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Survival of submerged blowfly species and their parasitoids: Implications for postmortem submersion interval

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ABSTRACT

Pupal survival of three blowfly species, *Chrysomya albiceps*, *Chrysomya megacephala*, and *Chrysomya putoria* (Diptera: Calliphoridae) and the parasitoid species *Nasonia vitripennis* (Hymenoptera: Pteromalidae) was studied after the pupae were experimentally submerged in water. Non-parasitized pupae at different developmental stages, 0, 24, 48, and 72 h, and parasitized pupae after 3, 8, 10, and 12 days of development were submerged for 6, 24, 48, or 72 h. Control groups for each pupal developmental stage (parasitized or not), which were not submerged, were also observed in order to compare the adult emergence rates. The survival of white pupae (0 h/age) decreased with time of submergence for all three blowfly species, showing the lowest rates compared with other experimental pupa groups. For the three blowfly species, non-parasitized pupae at 24 and 48 h of age showed survival rates above 60%. However, for pupae at 72 h of age, the survival rates decreased with increased underwater time, with less than 30% survival after 72 h in *C. putoria* and *C. albiceps*. The survival of parasitoids inside blowfly pupae that were submerged during their larval stage (3 days/age) decreased with the increase of submergence time. After the parasitoids reached the pre-pupal life stage, the survival was higher for all underwater periods. These observations can be useful in investigations of the decomposition of partially submerged bodies, or in cases of pupae found adhering to decaying flesh, hair, or clothes of corpses that were submerged after the larvae had developed and pupated.

1. Introduction

Forensic entomology is the science that applies the knowledge of the biology, ecology, and behavior of arthropods colonising corpses as a tool to aid in criminal trials in legal medicine [1]. The course of decomposition of corpses in terrestrial environments is well understood [2,3]. It is characterized by a succession of insect species, which can be divided into different waves of colonization [4]. Blowflies are among the main colonizer insect species [5,6]; however, during decomposition of the corpse, they may be parasitized by small wasps, the parasitoids. Female parasitoids use blowfly larvae and/or pupae for the development of their progeny, modifying the post-embryonic developmental period and interrupting the life cycle of their hosts, and thus altering the estimate of the PMI (Postmortem Interval) [7,8].

The wasp *Nasonia vitripennis* (Hymenoptera, Pteromalidae) is commonly found on carcasses and in bird nests [8–10]. This species is a generalist parasitoid that parasitizes a wide range of dipteran pupae, from ornithoparasitic blow flies to necrophagous dipterans [8–10]. The wasp parasitizes several species of the necrophagous dipteran families Calliphoridae and Sarcophagidae [10], using their pupae for feeding and reproduction [11]. Because of this association, some studies of the biology and behavior of *N. vitripennis* have demonstrated its potential as a forensic indicator [6,8,12,13].

Among the different types of crimes investigated by means of forensic entomology, perhaps the most poorly documented category is deaths involving submerged corpses [14]. Most forensic studies have emphasized only the terrestrial environment, with only 15% of investigations evaluating the postmortem ecology of bodies in aquatic environments [14]. Few studies have described the colonizing fauna of a partially submerged body [15–17], and none have considered parasitoids in this context. Studies involving the abilities of blowflies and parasitoids to survive after periods underwater can be useful in homicide cases in which immature stages of blowflies have colonized exposed parts of submerged corpses; in which pupae are found adhering in decaying flesh, hair, or clothes of corpses that were submerged after the larvae had developed and pupated [17]; or in which the decomposition has occurred in constantly flooded soil [18].

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Singh and Greenberg [17] compared the survival of pupae in different ages of development, and found low survival rates of white pupae and pharate adults of species of blowflies after 1–5 days of submergence in water. They found higher survival for pharate adults than for white pupae, but none of these pupal stages survived after 4 days. However, nothing is known about parasitoids subjected to submergence.

Our study aimed to investigate the survival of submerged pupae of *Chrysomya albiceps*, *Chrysomya megacephala*, and *Chrysomya putoria* and the parasitoid *N. vitripennis*. The results can contribute to improve estimates of the Postmortem Interval (PMI) and the Postmortem Submergence Interval (PMSI) in forensic investigations. The latter term refers to the period of time from when the body enters the water to the point of its discovery [19].

2. Materials and methods

2.1. The species studied

The blowflies and parasitoid specimens were collected in the vicinity of the campus of the Universidade Estadual Paulista, Botucatu, São Paulo, Brazil. The blowfly species used were *C. albiceps* (Wiedemann), *C. megacephala* (Fabricius), and *C. putoria* (Wiedemann) (Diptera: Calliphoridae). After collection, the blowfly adults were reared in the laboratory, in cages (30 cm^3), and females received water, sugar, and fresh beef liver [20]. After the laboratory colony was established, the newly hatched pupae were obtained from eggs of adult flies for the water-submergence experiments.

The blowfly-pupal parasitoid *N. vitripennis* was collected by placing pupae of *C. albiceps* in containers with ground beef on which these blowfly larvae had fed, and wood shavings. Each container was covered with mesh (2 mm diameter) to permit only small parasitoids to enter, and the containers were hung in trees. After four days, all puparia in the containers were separated individually in gelatin capsules and laboratory-reared for up to 35 days. After establishment of the parasitoid laboratory colonies, *C. albiceps* pupae were offered to females of *N. vitripennis* for a period of 24 h. These parasitized pupae were used for the water-submergence experiments. Because this experimental section aimed to analyze the survival of the parasitoids inside their host, only pupae of *C. albiceps* were used as the host.

2.2. Experimental design

2.2.1. Blowfly pupae survival

The pupal development in three blowfly species was analyzed with 0 (white pupae), 24 and 72 h old pupae. Each set of pupae was subjected to one of four periods of underwater submergence: 6, 24, 48 and 72 h. In each set, 30 pupae were submerged in open glass vials containing 100 ml of deionized tap water. To prevent the pupae from floating, the vial openings were covered with absorbent cotton that had been soaked in the same water, preventing air bubbles from forming under the cotton [17]. A control group of pupae that were not submerged in water also was set up.

After the period of submergence, the pupae were placed in Petri dishes covered with liquid-absorbing paper and observed for one week. After this period, the number of surviving adults was recorded. Ten replicates were used for each treatment.

2.2.2. Parasitoid survival

Four developmental stages of parasitoids were analyzed: larva, pre-pupa, pupa, and adult (3, 8, 10, and 12 days of age, respectively). For this, pupae of *C. albiceps* were offered to nulliparous female parasitoids for 24 h. Then, pupae from which adult blowflies had not emerged were placed individually in gelatin capsules. For each treatment, a sample of pupae was dissected to confirm the development of the parasitoid. Parasitoids were subjected to four treatments, i.e., were submerged in water for intervals of 6, 24, 48 or 72 h. A control group consisting of parasitoids that were not submerged also was setup, to compare with the other treatments.

The procedure to investigate the survival of parasitoids was the same as previously described. After the period submerged in water, the parasitized pupae were placed in gelatin capsules and observed for 30 days. Then, the number of pupae from which parasitoids emerged was recorded. Ten replicates of each treatment were carried out.

2.3. Statistical analyses

The proportion of pupae with flies and/or parasitoid emergence after underwater submergence was analyzed using ANOVA following arcsine transformation [21]. The data were subjected to a *t*-test ($p < 0.05$) for comparisons between the number of surviving insects of the control group (without water submergence) and the other treatments (different periods of pupa submergence).

In order to analyze pupa survival, the developmental stage of flies, period of water submergence, and blowfly species were considered as the explanatory variables. To investigate the parasitoid emergence, the developmental stage and the periods of water submergence were considered as the explanatory variables. The tests were computed by means of the R program (R Development Core Team, 2009).

3. Results

3.1. Blowfly pupal survival

The results indicated that there was a significant interaction among the three explanatory variables, blowfly species, pupal development stage, and period of water submergence (df = 4, $F = 4.68$, $p < 0.01$). The effects of the pupal development stage and submergence period were different for each blowfly species, and because of this, the effect of water submergence was analyzed for each species individually.

The survival of the pupae in the control, i.e., non-submerged white pupae (0 h), was statistically different from the other treatments: 6 h ($t = -7.05$, $p < 0.05$), 24 h ($t = -19.95$, $p < 0.05$), 48 h ($t = -21.2$, $p < 0.05$), and 72 h ($t = -21.2$, $p < 0.05$) for all three blowfly species. The survival of white pupae decreased with the increase in the underwater period, and the mortality reached 100% for periods of water submergence longer than 24 h for all three blowfly species (Fig. 1).

All experimental pupa groups of *C. albiceps*, with the exception of white pupae, showed survival rates above 85% and exhibited no significant difference from their respective controls if submerged for less than 24 h (Fig. 1). However, after submergence for 48 or 72 h, pupa survival decreased significantly, to as low as 30% for the 72 h developmental stage (Fig. 1).

Submerged *C. putoria* pupae at the 24 h developmental stage exhibited significant mortality compared to the control group (no

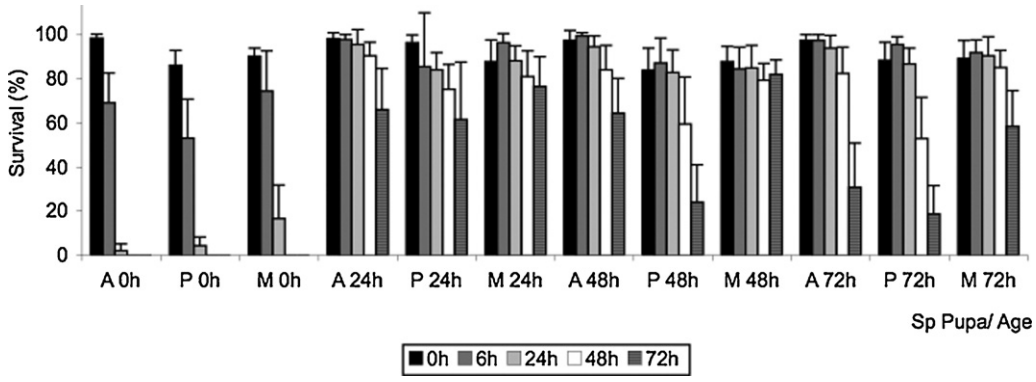

Fig. 1. Mean survival (%) of blowfly pupae of different developmental ages after submergence in water (no submergence, 6 h, 1, 2 and 3 days). * indicates statistical difference ($P < 0.05$) between control group (no water submergence) and water-submergence treatments for each developmental age and species of pupa. (A, *Chrysomya albiceps*; P, *Chrysomya putoria*; M, *Chrysomya megacephala*.)