From the total of 74,906 eggs observed for the fertility study, 99.13% (74,251) were fertile and only 0.87% (655) infertile. Most of oviposition (162) presented 99 to 100% of fertility and the others (51) not less than 94% (Fig. 1).

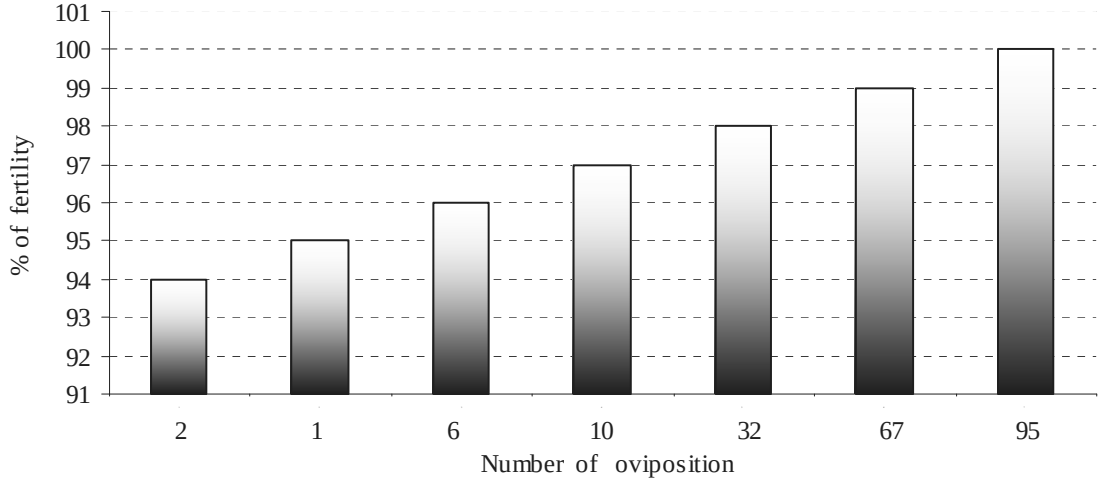

Figure 1 - Fertility percentual index of *Simulium* spp eggs collected at "Riacho dos Padres" river, Almirante Tamandaré, Paraná, Brazil.

Aspects of the life cycle

Egg hatching presented synchronic in the categories **a)** of ovipositions. Larva eclosions were observed occurring on the same day. On the other hand, assinchronic eclosions occurred particularly in the category **c)** of oviposition. Eclosions were observed starting in eggs located on the peripheral parts of the oviposition, i.e. exposed to the environment where more aeration is available. Delays were observed (two to three days) compared to the layers of inside where larvae started to come out by the time the gelatinous matrix was dissolving.

The 78 ovipositions engaged in the study of development from egg to adult were from categories **a)** and **b)** of oviposition. For the six *Simulium* species, the average of egg incubation was 4 to 6 days under room temperature from 22 to 28.5^{o}C and water 16.5 to 28^{o}C. As for the larval stage, the average duration for the six *Simulium* species was 22 to 39 days and the pupal stage was 4 to 5 days. More specifically ascending order, the total duration of the cycle from egg to adult was 30 to 32 days for *S. orbitale*, 32 to 35 days for *S. perflavum*, 33 to 39 days for *S. subnigrum*, 41 to 47 days for *S. inaequale*, 42 to 48 days for *S. pertinax* and the longest duration was observed for *S. distictum* to range 48 to 49 days.

DISCUSSION

Humidity constitutes an important environmental factor to embriogenesis and larvae development of blackflies. An example of this is the presence of gelatinous matrix totally or partially involving the ovipositions or simply used to fix eggs to substrate. In the "Riacho dos Padres" river, many different kinds of oviposition were observed. The three categories described reflected the diversity of strategies used by Simuliidae for adaptation to its environment. In this river, the females of *Simulium* laid their eggs in disperse or aggregated way and the ovipositons might or not involved in gelatinous matrix. Aggregated oviposition are normally associated to matrix. This can be characteristic for some species. Ovipositions of the African *S. damnosum s. l.* for example were aggregated and involved in a thick layer of matrix (McCall et al., 1997; Wilson et al., 2000). McCall et al. (1997) attributed aggregated oviposition to the presence of female pheromones synthesised in the ovaries during the gonotrophic process and released from eggs layed. Pheromones attracted gravid females to places with eggs laid. In the present study, aggregated ovipositions from different females were found associated to muss and strips substrates.

It was important to notice that attractive pheromones of aggregated ovipositions were also

involved in disperse ovipositions. This was detected in the present work for *S. distinctum* and *S. pertinax* that lay eggs in the same substrate.

A number of *Simulium* ovipositions in the river "Riacho dos Padres" occurred according to category **a)**, i.e., disperse eggs in small amounts and little gelatinous matrix. This phenomenon was observed before, without matrix, by Pegoraro (1993) when studied *S. pertinax* in laboratory conditions and observed that ovipositions were like the category **a)**, however, without matrix. The same oviposition pattern was observed by Rodriguez-Pérez *et al.* (2003) for *S. ochraceum*, an important vector of *Onchocerca volvulus* in Neotropical regions. However, in the present work the majority of ovipositions were observed to occur as in category **b)** such as noticed in *S. inaequale* and *S. subnigrum*.

The guarantee of the humidity depends largely on the female oviposition behaviour by the choice of a substrate which varies in Simuliidae. The substrate types observed in the present study did not show consistent preferences to a specific one, however, reflected a range of artificial and natural substrates that the female blackflies were able to exploit. As for the colour of the artificial substrate, the use of light colours were shown to be more effective for better visualization of ovipositions and egg hatching.

In nature, Simuliidae females lay their eggs in different ways. It can be straight into water like *Cnephia pecuarum* and *C. decotensis* from Canada (Davies and Peterson, 1956; Kettle, 1995) or using substrates like as *S. venustum* (Davies and Peterson, 1956; Kettle, 1995), *S. perflavum* (Hamada, 1998) and *S. inaequale* (Moreira and Sato, 1996). As observed in the present work, Simuliidae used moss surfaces to lay eggs. This has also been described for some other species such as *Prosimulium hirtipes*, *P. tomosvaryi* and *P. subrufipes* (Zwick and Zwick 1990).

The variety on oviposition types in Simuliidae also reflected a variety in the number of eggs. Aggregated ovipositions implicated in a large amount of eggs. Without considering extenses oviposition masses, it was observed that the number of eggs per oviposition was not less than 104. For an exact computation of egg number produced by a species, the study should be conduced using gravid females. The influence of gonotrophic cycle and age must be taken into account and it is known that females can live three to four weeks which is potentially enough for three gonotrophic cycles (Davies and Peterson, 1956; Crosskey, 1993).

Eggs collected in "Riacho dos Padres" rivers showed very high fertility rate compared to other studies. The fertility rate of *Simulium* spp was 94 to 100%. Under controlled conditions, fertility rates were much lower, possibly due to the difficulties to reproduce permissive conditions of a particular species in the laboratory. The literature showed *S. decorum* with 50 to 60% in fertility (Simmons and Edman, 1981), *S. bidentatum* 61.40% (Hadi and Takaoka, 1995), *S. ochraceum* 72.3% (Baba et al.,1988), *S. damnosum* 51 to 86% (Wilson et al., 2000), and *S. pertinax* 30% (Pegoraro, 1993). The high egg fertility rate could in part explain the reason why the frequency of *Simulium* spp through calendar year 2002-2003 in "Riacho dos Padres" river was that intense (Lozovei et al., 2004).

The length of time between egg and adult emergence was mainly given by the duration of larval stage that was 22 to 39 days. The egg incubation and pupal stage were similar in duration and fast compared to that in larvae. The incubation was normally rapid when eggs did not have diapause or other kind of hypobiosis. For *S. nigritarse*, for example, the incubation lasted from 3 to 13 days at 25^{o}C (Begemann, 1980) suggesting that intrinsic factors were implicated in incubation and in this case not exclusively temperature-dependent. In addition, considering aggregated eggs, the delays in two to three days observed in the present work was also noticed by other authors (Begemann, 1980; Lair, 1981).

Time duration of life cycle, particularly the larval forms are important to control blackflies by using biolarvicide (Bti). It helps on the establishment of a timetable of re-application of the product in the target-location. Based on the average of larval stage duration of *Simulium* spp (20 days), a blackfly control program by using Bti and mechanical management was designed to "Riacho dos Padres" river. The control program implemented in 2004 is so far showing great success on the control of Simuliidae in that region (unpublished data).

RESUMO

Foram estudados o índice de fertilidade de ovos de espécies de *Simulium* e o ciclo biológico das fases aquáticas em posturas trazidas de criadouro natural

localizado na área rural. A fertilidade de ovos mostrou-se altíssima, das 213 posturas coletadas, contendo 74.906 ovos, o índice médio percentual geral foi de 99,13%. Entre as diferentes posturas, a variação oscilou de 94% a 100%. O ciclo biológico a partir da incubação do ovo até a emergência de adulto em *Simulium orbitale* foi de 30 a 32 dias; *Simulium perflavum* de 32 a 35 dias; *Simulium subnigrum* de 33 a 39 dias, *Simulium inaequale* de 41 a 47 dias; *Simulium pertinax* de 42 a 48 dias e *Simulium distinctum* de 48 a 49 dias em temperaturas com variação de 16,5^{o}C a 28^{o}C.

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