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RESEARCH ARTICLE

Necrodes littoralis (Coleoptera: Silphidae) visiting and breeding on a carcass in Italy

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ABSTRACT

The community that progressively colonizes a decaying corpse can be considered a small ecosystem mostly composed of sarcosaprophagous arthropods belonging to the orders Diptera and Coleoptera. Studies on these species are often performed through animal models to obtain data on their succession, behaviour and life cycle, together with information on habitat, corpse conditions, season and association with other species. These data may be relevant for forensic investigations, especially concerning the estimation of Post Mortem Interval (PMI). An investigation on the sarcosaprophagous insect community in a rural area was set in Calabria (Southern Italy), using a pig, *Sus scrofa* Linnaeus, 1758 (Artiodactyla: Suidae) as experimental model. Analyses of the community of Diptera and Coleoptera revealed the massive presence of *Necrodes littoralis* (Linnaeus, 1758) (Coleoptera: Silphidae). Adults of this species reached the carcass during the bloated stage and a large amount of larvae was detected from the decay stage onwards, simultaneous to the sharp decrease in dipteran larvae and pupae. The occurrence and the activity of *N. littoralis* should be considered to avoid misinterpretation and errors in estimating PMI in forensic investigation.

Keywords: Animal model; interference; Italy; *Necrodes littoralis*; Silphidae.

INTRODUCTION

The community of organisms involved in the decomposition process of a corpse is typically composed of invertebrates (especially arthropods), large scavengers (other vertebrates) and microbes (bacteria and fungi) (Mondor *et al*., 2012). Among arthropods found on corpses, there are necrophagous, predator, parasite, and parasitoid species (Byrd & Castner, 2001; Rivers, 2016). Among them, insects are the most represented, and Diptera and Coleoptera the most frequent orders (Byrd & Castner, 2001; Mondor *et al*., 2012; Tuccia *et al*., 2018). In sarcosaprophagous communities, Diptera are usually represented by the families Calliphoridae, Sarcophagidae and Muscidae, and Coleoptera by the families Dermestidae, Silphidae, Histeridae, Cleridae, Nitidulidae and Staphylinidae (Goff & Catts, 1990; Byrd & Castner, 2001; Mondor *et al*., 2012). Studies on sarcosaprophagous arthropods are often performed on animal models to obtain data on community succession, behaviour and biology of the species colonizing a corpse in different situations. Many investigations with animal models aim to relate the insect life cycle to stages of decomposition, habitat and conditions of the corpse, and to the season and the presence of other species. Other studies may provide data on insect successional patterns in relation to decay stage of the animal model in specific conditions. All these data are relevant to provide schemes useful for forensic investigations when the main purpose is to estimate the Post Mortem Interval (PMI) (Tomberlin *et al*., 2012; Matuszewski *et al*., 2020). During an investigation on a sarcosaprophagous insect community in an animal model, *Sus scrofa* Linnaeus, 1758 (Artiodactyla: Suidae) conducted in Calabria (Southern Italy), a massive presence of *Necrodes littoralis* (Linnaeus, 1758) (Coleoptera: Silphidae) was detected. The peculiarity of the finding of *N. littoralis* was completed by a detailed description of the visiting, breeding and association with other species found on the carcass, providing interesting information about the role of this species in forensic investigations.

MATERIALS AND METHODS

The experimental study was conducted from 21 February to 28 May 2018 in a rural area near the Botanical Garden of the University of Calabria, Arcavacata di Rende (Cosenza, Italy) (39° 21’ 35.11” N 16° 13’ 52.73” E). The area is characterized by a wild meadow with few trees of *Quercus pubescens* Willd., 1805 (Fagales: Fagaceae), *Olea europaea* L., 1753 (Scrophulariales: Oleaceae) and *Populus alba* L., 1753 (Salicales: Salicaceae). A carcass of *Sus scrofa* Linnaeus, 1758 (Artiodactyla: Suidae) female, weighing about 130 kg, was purchased at a farm adjacent to the experimental site.

The sow was killed at the local slaughterhouse under veterinary control, according to the Regulation (EU) No. 1099/2009 on the protection of animals at the time of killing and Regulation (EU) No. 2017/625 on animal health and welfare.

About 1 hour after death, the carcass was placed on the right side in a sunny area on a wild meadow and was covered with a metal mesh to avoid interference from vertebrate scavengers.

The experimental procedure followed a previous method for forensic entomology studies (Amendt *et al*., 2007). The temperature and humidity data were recorded hourly by a data logger Escort Junior 10C16 (Escort, Washington, USA), placed at about 1 m from the carcass. The rain data were provided by the ARPACAL (Agenzia Regionale per la Protezione dell'Ambiente della Calabria - Centro Funzionale Multirischi) web site (https://www.cfd.calabria.it). The distance between the site of the experiment and the Cosenza 118 (Code 1017) weather station was 7.5 km.

The presence of insects on the carcass was checked every day from 9 am to 3 pm, carefully inspecting not only the carcass but also the ground to a distance of about 10 m in every direction. During the daily inspections, the decomposition stages were recorded until the end of the study. Insects were hand collected by entomological nets and tweezers and their activity was documented by photographs and video-recording using a Nikon D3100 (Nikon, Phra Nakhon Si Ayutthaya, Thailand). Adults of Coleoptera and Diptera were painlessly killed in a killing jar saturated with CO_2 and then preserved in 60% ethanol, and taxonomically identified at the maximum level using suitable keys (Porta, 1926; Porta, 1929; Szpila, 2012; Rochefort *et al*., 2015; Gregor *et al*., 2016; http://coleonet.de/coleo/index.htm; https://sites.google.com/view/mikes-insect-keys/mikes-insect-keys). Insect larvae found on the carcass were hand collected by tweezers and reared in the laboratory. The Diptera larvae were reared at 25°C, photoperiod L:D 12:12 and relative humidity 60-70%, in plastic boxes with 100 g of minced bovine liver until the pupal stage. The puparia were then transferred in boxes with sand until emerging of adults. The emerged adults were preserved in 60% ethanol and identified using the previously mentioned taxonomical keys (Szpila, 2012; Rochefort *et al*., 2015; Gregor *et al*., 2016). The larvae of Dermestidae were collected together with small pieces of the skin carcass, and reared on the same pieces in laboratory, in plastic boxes at 25°C, photoperiod L:D 12:12 and relative humidity 60-70%. The emerged adults were preserved in 60% ethanol and identified using suitable keys (Porta, 1929; http://coleonet.de/coleo/index.htm; https://sites.google.com/view/mikes-insect-keys/mikes-insect-keys). The larvae of Silphidae were reared in laboratory at 25°C, photoperiod L:D 12:12 and relative humidity 60-70%, in plastic boxes containing a layer of moist soil and 200 g of pork meat until pupation. At this stage, the food source was removed and the pupae reared until the adult stage in boxes with moisten soil. The adults of Silphidae were identified using taxonomical keys (Porta, 1929; http://coleonet.de/coleo/index.htm).

RESULTS

The air temperature, humidity and total rainfall were recorded all along the experimental period from 21 February to 28 May 2018. The daily air temperature (mean, maximum and minimum) is shown in Figure 1. Along the experimental period, the temperature ranged from a minimum of 3.4°C to a maximum of 34.3°C. The daily humidity, shown in Figure 2, ranged from a minimum of 41% to a maximum of 92%. The daily rainfall during the experimental period is shown in Figure 3. The total rainfall from 21 February to 28 May 2018 was 303.6 mm. The complete decay of the pig carcass lasted 98 days and five decomposition stages were observed: fresh (1 day), bloated (13 days), decay (32 days) and advanced decay (44 days) and dry (8 days).

A total of 7113 insects were collected, belonging to 10 families. During the fresh stage, the dipteran species visiting the carcass and collected were *Calliphora vicina* Robineau-Desvoidy, 1830, *Calliphora vomitoria* (L., 1758) (Diptera: Calliphoridae), *Hydrotaea dentipes* (Fabricius, 1805) (Diptera: Muscidae), *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae) and *Musca domestica* L., 1758 (Diptera: Muscidae) (Figure 4). During the bloated stage, adults belonging to

Figure 1. Daily air temperature (T) expressed in °C, recorded hourly by a data logger Escort Junior 10C16 on site during the experimental period from 21 February to 28 May 2018. T. mean, mean temperature; T. max, maximum temperature; T. min, minimum temperature.

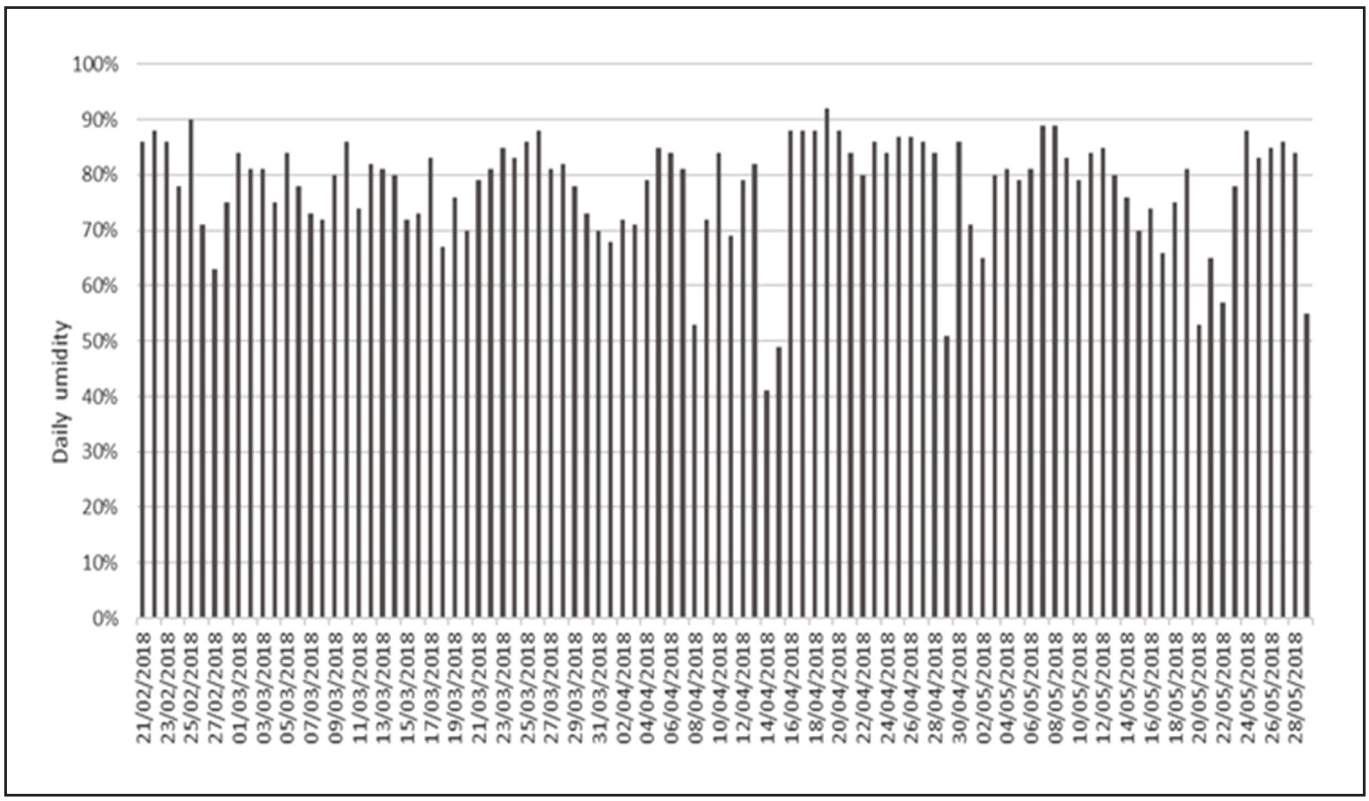

Figure 2. Daily mean humidity (%) recorded by the data logger Escort Junior 10C16 on site during the experimental period from 21 February to 28 May 2018.

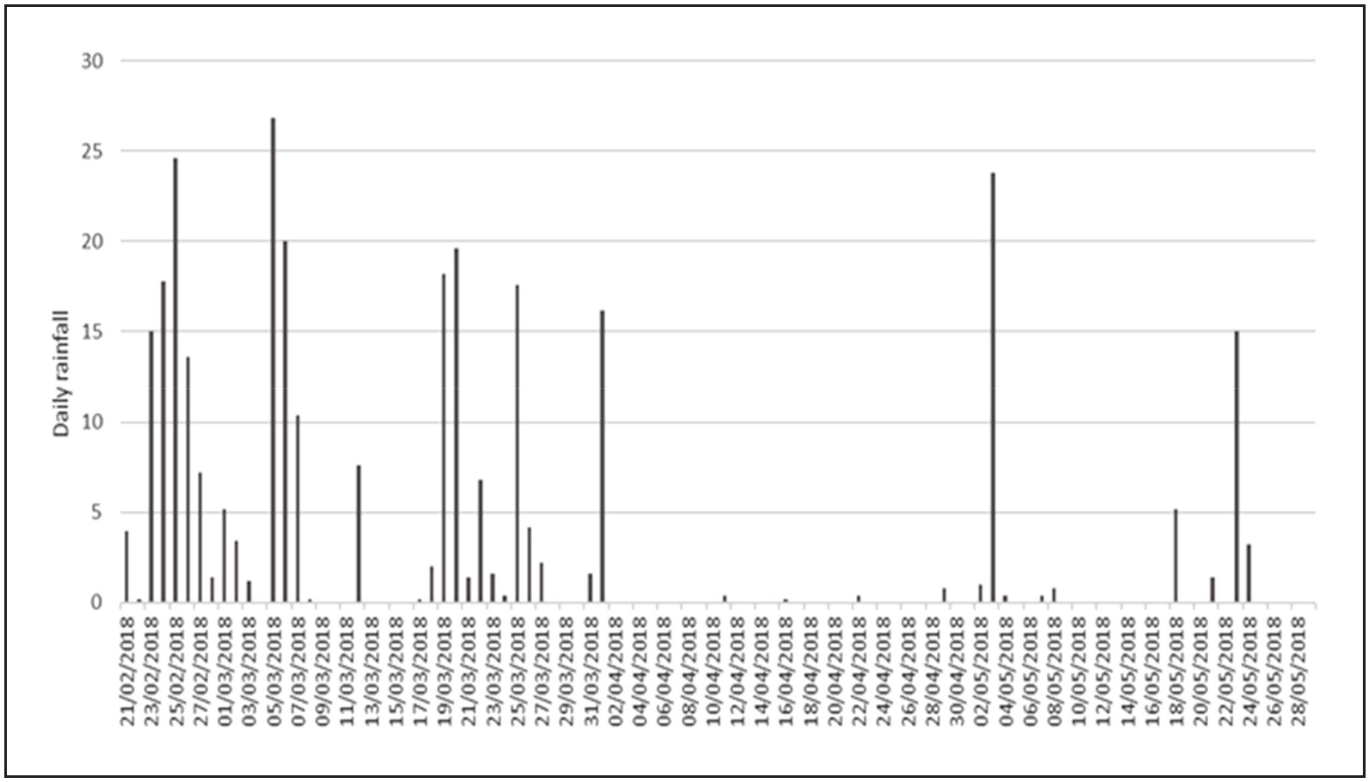

Figure 3. Daily rainfall (mm) recorded on site during the experimental period from 21 February to 28 May 2018. Data provided by ARPACAL (https://www.cfd.calabria.it) referring to station Cosenza 118 (code 1017).

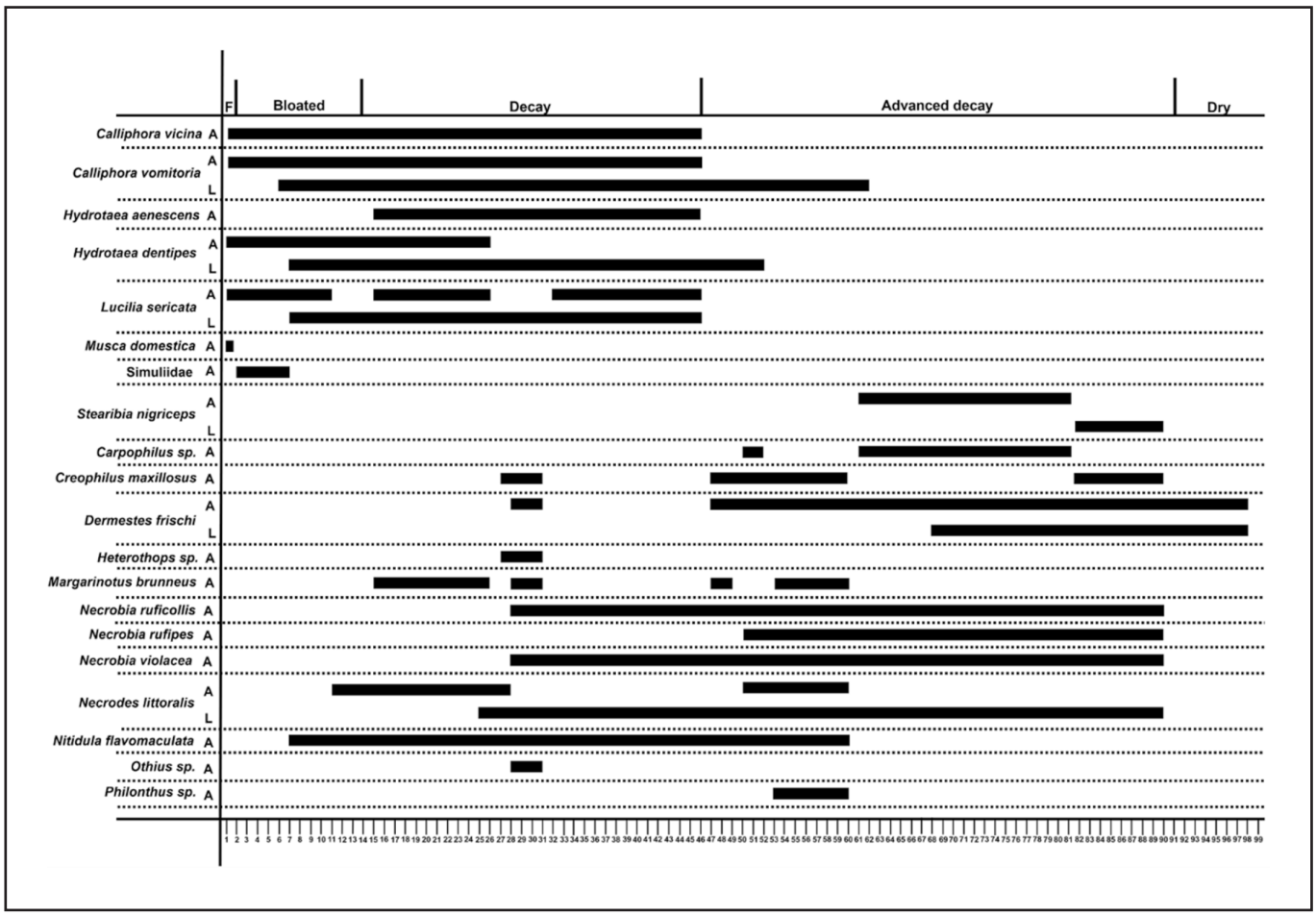

Figure 4. Presence (dark bars) of adults (A) and larvae (L) belonging to taxa of Diptera and Coleoptera during all the experimental period days. F, fresh stage.

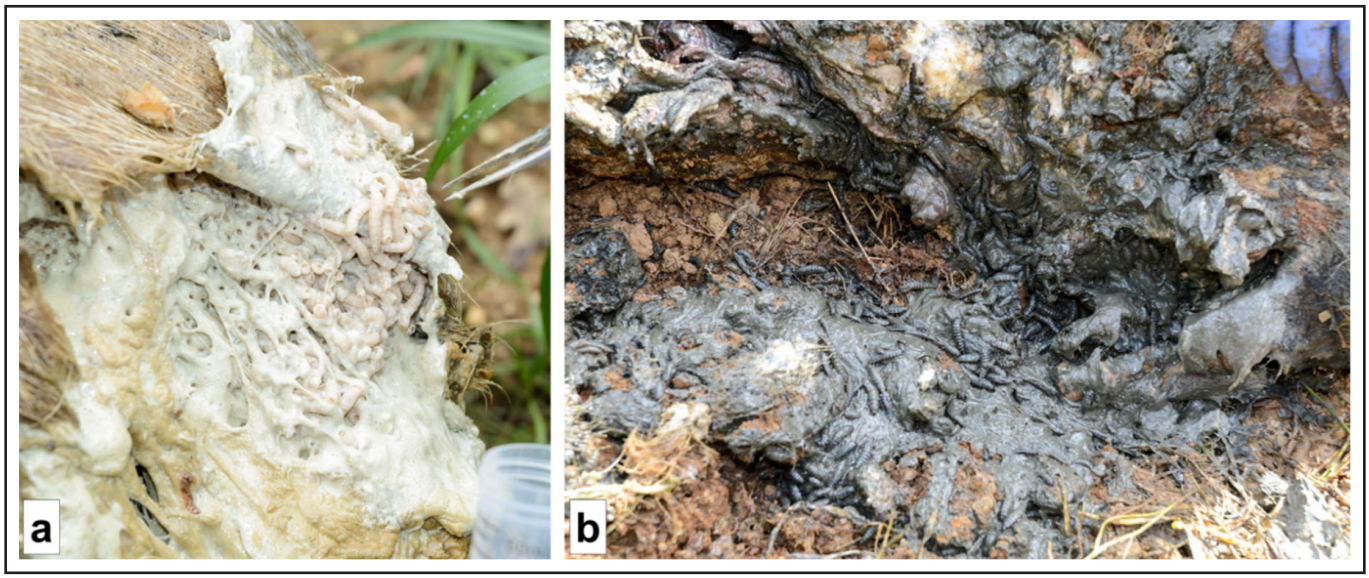

Figure 5. Massive presence of larvae during the decay stages of the experimental carcass of a pig, *Sus scrofa*. **(a)** Larvae of Diptera. **(b)** Larvae of Coleoptera, mostly belonging to the species *Necrodes littoralis*.

orders Diptera and Coleoptera were collected: the Diptera included *C. vicina*, *C. vomitoria*, *H. dentipes*, *L. sericata* and Simuliidae and the Coleoptera included *N. littoralis* and *Nitidula flavomaculata* Rossi, 1790 (Nitidulidae). During the decay stage, few Diptera adults of *C. vicina*, *C. vomitoria*, *Hydrotaea aenescens* (Wiedemann, 1830) (Muscidae), *H. dentipes* and *L. sericata* were collected. Concerning Coleoptera, during this stage six families were observed visiting the pig carcass and were collected: Cleridae, Dermestidae, Histeridae, Nitidulidae, Silphidae and Staphylinidae. The taxa detected were *Necrobia ruficollis* (Fabricius, 1775) and *Necrobia violacea* Linnaeus, 1758 (Cleridae); *Dermestes frischi* Kugelann, 1792 (Dermestidae); *Margarinotus brunneus* (Fabricius, 1775) (Histeridae); *Ni. flavomaculata* (Nitidulidae); *N. littoralis* (Silphidae); *Creophilus maxillosus* (L., 1758), *Heterothops* sp. Stephens, 1829 and *Othius* sp. Stephens, 1829 (Staphylinidae) (Figure 4). During the advanced decay stage, the only adults of Diptera collected were those of *Stearibia nigriceps* (Meigen, 1826) (Diptera: Piophilidae). Among Coleoptera, the adults collected were *Carpophilus* sp. Stephens, 1830 (Nitidulidae), *Cr. maxillosus*, *D. frischi*, *Ma. brunneus*, *Ni. flavomaculata*, *N. littoralis*, *Ne. ruficollis*, *Necrobia rufipes* (Fabricius, 1781) (Cleridae), *Ne. violacea*, and *Philonthus* sp. Stephens, 1829 (Staphylinidae). During the dry stage, many adults of *D. frischi* were collected. The first oviposition by Diptera occurred since the first day of carcass positioning. A great number of larvae (about 4000) of *C. vomitoria* were observed and collected from bloated to advanced decay stage (Figure 4 and 5a). High numbers of larvae of *S. nigriceps* were also collected at the end of the advanced decay stage. A few larvae of *H. dentipes* were also collected from bloated to advanced decay stage and a few of *L. sericata* in the bloated and decay stages.

Concerning Coleoptera, larvae of *N. littoralis* appeared during the decay stage, progressively increased during the advanced decay stage and sharply declined at the end of this stage (Figure 4 and 5b). The larvae of *D. frischi* appeared in the second half of the advanced decay stage and reached high numbers during the dry stage. Based on the immature stages collected, six species were found breeding on the carcass: *C. vomitoria*, *H. dentipes*, *L. sericata*, *S. nigriceps*, *D. frischi* and *N. littoralis* (Figure 4). After the appearance of larvae of *N. littoralis* on the carcass, no more larvae, pupae or empty puparia of Diptera were collected under or nearby the carcass, although necrotic tissues suitable for development of Calliphoridae larvae were still present. The ground around the carcass was checked on the surface and at the maximum depth of 15 cm for Diptera pupae and only about 20 of them were found.

DISCUSSION

Climatic factors, environment and corpse exposure are known to affect insect communities and successional patterns of forensic interest in different geographical areas, based on experimental investigations on animal models (Eberhardt & Elliot, 2008; Michaud & Moreau, 2009; Matuszewski, 2011; Al-Mekhlafi *et al*., 2020; Bonacci *et al*., 2021a). However, a relevant issue concerns species that compete or prey on populations of the first colonizers of the corpse. These species cause a disruption in successions of dipteran species of forensic interest and interfere with a correct estimation of PMI (Grassberger *et al*., 2003; Bonacci *et al*., 2011a; Bonacci *et al*., 2011b; Bonacci & Vercillo, 2015; Bonacci *et al*., 2019). Previous investigations reported that the delayed arrival of Silphidae species on corpses and their predatory activity on dipteran larvae could affect the succession of other insects (Bonacci *et al*., 2011a; Charabidze *et al*., 2016), as known to occur for *Chrysomya albiceps* Wiedemann, 1819 (Diptera: Calliphoridae) (Grassberger *et al*., 2003). Mixed competition events between *Necrodes* beetles and blowflies (Calliphoridae) were recently described (Matuszewski & Mądra-Bielewicz, 2021).

Adults of *N. littoralis* have been recently reported in Italy on a human corpse found indoors in a suburban area of the city of Cosenza (Calabria, Southern Italy) (Bonacci *et al*., 2021b). The experimental data obtained in the present study show that this species can be collected on carcasses from March to May in the province of Cosenza. Adults of *N. littoralis* made their first appearance 11 days after positioning of the carcass (bloated stage) in association with Diptera and other Coleoptera, and the larvae were detected for the first time 25 days after carcass positioning, gradually increasing in number until the advanced decay stage. Simultaneous to the increase of silphid larvae, a sharp decrease in the number of larvae of *C. vomitoria*, *H. dentipes* and *L. sericata* was observed. Probably, the decrease of *C. vomitoria* larvae and the disappearance of those of *H. dentipes* and *L. sericata* were not due to their typical behaviour of leaving the carcass to pupate, since the ground around the carcass was checked for pupae and only a few of them were found. Consequently, during the decay stage there was a shift of the predominant species from *C. vomitoria* to *N. littoralis* because of the competitive and predatory activity of the latter species, disrupting the structure of local insect communities. These data should be considered for a correct estimation of PMI in corpses found in environments and seasons suitable for *N. littoralis*, such as in Calabria, and are supported by previous experimental observations on the relationship between *N. littoralis* colonization and temperature in forest environments of central Europe (Matuszewski, 2011).

Our results also provide additional data on biology, phenology and ecological preferences and behavioural pattern of *N. littoralis*. Previous olfactometric studies suggest that adults of *Nicrophorus vespillo* (Linnaeus, 1758) and *Nicrophorus vespilloides* Herbst, 1783 (Coleoptera: Silphidae) may be attracted to carcasses by sulphur containing compounds, probably released during the decay process (Kalinová *et al*., 2009). Other signal molecules, probably related to the decay of large carcasses in specific conditions, could be involved in the aggregation of both adults and larvae of *N. littoralis*.

In this experimental investigation, *N. littoralis* was the only silphid collected on the carcass. Previous investigations on pig carcasses in the same region but in the winter season reported the activity of two other silphid species, *Thanatophilus rugosus* (Linnaeus, 1758) and *Thanatophilus sinuatus* (Fabricius, 1775) (Coleoptera: Silphidae), which interfered with the initial community of dipteran larvae in natural openings of the experimental carcasses (Bonacci *et al*., 2011a). The two species extended their colonization, becoming the predominant taxa until the dry stage.

Based on the results of this study, *N. littoralis* reached the carcass during the bloated stage and the intense predatory activity of adults and larvae on dipteran larvae was confirmed, together with the necrophagous habits on the decaying remains. The adults of *N. littoralis* use larger carcasses as trophic resource for breeding and larval development (Ratcliffe, 1996).

In conclusion, experimental studies on animal models may be relevant to identify complex relationships among different species that may affect insect communities of forensic interest, providing useful information for an appropriate crime scene analysis and a correct estimation of PMI by forensic investigators.

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Conflicts of interest statement

The author declares that they have no conflict of interests.

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