Nauplius

The Journal of The Brazilian Crustacean Society

e-ISSN 2358-2936
www.scielo.br/nau
www.crustacea.org.br

External marking of terrestrial isopods (Isopoda, Oniscidea): efficiency of materials and influence on feeding behavior

Diego Costa Kenne[1,2,3] orcid.org/0000-0003-1530-2637

Paula Beatriz Araujo[2] orcid.org/0000-0002-7587-3936

Geraldo Luiz Gonçalves Soares[1,3] orcid.org/0000-0002-8976-1935

1 Programa de Pós-graduação em Ecologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, Porto Alegre, RS, Brazil.
DCK E-mail: diegokenne@gmail.com

2 Laboratório de Carcinologia, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, Porto Alegre, RS, Brazil.
PBA E-mail: pabearaujo@gmail.com

3 Laboratório de Ecologia Química e Quimiotaxonomia, Departamento de Botânica, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, Porto Alegre, RS, Brazil.
GLGS E-mail: glgsoares@gmail.com

ZOOBANK http://zoobank.org/urn:lsid:zoobank.org:pub:CAA6F33A-8124-46DA-A401-B6BD1E9D838A

Abstract

Many monitoring studies use marking techniques to obtain dispersal data on individuals. Marking procedures may influence the vital activities of the terrestrial isopods, such as feeding behavior. The objective of this study was to identify the efficiency of different materials for external marking of woodlice and the influence on their feeding performance. Cyanoacrylate glue + glitter and nail polish were used as separate marking materials on three species: *Balloniscus glaber*, *Benthana picta* and *Armadillidium vulgare*. The material efficiency was compared using Kaplan-Meier curves and Log-Rank analysis. Individuals were fed with leaf discs of *Machaerium stipitatum* for feeding performance trials, and consumption rates were compared using ANOVA ($\alpha<0.05$). There was no difference in durability between the materials used for external markings, except between marked and unmarked *Benthana* specimens when the observation period exceeded 15 days (Log-Rank=8.446, $p=0.015$). Nail polish was considered more suitable for feeding experiments. Statistical differences in consumption rates were observed only between the marked individual treatments of *Benthana* (ANOVA, $F=7.5440$, $p=0.0002$). These results indicate that external marking does not affect the feeding habits of the animals and suggest that this technique can be used to monitor dispersal of terrestrial isopods in field conditions for approximately 15 days.

Keywords

Woodlice, consumption rates, material efficiency, Kaplan-Meier curve, nail polish

CORRESPONDING AUTHOR
Diego Costa Kenne
diegokenne@gmail.com

SUBMITTED 27 June 2019
ACCEPTED 30 September 2019
PUBLISHED 02 December 2019

DOI 10.1590/2358-2936e2019018

Introduction

Animal marking techniques are widely used in monitoring research, such as in density and population size studies, through the recognition of marked individuals in recapture events (Suzuki *et al.*, 2010; Drag *et al.*, 2011). This method is also used in animal dispersal observations, via periodic inspections of hotspots or by active searching for marked individuals in natural environments (Nazni *et al.*, 2005; Hamer *et al.*, 2014). Researchers can also employ marking technique for the evaluation of survivorship aspects and growth rate studies following the behavior of a specific animal by regular observations of this same specimen exposed to different conditions (Muir and Kay, 1998; Rose *et al.*, 2018). In biological control, species conservation and animal physiology experiments, this technique is also useful for differentiating individuals that have undergone an *a prior* process of distinct treatment (*i.e.*, exposure to volatiles or drugs) and then they go through the observation process all together in the same area (*i.e.*, arenas or chambers) (Baguette *et al.*, 2000; Horton *et al.*, 2009; Groner *et al.*, 2018).

There are a considerable number of marking and recapture techniques for invertebrates and vertebrates, such as internal and external dyes, mutilation and amputation, radio-telemetry, fluorescent powder, water-based and oil-based paints and labels (Hagler and Jackson, 2001). According to Hagler and Jackson (2001) the durability of the mark is considered the main aspect of a technique for the material to be considered "efficient". Failure to attach the material to the cuticle surface of some arthropods may compromise long-term studies (*i.e.*, more than one intermoult period for crustaceans) (Walker and Wineriter, 1981; Frisch and Hobbs, 2006; Hagler and Jackson, 2001). In addition, the mark can also be removed by the animal itself, due to chemical constituents of the cuticle (Lok *et al.*, 1975), abrasion with elements present in the environment (Tuf *et al.*, 2013), grooming behavior (Wojcik *et al.*, 2000), or even by simple loss through the molting process (Willows, 1987).

Depending on the goal to be achieved, these methods can show both positive and negative points. Sometimes though the researchers fail to describe or analyze the influence of marking techniques on animal behavior (Hagler and Jackson, 2001; De Souza *et al.*, 2012; Drahokoupilová and Tuf, 2012). For vertebrates, for example, toe clipping may compromise the animal's locomotor ability, as well as their foraging behavior (Schmidt and Schwarzkopf, 2010; Petit *et al.*, 2012). The use of colored materials in invertebrates can increase their visibility to predators and, consequently, their survivorship rate after the marking procedure (Janks and Barker, 2013). In addition, excessive marking can alter the individuals' vital activities and it can imply misrepresentation of the data and distort the analyses (Pardo *et al.*, 1996; Döge *et al.*, 2009; Lucía *et al.*, 2018). However, in consonance with Southwood and Henderson (2000), the animals' survivorship and behavior should not be affected by the marking process for the technique to be considered plausible and reliable.

Most animal tracking studies aim at home range mapping and to infer migration routes of vertebrates (Luschi, 2013; Gnanadesikan *et al.*, 2017; Child *et al.*, 2019). They usually discuss patterns of locomotion of predators and other organisms at the top of the food web (Phillips *et al.*, 2004; Morgan *et al.*, 2009; Pérez *et al.*, 2018). Generally, this information is linked with dispersion data about their prey, since these prey animals (smaller vertebrates and invertebrates) act as movement motivators of predators (Austin *et al.*, 2004; Block *et al.*, 2011; Gnanadesikan *et al.*, 2017). Some of these prey participate in the first trophic levels of many food chains, serving as a link between producer organisms and primary consumers (Bridgeland *et al.*, 2010; Wu *et al.*, 2014), like woodlice.

Woodlice are among the terrestrial arthropods with special notoriety, due to their mechanical fragmentation activity in the leaf litter and soil organic matter (Zimmer, 2006; Frouz *et al.*, 2008; Wood *et al.*, 2012). They therefore play an important role, with other detritivores, since they work together in soil nutrient cycles (Caseiro *et al.*, 2000; Quadros and Araujo, 2008). Observing terrestrial isopod movement in natural environments is a difficult process because of their small body size and how quickly some species shelter in refuges with difficult access (Paoletti and Hassall, 1999; Hassall and Tuck, 2007). Oniscidean isopod species are classified into ecomorphological types according to their anti-predatory strategies, which reflect their morphology (*i.e.*, cuticle thickness, antennae and leg length), behavior and habitat use (Schmalfuss, 1984; Csonka *et al.*, 2013). The three most common surface-active ecomorphological groups are: (1) runners, which have long legs and antennae and move quickly; (2) rollers, which have the thickest cuticle and are able to roll up into a ball to protect their ventral parts, and (3) clingers, which have short and strong legs and they can press down firmly to the substratum (Schmalfuss, 1984, Hornung, 2011; Csonka *et al.*, 2013).

Although isopods are considered to have low dispersive capacity (Lemos de Castro, 1971), some authors understand that woodlice food selection is driven by direct locomotion to a food source (Zidar *et al.*, 2002; Gerlach *et al.*, 2014). Monitoring isopod dispersal on a local scale is of paramount importance for the understanding of the relationships between the detritivores and the fauna and/or flora (food source) associated with the colonization process and the edaphic community establishment (Purger *et al.*, 2007; Quadros, 2010).

Although some studies do not recommend the use of external marking on woodlice (Drahokoupilová and Tuf, 2012; Tuf *et al.*, 2013), there are still many questions regarding intra-moult period observations, mortality rates, behavioral differences between ecomorphological groups and durability of the marks in terrestrial isopods. Thus, there were two objectives of this present study utilizing a runner, a roller and a clinger isopod species: 1) to test materials for external marking; and 2) to observe possible influences of this process on the feeding performance of woodlice, since this is an essential behavior in an animal's life. We hypothesized that the most efficient material will be the one with the highest frequency of weekly-marked animals remaining at the end of the 28 day experiment. We expected distinct responses from each different ecomorphological group of isopods based on the thickness of their cuticles in the material efficiency tests. For example, rollers should be more resistant to both materials tested, runners should be negatively affected by them and clingers should show intermediate sensitivity. Additionally, we hypothesize that the best material will not interfere in the ability of the isopod to ingest food and consumptions rates will be similar for the three tested ecomorphological groups, as well as between the treatments (number of marks on each animal).

Material and Methods

Woodlice species

Three terrestrial isopods species from three distinct ecomorphological groups (*sensu* Schmalfuss, 1984) were used for both experiments: *Balloniscus glaber* Araujo and Zardo, 1995 (Balloniscidae), an endemic clinger species from the state of Rio Grande do Sul and Santa Catarina, Brazil, associated with Atlantic Forest fragments (Quadros *et al.*, 2009; Kenne and Araujo, 2015; Wood *et al.*, 2017; Campos-Filho *et al.*, 2018); *Benthana picta* (Brandt, 1833) (Philosciidae), a runner species from the state of Espírito Santo to Rio Grande do Sul in Brazil and also recorded in Paraguay (Campos-Filho *et al.*, 2015); and *Armadillidium vulgare* (Latreille, 1804) (Armadillidiidae), our roller model, a Mediterranean species with a global distribution and commonly associated with anthropogenic areas (Paoletti and Hassal, 1999). To avoid ambiguity regarding species names they will be referred to by their genus denomination.

The *Balloniscus* and *Armadillidium* specimens were collected from a secondary forest in Porto Alegre city, state of Rio Grande do Sul (RS), Brazil (30°12'30"S, 51°10'12"W); and the *Benthana* specimens were collected at the Henrique Luiz Roesler Park in the municipality of Novo Hamburgo, RS, Brazil (29°41'15"S, 51°06'35"W). Additional specimens of *Armadillidium* were collected at 'Campus do Vale' at Universidade Federal do Rio Grande do Sul (UFRGS) (30°04'07"S, 51°07'10"W). The animals were taken to the 'Laboratório de Carcinologia' (UFRGS) and kept in a controlled temperature and photoperiod room (18 ± 1°C and 12:12 hr, respectively). The collection of the animals occurred several times, in order to supply enough specimens for the experiments. The largest body size woodlice were selected for both tests. After experiments, all specimens were returned to their original natural areas.

External marking materials

Two types of materials were used for the efficiency (durability) evaluation: cyanoacrylate glue (LOCTITE™ Super Bonder Power Flex Gel Control) mixed with silver glitter, and nail polish (Risqué™). These two materials were easy to handle and were chosen due to their superior adhesion to the animals' cuticle, as observed in previous pilot experiments.

Experiment 1: Material efficiency

For each isopod species we used five (*Armadillidium*, N = 150) or three (*Balloniscus*, N = 45; *Benthana*, N = 69) individuals per experimental unit (EU) (a circular plastic container, 8 cm in diameter and 4.5 cm in height) in order to maintain the minimum aggregation required for animal stability (Broly *et al.*, 2012). Each EU contained a shelter (a piece of 1 × 2 cm folded polypropylene paper), a moist cotton ball for internal moisture maintenance and leaf discs (1 cm in diameter) of *Machaerium stipitatum* (DC) Vogel (Fabaceae). This plant species is considered a good food source to feed to isopods (Quadros *et al.*, 2014).

All terrestrial isopods in two-thirds of the total number of EU's of each species were marked every seven days, one-third with nail polish and one-third with glue + glitter. The external marking procedure consisted of the application of a small drop of the material with a very small pointed brush, initially on the cephalothorax and later on the subsequent pereonites (Fig. 1). The remaining one third were considered the control treatment and the isopods were only handled and underwent the same marking process, but with distilled water. Before the animals were returned to the EU's after this step, they were left in a Petri dish (13cm in diameter) for approximately 5 minutes to dry the marking material on their cuticle. The observation period lasted 28 days.

Animals that showed any incidence of marking material inefficiency (*i.e.*, death or a non-durable marking event) were removed from the EU's. In these cases, the circumstance of death and the number of days the individuals had survived in the experiment were registered. The material durability was demonstrated through Kaplan-Meier curves and Log-Rank (Mantel-Cox) analysis ($\alpha<0.05$). The mortality rates were calculated weekly, observing the remaining number of animals in each event of marking.

Experiment 2: Influence on feeding performance

For the feeding activity test we selected the material which we observed the lowest mortality during the efficiency trials. New individuals of each species, who did not participate in Experiment 1, were fed with carrots in order to empty their digestive tract of any previous food source (Wood *et al.*, 2012). This is observed through the presence of orange fecal pellets in the EU. Intermoult animals of both sexes were used in the experiment, except for ovigerous females (Wood *et al.*, 2012). After this, each animal received either one, two, three, four or no (unmarked = control treatment) external marks and was placed individually in EU's (5 cm in diameter and 2.5 cm in height). Each EU contained three *M. stipitatum* leaf discs (1 cm in diameter) and a wet cotton ball.

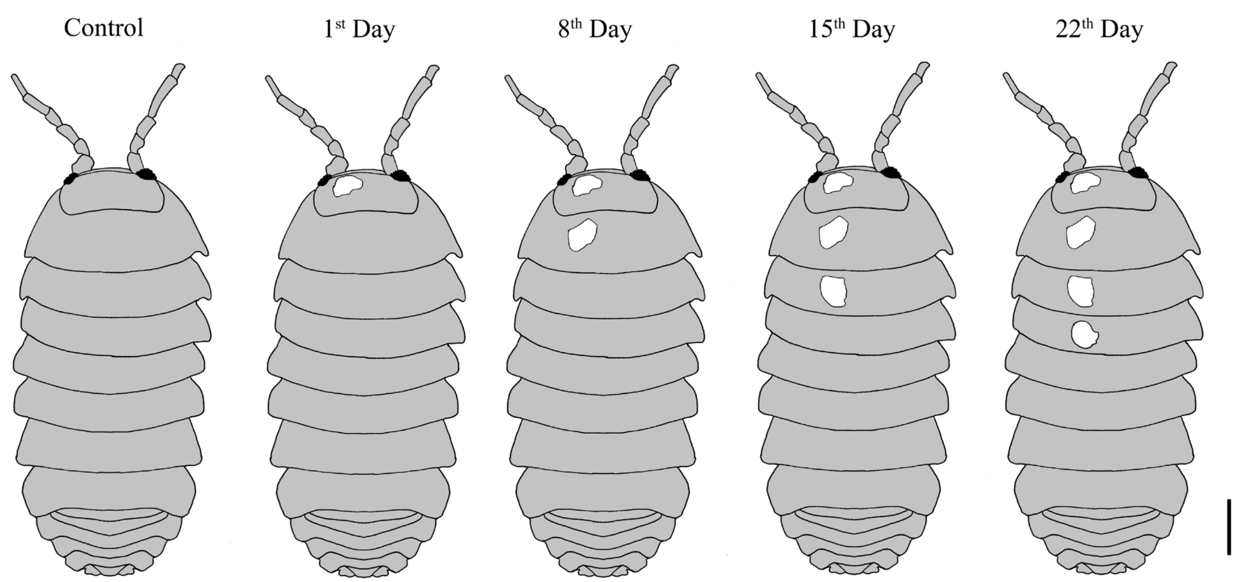

Figure 1. Woodlice (*Armadillidium vulgare*) external marking process during the 28 days of experiment.

All leaf discs were previously dried for 48 hours at 60°C, weighed (mg) and then re-hydrated before being offered to the animals (Boelter *et al.*, 2009).

The oniscidean isopods were weighed at the beginning, at every one or two day interval, and at the end of the experiment. For *Armadillidium* (50 individuals) and *Benthana* (47 individuals) the experiment lasted 10 days. For *Balloniscus* (32 individuals) it lasted seven days. The remaining plant material was dried and weighed at the end of the experiment. In order to obtain the autogenic mass loss percentage of the leaves, leaf disks were weighed from EU's without woodlice (Wood *et al.*, 2012). The relative consumption was calculated according to the adapted formula from Waldbauer (1968):

$$RC = [(M_{if} - M_{af}) - M_{ff}] / M_{isop} \times day$$

where (RC) is the Relative Consumption, (M_{if}) the initial food (three leaf discs) mass (mg), (M_{af}) the food autogenic mass loss (mg), (M_{ff}) the final food mass (mg) and (M_{isop}) the mean isopod mass (mg). Relative consumption was compared between treatments using ANOVA, and *post-hoc* Tukey test ($\alpha<0.05$).

Results

Experiment 1

The durability of the marks for the two materials used on the three oniscidean species showed no statistical difference, except for the occurrence of a significant higher frequency of remaining animals in the *Benthana* control group (Log-Rank: *Armadillidium* = 4.830, p = 0.089; *Balloniscus* = 3.706, p = 0.157; *Benthana* = 8.446, p = 0.015). At least until the 15th day of observation all species showed more than 50% survival of total individuals with the marks, and this fact indicates that the animals can tolerate three external marking events on a weekly frequency (Figs. 2–4).

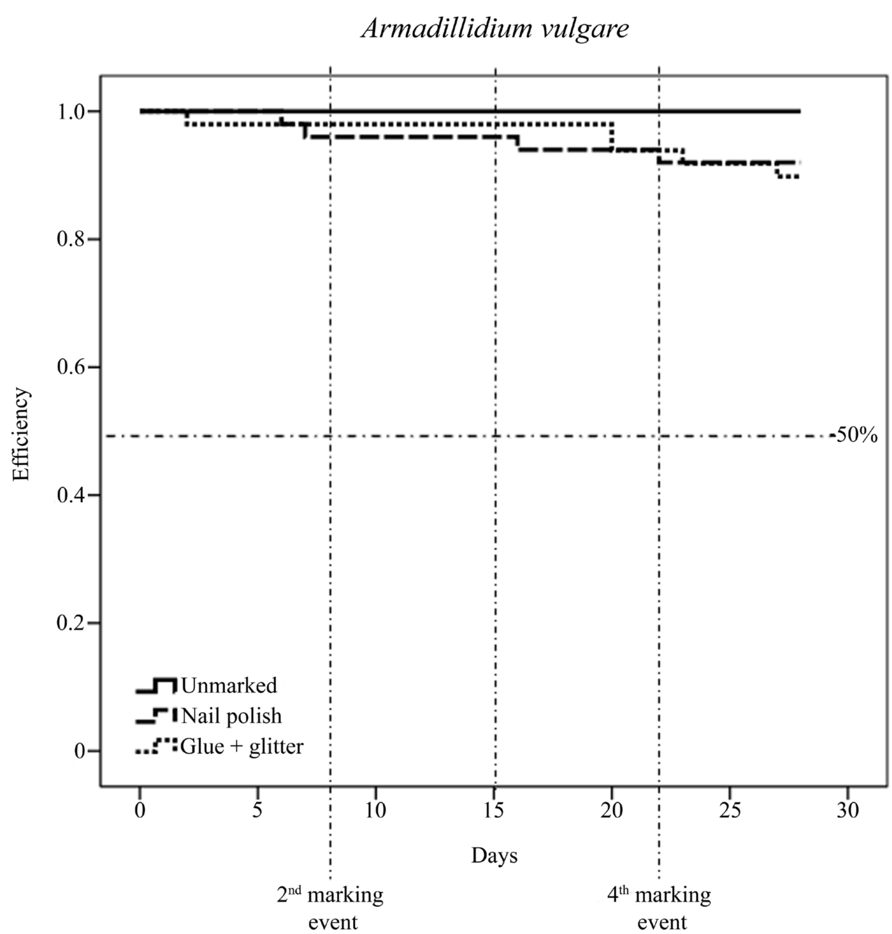

Figure 2. Kaplan-Meier curves for the external marking efficiency (durability) of the two materials tested on *Armadillidium vulgare* for 28 days of observation. Vertical dotted lines indicate the second, third and the forth event of external marking (8th, 15th and 22th days, respectively). Horizontal dotted line delimits the 50% of remaining marked individuals.

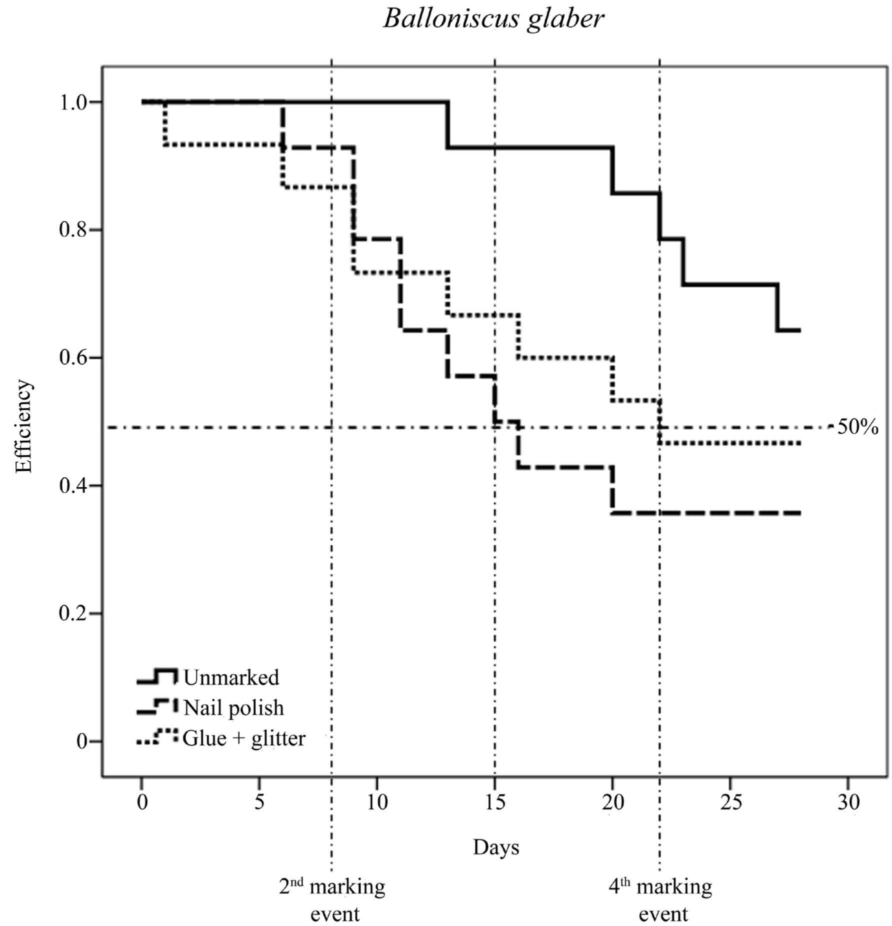

Figure 3. Kaplan-Meier curves for the external marking efficiency (durability) of the two materials tested on *Balloniscus glaber* for 28 days of observation. Vertical dotted lines indicate the second, third and the forth event of external marking (8th, 15th and 22th days, respectively). Horizontal dotted line delimits the 50% of remaining marked individuals.

Armadillidium showed the highest proportion of live animals bearing all marks reaching the 28th day, following by *Balloniscus* and then *Benthana* (Tab. 1).

The following events were observed during the experiment: lost mark (nail polish or glue+glitter drops in the EU), lost mark on exuvia (marks intact on old exoskeleton), natural death, death during ecdysis (dead woodlice partially attached to the old exoskeleton), cannibalism (dead individuals with no cephalothorax and/or pleon, probably consumed by the other animals in the same EU) and presumed drowning (dead animals trapped in the cotton ball). The drowning and cannibalism occurrences were not considered for efficiency comparison between materials. They most likely had no direct relation to the external marking process or with the material composition used in this process.

Armadillidium showed the lowest mortality rates in all treatments, regardless of the events of marking. Unexpectedly, unmarked *Balloniscus* exhibited a crescent mortality rate during the experiment, while the nail polish treatment fell to 0% mortality after the third event. The highest mortality rate was observed in *Benthana* for the last week of experiment (33.3%, nail polish treatment) (Fig. 5).

Although glue+glitter exhibited a higher durability but not significant for *Balloniscus* and indicated statistically different for *Benthana*, this material combination was the only one where we observed death incidents during the ecdysis process (Fig. 6). Thus, nail polish was elected for the feeding behavior experiments. Animal death during ecdysis process (a crucial arthropod event) indicates a greater risk for oniscidean survivorship and for the efficient use of these materials in field studies.

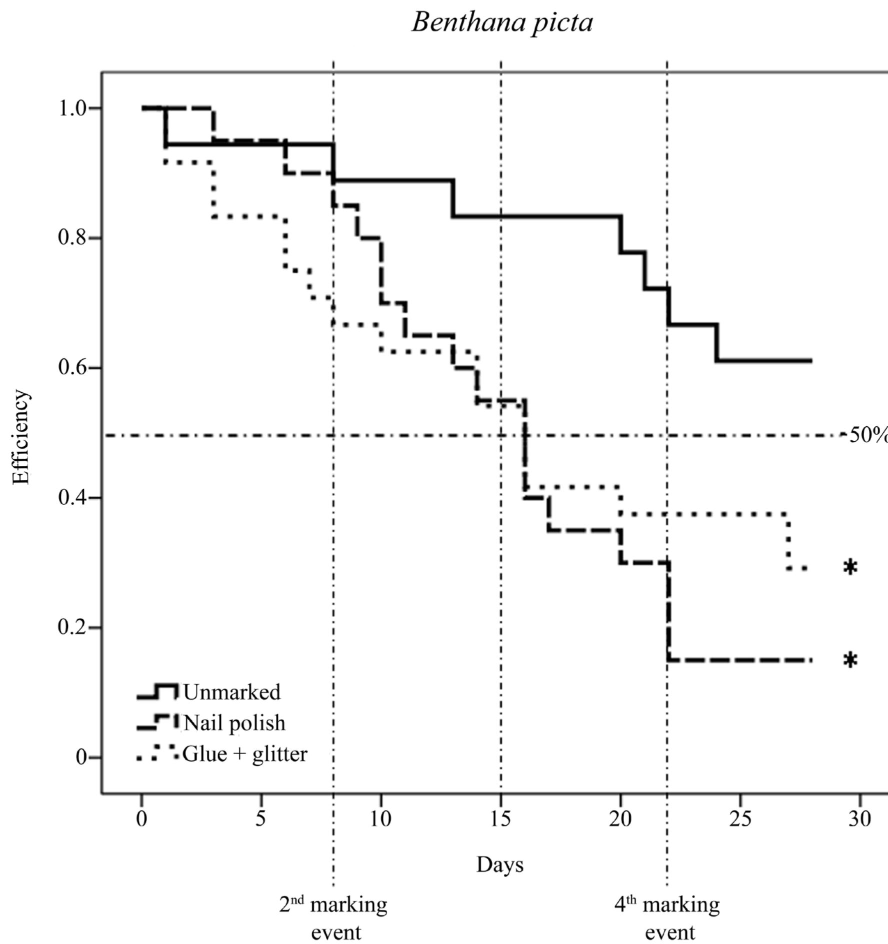

Figure 4. Kaplan-Meier curves for the external marking efficiency (durability) of the two materials tested on *Benthana picta* for 28 days of observation. Vertical dotted lines indicate the second, third and the forth event of external marking (8th, 15th and 22th days, respectively). Horizontal dotted line delimits the 50% of remaining marked individuals. * indicates statistical significance.

Table 1. Percentage of the remaining animals bearing all four marks in the end of 28 days of experiments.

Material	*Armadillidium*	*Balloniscus*	*Benthana*
Nail polish	92.0%	35.7%	15.0%
Glue + glitter	89.8%	46.7%	29.2%
Unmarked	100%	64.3%	61.1%
Total	93.9%	48.8%	33.9%

Experiment 2

The highest consumption of *M. stipitatum* leaves was observed in *Armadillidium* and *Balloniscus* control treatments (0.518 mg/mg*day and 0.816 mg/mg*day, respectively), and in the four-mark treatment for *Benthana* (1.015 mg/mg*day). There was no difference in the mean relative consumption of *M. stipitatum* leaf discs between treatments with marked and unmarked animals in the *Armadillidium* and *Balloniscus* experiments (ANOVA, $F_{Armadillidium} = 1.8496$, $p = 0.1351$; $F_{Balloniscus} = 0.8160$, $p = 0.5279$) (Figs. 7, 8). *Benthana*, on the other hand, showed a distinction in the mean relative consumption of the leaf discs, only in the four-marks treatment when compared between marked animal treatments (ANOVA, $F_{Benthana} = 7.5440$, $p = 0.0002$) (Fig. 9).

Discussion

Both materials used herein followed most basic premises of viable techniques for marking and recapture studies (Southwood and Henderson, 2000; Wang *et al.*, 2010). They are easy to apply and the materials have low cost. Even though glue+glitter has made the ecdysis

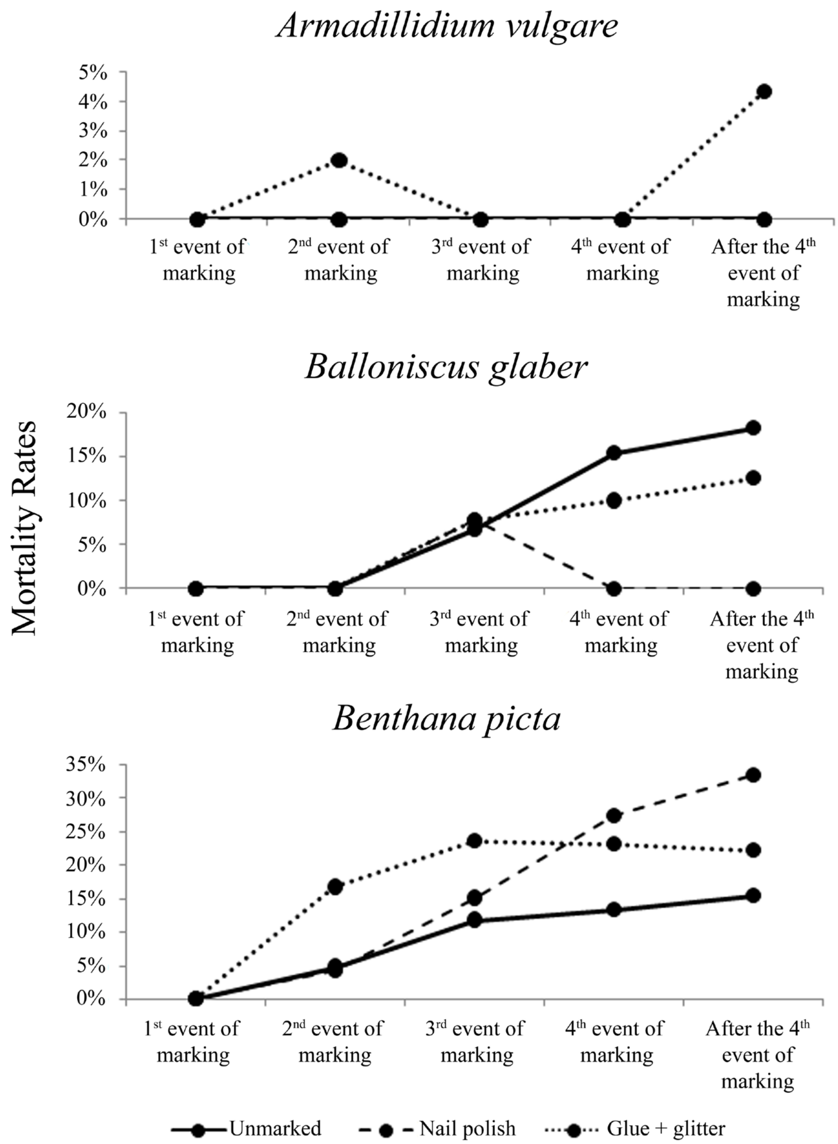

Figure 5. Mortality rates for the three woodlice species, unmarked and marked with nail polish and glue + glitter. The events of marking corresponding to the 1st, 8th, 15th, 22th and 28th day, respectively.

difficult, it showed no significant influence on the mortality rates for almost all isopods species tested in this work. Studies with aeglid crabs, beetles, stink bugs, *Limulus* and scorpions report the use of cyanoacrylate glue, and none of them have mentioned any effect on mortality rates (Bueno *et al.*, 2007; Backlund *et al.*, 2008; Lee *et al.*, 2013; Bibbs *et al.*, 2014).

Regarding the nail polish, it did not negatively interfere in the animals feeding activity. Its efficiency, durability and ease of application are also commented with freshwater crustaceans, spiders, beetles and stink bugs (Parmenter *et al.*, 1989; Dreyer and Baumgartner, 1997; Zambonato *et al.*, 2010; Ramalho *et al.*, 2010). Castillo and Kight (2005) used nail polish in prey-predation behavioral experiments with two terrestrial isopods species, and they do not report any external marking influence in relation to the parameters analyzed. Recently, Leclercq-Dransart *et al.* (2019) performed the same technique in physiology experiments with four woodlice species. They also did not describe any negative aspects about nail polish's external marks. However, Drahokoupilová and Tuf (2012) and Tuf *et al.* (2013) do not recommend nail polish for external marks on woodlice because their results indicated high mortality rates through the marking process. In addition, the authors also observed low nail polish durability. Our results showed there is little relation between death and the external marking procedure, since dead animals also

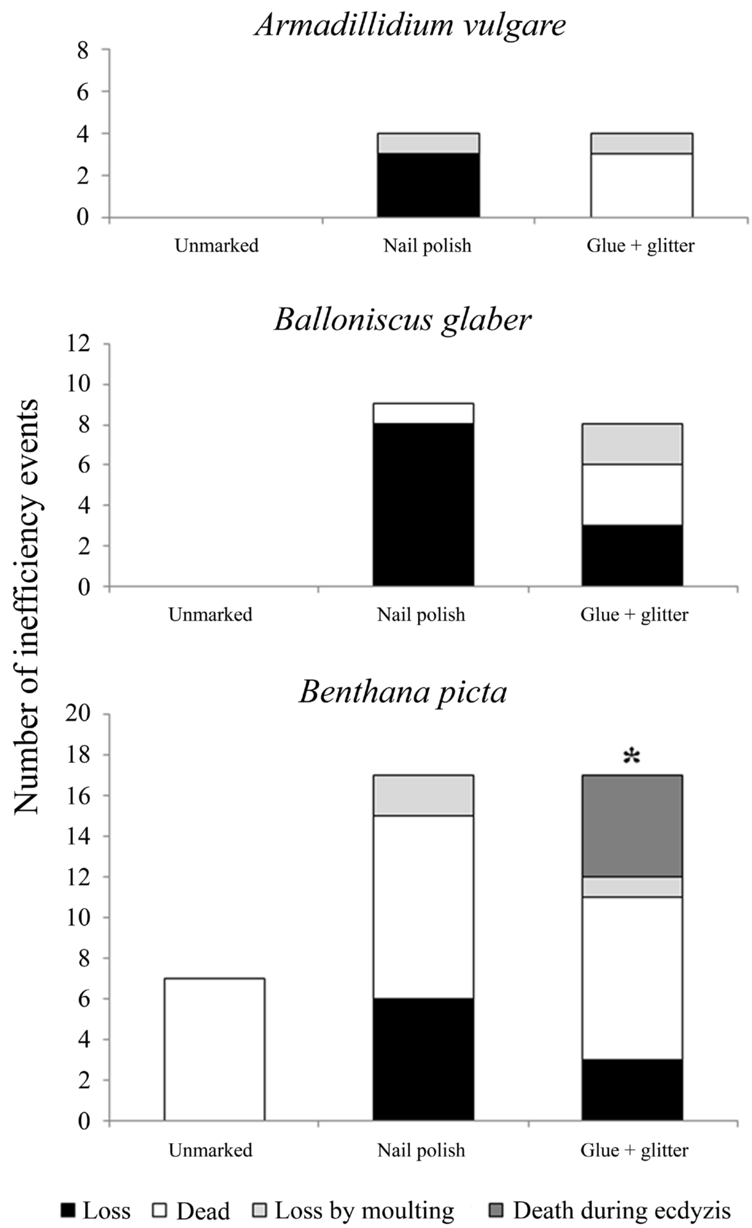

Figure 6. Number of inefficiency events during the 28 days experimental observation on three terrestrial isopods marked with glue + glitter, nail polish and the unmarked treatment. * indicates death event during ecdysis.

were observed in the unmarked treatments. Besides, we emphasized that nail polish did not affect ecdysis success and the woodlice survivorship during this process. Nail polish's durability observed in the present study was longer than cited by Drahokoupilová and Tuf (2012) (9 days) and some individuals reached 28 days with the external mark, as observed by Tuf *et al.* (2013).

According to our observations, direct marking on the woodlice cuticle may have influenced the woodlice survivorship and the mark's adhesion due to particular characteristics of each oniscidean ecomorphological group, regardless the material used. Unlike what is described for other terrestrial arthropods (Roer *et al.*, 2015), the woodlice cuticle thickness varies among different groups, and this characteristic provides to rollers, runners and clingers' species distinct permeability degrees (Greenaway and Warburg, 1998; Csonka *et al.*, 2013; Broly *et al.*, 2015; Wood *et al.*, 2017; Khemaissia *et al.*, 2018). *Armadillidium* showed low sensitivity to external marking and higher frequency of marked individuals until the end of the 28 days experiment. This can be explained as a result of their cuticle characteristics: thickness, low permeability and low water loss (Broly *et al.*, 2015; Nako *et al.*, 2018;

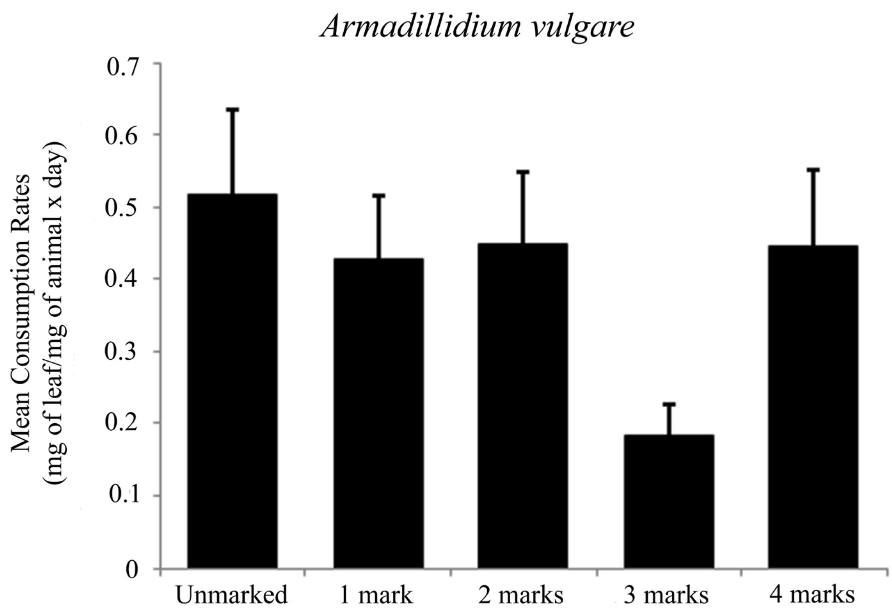

Figure 7. Mean relative consumption of *Armadillidium vulgare* fed with three leaf disks of *M. stipitatum* during 10 days of experiment. Each treatment corresponding to the number of nail polish external marks on animals' cuticle (zero – unmarked, one, two, three and four). Vertical bars indicate standard error.

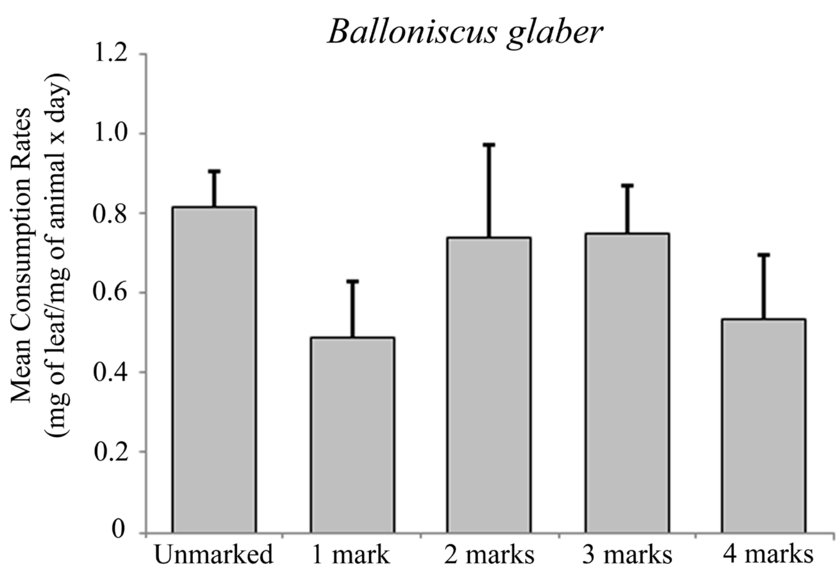

Figure 8. Mean relative consumption of *Balloniscus glaber* fed with three leaf disks of *M. stipitatum* during seven days of experiment. Each treatment corresponding to the number of nail polish external marks on animals' cuticle (zero – unmarked, one, two, three and four). Vertical bars indicate standard error.

Csonka *et al.*, 2018). *Armadillidium* can increase its resistance to desiccation in dry microhabitats or when exposed to high temperatures (Csonka *et al.*, 2018) and additionally, as we observed, to the possible nail polish toxicity.

The *Benthana* species has a delicate, lighter and flexible cuticle, which can be highly affected by fluctuation in temperature and humidity and less tolerance to environmental changes, as described for another runner species, *Atlantoscia floridana* (Van Name, 1940) (Araujo and Bond-Buckup, 2005; Sokolowicz and Araujo, 2013; Wood *et al.*, 2017, Csonka *et al.*, 2018). The high permeability and thinnest cuticle compared to the species we tested in

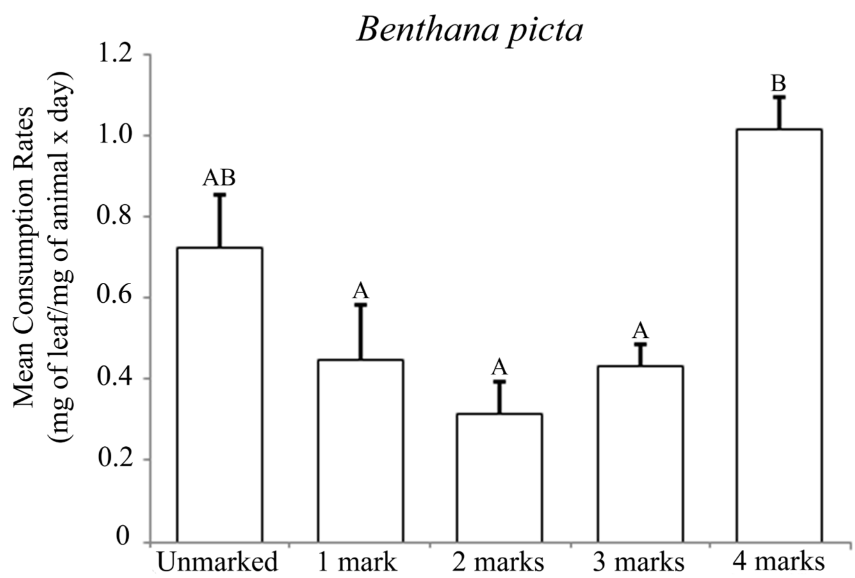

Figure 9. Mean relative consumption of *Benthana picta* fed with three leaf disks of *M. stipitatum* during 10 days of experiment. Each treatment corresponding to the number of nail polish external marks on animals' cuticle (zero – unmarked, one, two, three and four). Vertical bars indicate standard error. Different letters above the bars show statistical difference through analysis of variance (ANOVA) and *post-hoc* Tukey's test ($\alpha<0.05$).

the present study may explain the higher number of death events and higher frequency in cases of mark loss. Even though we observed individuals trapped in the old exoskeleton only when they were marked with glue+glitter, *Benthana* was the most fragile to both external marking materials.

As described by Wood *et al.* (2017), the *Balloniscus*' cuticle has lighter thickness in comparison to the *Armadillidium* one and it has many curved scales. In relation to runner species, clingers demand a tougher cuticle because the individuals need to adhere to the substrate to protect their soft parts from predators (Wood *et al.*, 2017). In the present study, material's external marking efficiency on *Balloniscus*' cuticle was intermediate when compared with the other two species. This result is supported by the clinger's intermediary cuticle attributes between rollers and runners discussed above.

Taking into consideration that the bigger the woodlouse the longer the intermoult interval (Lawlor, 1976), we selected the largest animals for both tests. This strategy was adopted to avoid the chances of the loss of the mark after ecdysis (Frisch and Hobbs, 2006; Bueno *et al.*, 2007; Zambonato *et al.*, 2010; Tuf *et al.*, 2013). Even attempting to minimize the effect in case of ecdysis, the information about the animal location, for example, may be not totally lost. The exuvia still maintains the last marking data, as we observed, and it can be found in the field if the animal monitoring studies use periodic trap observations. However, this will be that individual's last dispersion record. Thus we recommend the use of nail polish only on the biggest adult animals and implement the marking as soon as possible after the ecdysis. Intermoult period has a crucial role in this process.

There is little quantitative information about marking techniques' effects on feeding activity of invertebrates. In general, the studies discuss some qualitative data along with other behavioral approaches (Kobelt *et al.*, 2009; Drahokoupilová and Tuf, 2011, 2012; De Souza *et al.*, 2012; Wells and Sebens, 2017). A great number of studies that use marking techniques only report their main application for distinct treatments identification in experiments (Rieske and Raffa, 1990; Loreto *et al.*, 2009; Perry *et al.*, 2017; Leclercq-Dransart *et al.*, 2019). Bueno *et al.* (2007) observed marked and unmarked aeglid crabs in baited traps, which means the animals were attracted to food in spite of the external marking process. In a study with another detritivore, the millipede *Glomeris tetrasticha* Brandt, 1833, the authors observed unmarked animals feeding more often than the marked individuals (Drahokoupilová and Tuf, 2011). They also compared other behavioral parameters, and it was suggested this marking technique could provide biased or false results (Drahokoupilová and Tuf, 2011). Drahokoupilová and Tuf (2012) recorded a decrease in the feeding activity of marked terrestrial isopods compared to unmarked animals. Our data showed the marked and unmarked woodlice feeding similarly, quantitatively. Only four-marked *Benthana* exhibited higher mean relative consumption of *M. stipitatum* leaf discs than the other marked animal treatments. This probably indicates the three oniscidean species are not deprived of the ability to feed, since none of them showed significant statistical difference in comparison to control treatments (unmarked woodlice).

Considering our results, the external marking did not show negative influence on feeding activity of the terrestrial isopods and this method using nail polish can be effective for mark-recapture and monitoring studies in the field, taking into account the whole species intermoult period and other behavioral aspects. More studies about these topics are being conducted to enhance these techniques.

Acknowledgements

The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for granting scholarship to DCK, fellowship to PBA and GLGS, Instituto de Biociências of UFRGS for the assistance, colleagues from Laboratório de Carcinologia and Laboratório de Ecologia Química e Quimiotaxonomia (Leqtax) for the help on performing the experiments and Augusto F. Huber for editing the image of marked animals.

References

Araujo, P.B. and Bond-Buckup, G. 2005. Population structure and reproductive biology of *Atlantoscia floridana* (van Name, 1940) (Crustacea, Isopoda, Oniscidea) in southern Brazil. *Acta Oecologica*, 28: 289–298.

Austin, D.; Bowen, W.D. and McMillan, J.I. 2004. Intraspecific variation in movement patterns: modeling individual behaviour in a large marine predator. *Oikos*, 105: 15–30.

Backlund, D.C.; Marrone, G.M.; Williams, C.K. and Tilmon, K. 2008. Population estimate of the endangered American Burying Beetle, *Nicrophorus americanus* Olivier (Coleoptera: Silphidae) in South Dakota. *The Coleopterists Bulletin*, 62: 9–15.

Baguette, M.; Petit, S. and Queva, F. 2000. Population spatial structure and migration of three the same habitat butterfly species within network: consequences for conservation. *Journal of Applied Ecology*, 37: 100–108.

Bibbs, C.S.; Bengston, S.E. and Dawn, H.G. 2014. Exploration of refuge preference in the Arizona Bark Scorpion (Scorpiones: Buthidae). *Environmental Entomology*, 43: 1345–1353.

Boelter, J.F.; Quadros, A.F. and Araujo, P.B. 2009. The feeding rates and preferences of a Neotropical terrestrial isopod (Oniscidea). *Nauplius*, 17: 107–113.

Block, B.A.; Jonsen, I.D.; Jorgensen, S.J.; Winship, A.J.; Shaffer, S.A.; Bograd, S.J.; Hazen, E.L.; Foley, D.G.; Breed, G.A.; Harrison, A.L.; Ganong, J.E.; Swithenbank, A.; Castleton, M.; Dewar, H.; Mate, B.R.; Shillinger, G.L.; Schaefer, K.M.; Benson, S.R.; Weise, M.J.; Henry, R.W. and Costa, D.P. 2011. Tracking apex marine predator movements in a dynamic ocean. *Nature*, 475: 86–90.

Bridgeland, W.T.; Beier, P.; Kolb, T. and Whitham, T.G. 2010. A conditional trophic cascade: birds benefit faster growing trees with strong links between predators and plants. *Ecology*, 91: 73–84.

Broly, P.; Devigne, C. and Deneubourg, J. 2015. Body shape in terrestrial isopods: A morphological mechanism to resist desiccation? *Journal of Morphology*, 276: 1283–1289.

Broly, P.; Mullier, R.; Deneubourg, J. and Devigne, C. 2012. Aggregation in woodlice: social interaction and density effects. *ZooKeys*, 176: 133–144.

Bueno, S.L.S.; Shimizu, R.M. and Da Rocha, S.S. 2007. Estimating the population size of *Aegla franca* (Crustacea: Decapoda: Anomura) by mark-recapture technique from an isolated section of Barro Preto stream, county of Claraval, state of Minas Gerais, southeastern Brazil. *Journal of Crustacean Biology*, 27: 553–559.

Campos-Filho, I.S.; Cardoso, G.M. and Aguiar, J.O. 2018. Catalogue of terrestrial isopods (Crustacea, Isopoda, Oniscidea) from Brazil: an update with some considerations. *Nauplius*, 26: e2018038.

Campos-Filho, I.S.; Taiti, S. and Araujo, P.B. 2015. Taxonomic revision of the genus *Benthana* Budde-Lund, 1908 (Isopoda: Oniscidea: Philosciidae). *Zootaxa*, 4022: 1–73.

Caseiro, I.; Santos, S.; Sousa, J.P.; Nogueira, A.J.A. and Soares, A.M.V.M. 2000. Optimization of culture conditions of *Porcellio dilatatus* (Crustacea, Isopoda) for laboratory test development. *Ecotoxicology and Environmental Safety*, 47: 285–291.

Castillo, M.E. and Kight, S. 2005. Response of terrestrial isopods, *Armadillidium vulgare* and *Porcellio laevis* (Isopoda: Oniscidea) to the ant *Tetramorium caespitum*: Morphology, behavior and reproductive success. *Invertebrate Reproduction and Development*, 47: 183–190.

Child, M.F.; Selier, S.A.; Radloff, F.G.; Taylor, W.A.; Hoffmann, M.; Nel, L.; Power, R.J.; Birss, C.; Okes, N.C.; Peel, M.J.; Mallon, D. and Davies-Mostert, H. 2019. A framework to measure the wildness of managed large vertebrate populations. *Conservation Biology*, 33: 1106–1119.

Csonka, D.; Halasy, K.; Buczkó, K. and Hornung, E. 2018. Morphological traits – desiccation resistance – habitat characteristics: A possible key for distribution in woodlice (Isopoda, Oniscidea). *Zookeys*, 801: 481–499.

Csonka, D.; Halasy, K.; Szabó, P.; Mrak, P.; Štrus, J. and Hornung, E. 2013. Eco-morphological studies on pleopodal lungs and cuticle in *Armadillidium* species (Crustacea, Isopoda, Oniscidea). *Arthropod Structure and Development*, 42: 229–35.

De Souza, A.R.; Ribeiro, B.; José, N. and Prezoto, F. 2012. Paint marking social wasps: An evaluation of behavioral effects and toxicity. *Entomologia Experimentalis et Applicata*, 144: 244–247.

Döge, J.S.; Hochmüller, C.J.C.; Valente, V.L.S. and Tidon, R. 2009. Potential use of marker pen ink as a marking method for drosophilids. *Drosophila Information Service*, 92: 123–126.

Drag, L.; Hauck, D.; Pokluda, P.; Zimmermann, K. and Cizek, L. 2011. Demography and dispersal ability of a threatened Saproxylic beetle: A mark-recapture study of the Rosalia Longicorn (*Rosalia alpina*). *PLoS ONE*, 6: e21345.

Drahokoupilová, T. and Tuf, I.H. 2011. Behaviour of pill millipedes can be affected by external marking. *International Journal of Myriapodology*, 6: 51–60.

Drahokoupilová, T. and Tuf, I.H. 2012. The effect of external marking on the behaviour of the common pill woodlouse *Armadillidium vulgare*. *Zookeys*, 176, 145–154.

Dreyer, H. and Baumgartner, J. 1997. Adult movement and dynamics of Clavigralla tomentosicollis (Heteroptera: Coreidae) populations in Cowpea Fields of Benin, West Africa. *Journal of Economic Entomology*, 90: 421–426.

Frisch, A.J. and Hobbs, J.P.A. 2006. Long-term retention of internal elastomer tags in a wild population of painted crayfish (*Panulirus versicolor* [Latreille]) on the Great Barrier Reef. *Journal of Experimental Marine Biology and Ecology*, 339: 104–110.

Frouz, J.; Lobinske, R.; Kalcik, J. and Ali, A. 2008. Effects of the exotic Crustacean, *Armadillidium vulgare* (Isopoda), and other macrofauna on organic matter dynamics in soil microcosms in a hardwood Forest in Central Florida. *Florida Entomologist*, 91: 328–331.

Dreyer, H. and Baumgartner, J. 1997. Adult movement and dynamics of *Clavigralla tomentosicollis* (Heteroptera: Coreidae) populations in Cowpea Fields of Benin, West Africa. *Journal of Economic Entomology*, 90: 421–426.

Gerlach, A.; Russell, D.J.; Jaeschker, B. and Rombke, J. 2014. Feeding preferences of native terrestrial isopod species (Oniscidea, Isopoda) for native and introduced leaf litter. *Applied Soil Ecology*, 83: 95–100.

Gnanadesikan, G.E.; Pearse, W.D. and Shaw, A.K. 2017. Evolution of mammalian migrations for refuge, breeding, and food. *Ecology and Evolution*, 7: 5891–5900.

Greenaway, P. and Warburg, M.R. 1998. Water fluxes in terrestrial isopods. *Israel Journal of Entomology*. 44: 473–486.

Groner, M.L.; Shields, J.D.; Landers, D.F.; Swenarton, J. and Hoenig, J.M. 2018. Rising temperatures, molting phenology, and epizootic shell disease in the American Lobster. *The American Naturalist*, 192: E163–E177. doi:10.1086/699478.

Hagler, J.R. and Jackson, C.G. 2001. Methods for Marking Insects: Current techniques and future prospects. *Annual Review of Entomology*, 46: 511–543.

Hamer, G.L.; Anderson, T.K.; Donovan, D.J.; Brawn, J.D.; Krebs, B.L.; Gardner, A.M.; Ruiz, M.O.; Brown, W.M.; Kitron, U.D.; Newman, C.M.; Goldberg, T.L. and Walker, E.D. 2014. Dispersal of adult *Culex* mosquitoes in an urban West Nile virus hotspot: A mark-capture study incorporating stable isotope enrichment of natural larval habitats. *PLoS Neglected Tropical Diseases*, 8: 6–12.

Hassall, M. and Tuck, J.M. 2007. Sheltering behavior of terrestrial isopods in grasslands. *Invertebrate Biology*, 126: 46–56.

Hornung, E. 2011. Evolutionary adaptation of oniscidean isopods to terrestrial life: Structure, physiology and behavior. *Terrestrial Arthropod Reviews*, 4: 95–130.

Horton, D.R.; Jones, V.P. and Unruh, T.R. 2009. Use of a new immunomarking method to assess movement by generalist predators between a cover crop and tree canopy in a pear orchard. *American Entomologist*, 55: 49–56.

Janks, M.R. and Barker, N.P. 2013. Using mark-recapture to provide population census data for use in Red Listing of invertebrates: The rare terrestrial snail *Prestonella bowkeri* as a case study. *Biodiversity and Conservation*, 22: 1609–1621.

Janks, M.R. and Barker, N.P. 2013. Using mark-recapture to provide population census data for use in Red Listing of invertebrates: The rare terrestrial snail *Prestonella bowkeri* as a case study. *Biodiversity and Conservation*, 22: 1609–1621.

Kenne, D.C. and Araujo, P.B. 2015. *Balloniscus glaber* (Crustacea, Isopoda, Balloniscidae), a habitat specialist species in a disturbed area of Brazil. *Iheringia, Série Zoologia*, 105: 430–438.

Khemaissia, H.; Raimond, M.; Ayari, A.; Jelassi, R.; Souty-Grosset, C. and Nasri-Ammar, K. 2018. Cuticular differences of the exoskeleton relative to habitat preferences among three terrestrial isopods. *Biologia*, 73: 447–483.

Kobelt, A.J.; Yen, A.L. and Kitching, M. 2009. Laboratory validation of rubidium marking of herbivorous insects and their predators. *Australian Journal of Entomology*, 48: 204–209.

Lawlor, L.R. 1976. Molting, growth and reproductive strategies in the terrestrial isopod, *Armadillidium vulgare*. *Ecology*, 57: 1179–1194.

Leclercq-Dransart, J.; Pernin, C.; Demuynck, S.; Grumiaux, F.; Lemière, S. and Leprêtre, A. 2019. Isopod physiological and behavioral responses to wet and drier conditions: an experimental study with four species in the context of global warming. *European Journal of Soil Biology*, 90: 22–30.

Lee, D.; Wright, S.E.; Boiteau, G.; Vincent, C. and Leskey, T.C. 2013. Effectiveness of glues for harmonic radar tag attachment on *Halyomorpha halys* (Hemiptera: Pentatomidae) and their impact on adult survivorship and mobility. *Environmental Entomology*, 42: 515–523.

Lemos de Castro, A. 1971. Isópodos terrestres introduzidos no Brasil (Isopoda, Oniscoidea). *Boletim Museu Nacional Rio de Janeiro*, 282: 1–14.

Lok, J.B.; Cupp, E.W. and Blomquist, G.J. 1975. Cuticular lipids of the imported fire ants, *Solenopsis invicta* and *Richteri james*. *Insect Biochemestry*, 5: 821–829.

Loreto, R.G.; Desouza, O. and Elliot, S.L. 2009. Colored glue as a tool to mark termites (*Cornitermes cumulans*; Isoptera. Termitidae) for ecological and behavioral studies. *Sociobiology*, 54: 351–360.

Lucía, M.; Ricardo, P.A. and Stella, Z.M. 2018. Dispersión de adultos de *Piezodorus guildinii* (Hemiptera: Pentatomidae) entre cultivos de soja y de alfalfa. *Agrociencia Uruguay*, 22: 1–10.

Luschi, P. 2013. Long-distance animal migrations in the oceanic environment: orientation and navigation correlates. *ISRN Zoology*, Article ID 631839.

Morgan, S.A.; Hansen, C.M.; Ross, J.G.; Hickling, G.J.; Ogilvie, S.C.; Paterson, A.M.; 2009. Urban cat (*Felis catus*) movement and predation activity associated with a wetland reserve in New Zealand. *Wildlife Research*, 36, 574–580.

Muir, L.E. and Kay, B.H. 1998. *Aedes aegypti* survival and dispersal estimated by mark-release-recapture in northern Australia. *American Journal of Tropical Medicine and Hygiene*, 58: 277–282.

Nako, J.; Lee, N.S. and Wright, J.C. 2018. Water vapor absorption allows for volume expansion during molting in *Armadillidium vulgare* and *Porcellio dilatatus* (Crustacea, Isopoda, Oniscidea). *ZooKeys*, 801: 459–479.

Nazni, W.A.; Luke, H.; Wan Rozita, W.M.; Abdullah, A.G.; Sa'diyah, I.; Azahari, A.H.; Zamree, I.; Tan, S.B.; Lee, H.L. and Sofian, M.A. 2005. Determination of the flight range and dispersal of the house fly, *Musca domestica* (L.) using mark release recapture technique. *Tropical Biomedicine*, 22: 53–61.

Paoletti, M.G. and Hassall, M. 1999. Woodlice (Isopoda: Oniscidea): Their potential for assessing sustainability and use as bioindicators. *Agriculture, Ecosystems and Environment*, 74: 157–165.

Pardo, R.H.; Torres, M.; Morrison, A.C. and Ferro, C. 1996. Effect of fluorescent powder on *Lutzomyia longipalpis* (Diptera: Psychodidae) and a simple device for marking sand flies. *Journal of the American Mosquito Control Association*, 12: 235–242.

Parmenter, R.R.; Macmahon, J.A. and Anderson, D.R. 1989. Animal density estimation using a trapping web design: Field validation experiments. *Ecology*, 70: 169–179.

Perry, K.I.; Wallin, K.F.; Wenzel, J.W. and Herms, D.A. 2017. Characterizing movement of ground-dwelling arthropods with a novel mark-capture method using fluorescent powder. *Journal of Insect Behavior*, 30: 32–47.

Pérez, G.E.; Conte, A.; Garde, E.J.; Messori, S.; Vanderstichel, R. and Serpell, J. 2018. Movement and home range of home range of owned free-roaming male dogs in Puerto Natales. *Chile Applied Animal Behavior Science*, 205: 74–82.

Petit, S.; Waudby, H.P.; Walker, A.T.; Zanker, R. and Rau, G. 2012. A non-mutilating method for marking small wild mammals and reptiles. *Australian Journal of Zoology*, 60: 64–71.

Phillips, M.L.; Clark, W.R.; Nusser, S.M.; Sovada, M.A. and Greenwood, R.J. 2004. Analysis of predator movement in prairie landscapes with contrasting grassland composition. *Journal of Mammalogy*, 85: 187–195.

Purger, J.J.; Farkas, S. and Dányi, L. 2007. Colonization of post-mining recultivated area by terrestrial isopods (Isopoda: Oniscoidea) and centipedes (Chilopoda) in Hungary. *Applied Ecology and Environmental Research*, 5: 87–92.

Quadros, A.F; Zimmer, M.; Araujo, P.B. and Kray, J.G. 2014. Litter traits and palatability to detritivores: a case study across bio-geographical boundaries. *Nauplius*, 22: 103–111.

Quadros, A.F. and Araujo, P. B. 2008. An assemblage of terrestrial isopods (Crustacea) in southern Brazil and their contribution to leaf litter processing. *Revista brasileira de Zoologia*, 25: 58–66.

Quadros, A.F. 2010. Os isópodos terrestres são boas ferramentas para monitorar e restaurar áreas impactadas por metais pesados no Brasil? *Oecologia Australis*, 14: 569–583.

Quadros, A.F.; Caubet, Y. and Araujo, P.B. 2009. Life history comparison of two terrestrial isopods in relation to habitat specialization. *Acta Oecologica*, 35: 243–249.

Ramalho, R.O.; Mcclain, W.R. and Anastácio, P.M. 2010. An effective and simple method of temporarily marking crayfish. *Freshwater Crayfish*, 17: 57–60.

Rieske, L.K. and Raffa, K.F. 1990. Dispersal patterns and mark-and-recapture estimates of two Pine Root Weevil Species, *Hylobius pales* and *Pachylobius picivorus* (Coleoptera: Curculionidae) in Christmas Tree Plantations. *Environmental Entomology*, 19: 1829–1836.

Roer, R.; Abehsera, S. and Sagi, A. 2015. Exoskeletons across the pancrustacea: Comparative morphology, physiology, biochemistry and genetics. *Integrative and Comparative Biology*, 55: 771–791.

Rose, J.P.; Wylie, G.D.; Casazza, M.L. and Halstead, B.J. 2018. Integrating growth and capture-mark-recapture models reveals size-dependent survival in an elusive species. *Ecosphere*, 9: e02384.

Schmalfuss, H. 1984. Eco-morphological strategies in terrestrial isopods. *Symposia of the Zoological Society of London*, 53: 49–63.

Schmidt, K. and Schwarzkopf, L. 2010. Visible implant elastomer tagging and toe-clipping: effects of marking on locomotor performance of frogs and skinks. *Herpetological Journal*, 20: 99–105.

Sokolowicz, C.C. and Araujo, P.B. 2013. Reproductive pattern of the Neotropical terrestrial isopod *Benthana cairensis* (Isopoda: Philosciidae). *Journal of Crustacean Biology*, 33: 210–217.

Southwood, T.R.E. and Henderson, P.A. 2000. Ecological Methods (3rd edition). Oxford, Blackwell Science Ltd. 576p.

Suzuki, F.M.; Zambaldi, L.P. and Pompeu, P.S. 2010. Uso de marcação e recaptura para estimar a abundância e densidade de *Trichomycterus brasiliensis* (Siluriformes, Trichomycteridae) em poções do córrego da Bexiga, Carrancas, Minas Gerais, Brasil. *Boletim do Museu de Biologia Mello Leitão*, 28: 89–104.

Tuf, I. H.; Petr, H.; Mačát, Z.; Machač, O.; Rendoš, M.; Trnka, F. and Vokálová, A. 2013. Suitability of nail polish for marking the common rough woodlouse, *Porcellio scaber* (Oniscidea). *Acta Societatis Zoolocae Bohemicae*, 77: 159–163.

Waldbauer, G.P. 1968. The consumption and utilization of food by insects. *Advances in Insect Physiology*, 5: 229–288.

Walker, T.J. and Wineriter, S.A. 1981. Marking techniques for recognizing individual insects. *The Florida Entomologist*, 64: 18–29.

Wang, X.; Chen, H.; Ma, C. and Li, Z. 2010. Chinese white pine beetle, *Dendroctonus armandi* (Coleoptera: Scolytinae), population density and dispersal estimated by mark-release-recapture in Qinling Mountains, Shaanxi, China. *Applied Entomology and Zoology*. 45: 557–567.

Wells, C.D. and Sebens, K.P. 2017. Individual marking of soft-bodied subtidal invertebrates *in situ* – A novel staining technique applied to the giant plumose anemone *Metridium farcimen* (Tilesius, 1809). *PLoS ONE*, 12: e0188263.

Willows, R.I. 1987. Population dynamics and life history of two contrasting populations of *Ligia oceanica* (Crustacea: Oniscidea) in the Rocky Supralittoral. *Journal of Animal Ecology*, 56: 315–330.

Wojcik, D.P.; Burges, R.J.; Blanton, C.M. and Focks, D.A. 2000. An improved and quantified technique for marking individual fire ants. *Florida Entomologist*, 83: 74–78.

Wood, C.T.; Kostanjšek, R.; Araujo, P.B. and Štrus, J. 2017. Morphology, microhabitat selection and life-history traits of two sympatric woodlice (Crustacea: Isopoda: Oniscidea): A comparative analysis. *Zoologischer Anzeiger*, 268, 1–10.

Wood, C.T.; Schlindwein, C.C.D.; Soares, G.L.G. and Araujo, P.B. 2012. Feeding rates of *Balloniscus sellowii* (Crustacea, Isopoda, Oniscidea): the effect of leaf litter decomposition and its relation to the phenolic and flavonoid content. *ZooKeys*, 176: 231–245.

Wu, X.; Griffin, J.N. and Sun, S. 2014. Cascading effects of predator–detritivore interactions depend on environmental context in a Tibetan alpine meadow. *Journal of Animal Ecology*. 83: 546–556.

Zambonato, B.P.; Daemon, E. and Prezoto, F. 2010. An alternative technique for individual marking of orb-web spiders. *Etologia*, 9: 3–5.

Zidar, P.; Kaschl, U.I.; Drobne, D.; Bozic, J. and Štrus, J. 2002. Behavioural response in paired food choice experiments with *Oniscus asellus* (Crustacea, Isopoda) as an indicator of different food quality. In: *1st SloTOX Workshop on Environmental Bioindicators and Refreshment in Basic Toxicology*, ArhHig Rada Toksikol. Lubjana, Slovenia, 177–181.

Zimmer, M. 2006. The role of animal-microbe interactions in isopod ecology and evolution. *Acta Biolica Benrodis*, 13: 127–168.