Diptera, Ceratopogonidae, *Dasyhelea necrophila*
Spinelli and Rodriguez, 1999: Detection of eggs in ovitraps, in Uruguay

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**ABSTRACT:** Ovitraps with eggs of *Dasyhelea necrophila* were detected at five localities in Uruguay during surveillance and control of *Aedes aegypti*. Relevant dates of oviposition in wild of this Ceratopogonidae species are included in addition to previous laboratory work on the species. Eggs of *D. necrophila* were deposited together with eggs of Culicidae and Psychodidae. These are the first records of the species from Uruguay.

Subfamily Dasyheleinae includes only the genus *Dasyhelea* Kieffer, species of which are known to be cacao pollinators in tropical zones. Adults feed on nectar and vegetable fluids. Knowledge of their immature stages, particularly eggs and first larval instars, is poor compared to the adults. Larvae live associated with algae in shallow water in rocks, tree hollows, and phytotelmic environments, also in decomposing vegetable mass and saline environments (Spinelli and Wirth 1993). Some species such as *D. necrophila* are found in artificial containers and can resist habitat desiccation for about 48 hours (Ronderos et al. 2006) and *D. pseudooincisurata* Waugh and Wirth, 1976 has been collected from artificial containers, in Florida, USA (Hribar 1998; Hribar et al. 2004).

*Dasyhelea necrophila* was described by Spinelli and Rodriguez (1999) based on adults, pupae and fourth instar larva. Ronderos et al. (2003) redescribed and illustrated the fourth instar larva and pupa based on ultrastructural observations and Ronderos et al. (2006) gave details of laboratory observations on morphology and biology under different conditions. De Villalobos and Ronderos (2003) experimentally infected larvae and pupae of *D. necrophila* with larvae of *Paragordius varius* (Leidy, 1851), as a potentially paratenic host.

Among the investigations and actions for control of *Aedes aegypti* (Linnaeus, 1762) which are carried out nationally by the Zoonosis and Vectors section of the Epidemiology Department in the Ministry of Public Health of Uruguay, ovitraps are used to collect eggs of container-inhabiting mosquitoes. During these investigations, eggs of *D. necrophila* were encountered in some ovitraps.

The objective of this work is to provide information about the egg posture registry of *D. necrophila*, detected in ovitraps at five localities in Uruguay, complementing previous laboratory work recently done on this species.

A total of 8,640 paddles from ovitraps was examined from 5 localities of Uruguay (Figure 1) during Spring, Summer and Fall: Flores and Montevideo (XII/2006-V/2007 and X/2007-V/2008); Florida (II/2007-V/2007 and X/2007-V/2008); Canelones (XII/2006-V/2007 and X/2007-V/2008); Maldonado (XII/2006-V/2007).

The ovitraps consist of a black plastic bottle with a wide mouth (15 cm high, 500 ml) one third filled with water and a small wooden paddle where different species of aquatic Diptera can deposit their eggs (Hernández et al. 2002; García-da-Rosa et al. 2003). Traps were placed at or near ground level in the shade, in quiet places far from noisy areas and zones with intense vehicular traffic, and without competition from co-occurrence of other potential breeding sites. The paddles in the ovitraps were placed in...
a tilted position (Figure 2). Paddle dimensions were varied in height (23.6 cm – 14.0 cm), width (1.4 cm – 1.0 cm), and thickness (1.0 cm – 0.2 cm). Paddles were replaced by new ones weekly and were sent to the lab with their corresponding data, and were observed using stereoscopic microscopy, registering presence or absence of eggs. Some of the eggs collected were raised to the adult stage in the lab, for specific determination. From each paddle the number of eggs in each group, the quantity of egg masses, and their minimum and maximum height, in centimeters, were recorded. These samples were photographed with a Canon A520 digital camera and processed in Photoshop 7.0, and then extracted from the paddles with the aid of a scalpel, and preserved in 70 % ethanol. The examined material is deposited in Entomological Collection of the Facultad de Ciencias, Universidad de la Repúbllica, Uruguay.

The eggs observed in the sampling of the paddles from ovitraps belong to three dipteran families: Ceratopogonidae, Culicidae, and Psychodidae. The Ceratopogonidae identified species was *D. necrophila* representing the first record of this species from Uruguay. Prior to this study it was recorded only from Argentina and Mexico (Borkent and Spinelli 2007).

From 8,640 paddles examined, eggs of this species were detected on 26 of them (Table 1). The eggs are C-shaped, grouped and glued with some kind of substance (Figure 3). The egg mass is glued to the substratum and in this way they are adequately incubated near water. It is possible that the sticky substance protects the eggs from natural enemies and weather changes. The egg masses were irregularly shaped and were found on both sides of the paddle, (front side = 14, reverse = 17; edges = 5); 76.9 % of egg masses were deposited parallel to the water; 15.4 % were deposited in an L-shaped mass, 7.7 % in a band of 3.5 to 4 cm wide (Figure 4).

Eight paddles had only one egg mass, three of them with 10 to 12 eggs each, one with 59 and 4 paddles showed a variable number of eggs, between 92 and 132. Only one paddle had a maximum of 22 groups of eggs, and the total amount of eggs counted was 1076 (Table 2).

The number of eggs groups per paddle varied as follows: 30.8% of paddles had only one egg group; 23.2 % of paddles had 2 groups; 19.3 % had 3 groups; 7.7 % had 6 groups and 3.8 % had 5, 9, 10, 11 or 22 groups. The eggs were deposited at 5.4 cm minimum average high (1-12.4) and 6 cm maximum average high (1-12.4) from the lower tip. Height of oviposition above the waterline was not recorded, but the records allow us to suppose that the water level was very variable in the ovitraps in the different trap localities. These observations allow us to broaden awareness of the egg masses of *D. necrophila* in nature and geographical distribution of this species.

| Department | Latitude Capital of Department | Longitude Capital of Department | Paddles total for localities | Average weekly Nº of paddles | Paddles with eggs of *D. necrophila* |
|------------|--------------------------------|--------------------------------|----------------------------|-------------------------------|---------------------------------|
| Flores     | 33°32'20''S                    | 56°53'19''W                    | 964                        | 16                            | 12                              |
| Florida    | 34°05'44''S                    | 56°12'51''W                    | 540                        | 13                            | 1                               |
| Canelones  | 34°31'22''S                    | 56°16'40''W                    | 4141                       | 69                            | 4                               |
| Maldonado  | 34°54'00''S                    | 54°57'00''W                    | 779                        | 30                            | 6                               |
| Montevideo | 34°51'29''S                    | 56°10'15''W                    | 2216                       | 36                            | 3                               |
| Total      |                                |                                | 8640                       | 164                           | 26                              |

*a tilted position (Figure 2). Paddle dimensions were varied in height (23.6 cm – 14.0 cm), width (1.4 cm – 1.0 cm), and thickness (1.0 cm – 0.2 cm). Paddles were replaced by new ones weekly and were sent to the lab with their corresponding data, and were observed using stereoscopic microscopy, registering presence or absence of eggs. Some of the eggs collected were raised to the adult stage in the lab, for specific determination. From each paddle the number of eggs in each group, the quantity of egg masses, and their minimum and maximum height, in centimeters, were recorded. These samples were photographed with a Canon A520 digital camera and processed in Photoshop 7.0, and then extracted from the paddles with the aid of a scalpel, and preserved in 70 % ethanol. The examined material is deposited in Entomological Collection of the Facultad de Ciencias, Universidad de la República, Uruguay.

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*Figure 2. Ovitrap with paddle.*

*Figure 3. Egg mass of *Dasyhelea necrophila.*

*Table 1. Paddles of ovitraps examined: for localities with their coordinates, absolute number, average weekly and total of paddles with eggs of *Dasyhelea necrophila.*
Table 2. Total of paddles with eggs of Dasyhelea necrophila, total of egg masses and total of eggs for each paddles.

| Nº of paddle | Nº of egg mass | Nº of eggs. | Nº of paddle | Nº of egg mass | Nº of eggs | Nº of egg mass | Nº of eggs | Nº of egg mass | Nº of eggs |
|--------------|----------------|-------------|--------------|----------------|------------|----------------|------------|----------------|------------|
| I            | 1              | 10          | X            | 2              | 36         | XIX            | 3          | 195            |
| II           | 1              | 12          | XI           | 2              | 44         | XX             | 5          | 117            |
| III          | 1              | 12          | XII          | 2              | 62         | XXI            | 6          | 54             |
| IV           | 1              | 59          | XIII         | 2              | 64         | XXII           | 6          | 146            |
| V            | 1              | 92          | XIV          | 2              | 70         | XXIII          | 9          | 179            |
| VI           | 1              | 110         | XV           | 3              | 24         | XXIV           | 10         | 109            |
| VII          | 1              | 111         | XVI          | 3              | 98         | XXV            | 11         | 111            |
| VIII         | 1              | 132         | XVII         | 3              | 98         | XXVI           | 22         | 1076           |
| IX           | 2              | 28          | XVIII        | 3              | 99         |                |            |                |

Figure 4. Egg mass of Dasyhelea necrophila in a paddle.

The knowledge of the breeding sites of different Diptera in the same habitat is very important to application of methods for biological integrative control, especially species with economical and sanitary relevance which share the same habitat for ontogenetic development. Ovitraps are a good entomological instrument for surveillance and control of dengue vectors. They make favorable conditions for the rearing of Aedes aegypti and permit early detection of its presence, so knowledge of related biological groups (with economical or sanitary relevance or as indicator taxa for environmental monitoring) would be important for future studies.

Acknowledgments: To Dra. G. Willat and persons and institutions who participated in detection and control of Aedes aegypti in Uruguay, to Dr. L. Hribar who give us bibliographic references and to Alba Bentos Pereira for suggestions.

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