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The behaviour of the terrestrial isopods

Ph.D. Thesis

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Abstract

Terrestrial isopods (Oniscidea) are a diverse group of land crustaceans that have successfully adapted to life on land. Their expansion into terrestrial habitats involved numerous behavioural and morphological adaptations, including changes in reproduction, diet, protection against desiccation, and defences against new terrestrial predators. The presented work includes current knowledge about the behaviour of terrestrial isopods and related experiments, supplemented by our research focusing on their antipredatory behaviour. We conducted experiments with avian and reptilian predators to investigate predator-prey interactions, as well as individual defence mechanisms of terrestrial isopods, such as aggregation, turn alternation, or the use of defence glands. The usage of other possible behavioural and morphological adaptations for defence against predators is discussed.

Key words: antipredatory strategies, behavioural traits, defence, Oniscidea

Declaration

I declare that I authored the Ph.D. thesis myself, utilizing results from my own research or collaborative work with colleagues, and supported by other publication resources, which are properly cited.

Olomouc, 12 June 2025 Barbora Ďurajková *m.p.*

Content

List of Appendices

List of publications

The thesis is based on the following papers. These are designated and cited as Appendices I–VI.

Appendix I

Ďurajková, B., Hladký, R. & Tuf, I. H. (2022): Higher temperature and substrate vibrations as stress factors for terrestrial isopods – model species matter. *ZooKeys*, 1101, 71–85.

Appendix II

Tuf, I. H. & **Ďurajková, B.** (2022): Antipredatory strategies of terrestrial isopods. *ZooKeys*, 1101, 109–129.

Appendix III

Skočková, L., **Ďurajková, B.** & Tuf, I. H. (2025): Predator responses to artificial aposematic and cryptic colouration in terrestrial isopods (Isopoda, Oniscidea). *ZooKeys*, 1225, 141–153.

Appendix IV

Ďurajková, B., Tuf, I. H. & Vittori, M. (2025): Repugnatorial glands and aposematism in terrestrial isopods (Isopoda: Oniscidea: Armadillidiidae, Porcellionidae). *Journal of Crustacean Biology*, 45, ruaf014.

Appendix V

Ďurajková, B., Veselý, P. & Tuf, I. H.: Function of vivid coloration of terrestrial isopods from the point of view of an avian predator. *Insects*. (in rev.)

Appendix VI

Ďurajková, B., Veselý, P. & Tuf, I. H.: Different responses of insectivorous birds to terrestrial isopods. (in prep.)

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Terrestrial isopods are crustaceans

Crustaceans (paraphyletically, therefore, except Hexapoda) are mainly aquatic animals. The order Isopoda includes ten marine and freshwater suborders and just one terrestrial (Cloudsley-Thompson 1977). The suborder Oniscidea, also known as terrestrial isopods, woodlice, or pill bugs, is one of eleven suborders within the order Isopoda (Pericarida, Crustacea), which first colonised land during the lower Carboniferous period (Schram 1982; Fu et al. 2010; Broly et al. 2013). Their terrestrial habitats range from sea level to high mountains up to almost 5,000 m a.s.l. (Hornung 2011) and include over 4,100 species belonging to more than 500 genera and 38 families (Javidkar et al. 2015; Sfenthourakis and Taiti 2015; Dimitriou et al. 2019; Tuf et al. 2025). Representing the most successful group of crustaceans ever to colonise dry land (Warburg 1993; Hornung 2011), Oniscidea underwent a range of ecological and physiological adaptations necessary for survival in their novel terrestrial environment. These adaptations addressed challenges related to respiration, feeding, locomotion, reproduction and desiccation in these new habitats (Hornung 2011). When terrestrial isopods colonised land some 300 million years ago, they faced a variety of predators, including centipedes, scorpions, spiders, amphibians and reptiles. Thus, predator-prey relationships have also been the source of morphological and behavioural adaptations.

The aim of the work

The work aims to provide an overview of the current knowledge about terrestrial isopods' behaviour, focusing on testing in laboratory conditions, supplemented by my own research on predator-prey interactions.

How to avoid desiccation

Over evolutionary time, colonisation of contrasting terrestrial habitats, where soil water regimes range from semi-aquatic to desert conditions, has led to substantial variation in desiccation resistance among isopod species (Warburg 1993; Schmalfuss 2003). Although their physiological adaptations to life on land appear incomplete - notably the lack of a waxy cuticle to prevent desiccation (Hadley and Quinlan 1984) - they can be considered good land colonisers (Dias et al. 2013).

Terrestrial isopods are equipped with a water-conducting system (Hoese 1981). This consists of shallow canals filled with fine pin-like structures. The main canals run along the body on the ventral side near the base of the limbs. The canal begins at the outlet of the maxillary glands and ends at the first pleopods (Carefoot 1993). All isopods are probably able to ingest water by drinking and also by immersing their uropods in water. Water rises from the uropods to irrigate the pleopods, or is absorbed through the anus (Spencer and Edney 1954). Uropod water intake is more important than oral water ingestion (Drobne and Fajgelj 1993). Isopods can also absorb water vapour or extract water from air with sufficient humidity thanks to hyperosmotic solutions in the cuticle. The efficiency of water absorption is also increased by rhythmic movements of the pleopods, which help to circulate moist air. Wright and Machin discovered that all tested species from the groups Crinocheta and Diplocheta can absorb water from the air, this ability was not found in species belonging to the Synocheta group (Wright and Machin 1990, 1993). However, Synocheta isopods are usually small and live in the upper layers of the soil where the soil humidity is constantly around 100% (Tuf and Jeřábková 2008) and where there is therefore no water loss by evapotranspiration; similarly, the largest species of Synocheta (ca 15 mm) are living in caves only. Nevertheless, some desert isopods, such as *Hemilepistus reaumurii* (Milne Edwards, 1840), can withstand extremely high temperatures and have an effective defence mechanism against water loss through the cuticle. However, these physiological and morphological adaptations do not adequately protect against harsh climatic conditions. Desert woodlice can only survive the extreme summer heat in the microclimate of their burrows, which they dig themselves. During the hot season, only deep burrows (between 40 cm and 1 m deep) provide sufficient protection. Only in the deepest parts does the temperature remain below the critical maximum of 36–37 °C, and the relative humidity stays within the required range of 93–100%. This balances water losses, which occur partly through the absorption of water vapour and partly through the consumption of humid soil from the deepest parts of the burrow. Alongside its digging ability, the desert woodlouse exhibits highly developed social behaviour. This combination illustrates how a woodlouse, which is not physiologically suited to its environment, can become one of the most successful species in a hot, dry habitat at the extreme edge of its range (Linsenmair 1974, 2007).

The aggregation behaviour of woodlice may be key to their survival on land, as it reduces desiccation (Allee 1926; Edney 1968). Aggregation by body stacking can be thought of as a mechanism that reduces the exposure of the body surface to air for individuals within the aggregate. In this way, a dense aggregate creates a locally moist microclimate for all its individuals (Waloff 1941; Schliebe 1988), similar to the use of huddling by musk oxes and penguins to combat cold temperatures. Isopods can detect each other's presence (Yao et al. 2009) and use the integration of social information to adapt their behaviour according to physiological conditions i.e. degree of dehydration (Kuenen and Nooteboom 1963) and habitat quality (Robinson et al. 2011). Terrestrial isopods can acclimate to a slow and gradual decrease in temperature (Edney 1968; Sutton 1980). However, they are sensitive to sudden drops in temperature, which can cause significant mortality levels (Brody et al. 1983). This may be one explanation for their sheltering in the more buffered microclimate of dense grass clumps (Davis 1984). Another reason may be the avoidance of desiccation by sheltering under a thick layer of litter in higher relative humidity conditions to avoid excessive water loss to the drier air above ground (Hassall and Tuck 2007). Although it is difficult to define the selection pressures that are actually at work on ecomorphotypes (Schmalfuss 1984), each of these strategies can be effective in limiting water loss (Broly et al. 2013). Runners, such as *Philoscia muscorum* (Scopoli, 1763), are excellent walkers. They can explore their environment intensively and quickly find microhabitats or aggregations of conspecifics. Clingers, such as *Porcellio scaber* Latreille, 1804 or *Oniscus asellus* Linnaeus, 1758, can press down firmly on the substrate and reduce evapotranspiration by hiding the ventral side with a soft cuticle (Constantinou and Cloudsley-Thompson 1987). Using such tactics, runners, and clingers are environmentally dependent because they require shelter or a moist, flat substrate (Broly et al. 2013). In contrast, rollers like *Armadillidium vulgare* (Latreille, 1804) can be more independent of the environment thanks to their rolling-up behaviour, which reduces the size of the ventral surface exposed to air (Smigel and Gibbs 2008). This individual strategy of sphere-forming (minimum surface/volume ratio) may explain the xeric tolerance of *A. vulgare* (Vandel 1962). Furthermore, different roller species have different limits when it comes to their tolerance of environmental conditions (Csonka et al. 2013). Various morphological adaptations, including cuticle thickness, are related to these moisture preference values. Csonka et al. (2013) showed that the thickness of the cuticle of the tergites varies among *Armadillidium* species and is an important determinant of their distribution within habitats while influencing geographical patterns of species occurrence.

How to avoid starving

Other major changes in the lifestyle of terrestrial isopods involved a shift in food specialisation from algal mats to plant tissues characterised by the presence of complex resistant (poorly digestible) substances such as cellulose or lignin. The acquisition of endosymbiotic bacteria in the digestive tract proved to be a very useful adaptation for cellulose digestion (Zimmer and Topp 1998). Research indicates that nearly one tenth of an isopod's diet may consist of its own waste products. These are believed to replenish the digestive microorganisms that the animal requires and to provide some nutrition. If coprophagy is not an option, the growth rate of isopods is slower than normal (Hutchins et al. 2004). The successful invasion of land may therefore have been made easier by the uptake of symbionts from the surrounding environment (Horváthová et al. 2015). Other nutritional requirements were related to elemental deficiencies (e.g. Ca, Mg), which marine isopods obtain in excess directly from the water (Orsavová and Tuf 2018). Laboratory experiments with juvenile *A. vulgare* showed that growth and survival of individuals was greatest on a diet of an excess of fresh leaves of dicotyledonous plants but declined as the supply of this food decreased, even when alternative, lower quality foods were provided in excess. Growth in the laboratory was positively related to temperature. However, mortality was not significantly related to temperature over the range observed in nature. During the period of rapid growth in spring and early summer, when the supply of leaf litter from dicotyledonous plants was minimal, the demand for high-quality food by the field population peaked. Experiments showed that competition for high-quality food during this period could reduce the growth and survival of the isopod population (Rushton and Hassall 1987). In long-term laboratory studies, *P. scaber* and *O. asellus* prospered better on litter with lower C/N ratios. In general, both isopod species reproduced more successfully on litter with higher pH and half the amount of tannins and other phenolics. Interspecific differences were evident concerning the importance of litter-colonising microbiota and the dependence of juveniles on certain dietary parameters. *Porcellio scaber* was influenced by both the litter and its physicochemical characteristics, while *O. asellus* was affected only by the litter characteristics. Juvenile mortality was influenced by food parameters, but not in *P. scaber*. The physicochemical characteristics of leaf litter also affected the growth rate. Furthermore, leaf litter microbiota had a significant effect on the growth rate of *P. scaber* but not *O. asellus*. The sympatric coexistence of two species belonging to the same guild of saprophagous soil animals may be explained by these similarities and differences between the observed species (Zimmer and Topp 2008). Lower quality food reduced isopod feeding performance as shown in a laboratory study with *Porcellionides pruinosus* (Brandt, 1833) fed on heavy metal contaminated food (Loureiro et al. 2006). Furthermore, Zidar et al. (2012) reported that terrestrial isopods show avoidance behaviour when exposed to pyrethrins in soil or food. By avoiding contaminated food or soil, woodlice may reduce toxic effects like copper intake, but only to a limited extent (Zidar et al. 2004). Thus, isopods may be used in the future as a bioindicator of soil contamination or in the assessment of contaminated sites or remediation processes (Loureiro et al. 2006). On the other hand, new studies have reported some species, such as *Armadillidium arcangelii* Strouhal, 1929, as crop pests (Fusaro et al. 2024).

How to move on

The preadaptation of the marine ancestor of terrestrial isopods to life on land was its dorsoventral flattening, which enabled it to have relatively low energy-demanding locomotion (Orsavová and Tuf 2018). The body size and abiotic factors significantly affect the mobility of terrestrial isopods (Dailey et al. 2009). Dailey et al. (2009) conducted a study on *Porcellio laevis* Latreille, 1804 to test the effects of temperature, desiccation, and body mass on the isopod's locomotion. They measured the speed of isopods of different masses on a linear racetrack at temperatures ranging from 15 to 35 °C. In addition, a circular thermal gradient was used to investigate the temperature preferences of isopods and to measure the impact of desiccation on their locomotion. The results showed that the measured speed of the isopods increased progressively with temperature. The isopods did not show a clear preference for any particular temperature within the gradient, but they generally avoided temperatures above 25 °C. Moderate desiccation had no apparent effect on locomotor performance. However, there was a progressive decrease in speed once the animals had lost over 10% of their initial body mass (Dailey et al. 2009). Locomotion is also more or less influenced by the isopod's circadian rhythm (Refinetti 2000). Moreover, females' mobility can be influenced by the brooding period. In a negative-phototaxis experiment, in which females moved away from a light source along a graduated surface, non-brooding females moved significantly farther and faster than brooding females. This suggests that the additional mass of eggs and mancas restricts locomotion (Kight and Ozga 2001). Thus, isopod locomotion is an intrinsic component of many complex behaviours, including predator avoidance, migration, and mate-seeking. However, it is also closely related to an animal's physiological state (Bayley 1995).

How to produce offspring

A very useful preadaptation, shared by all Peracarida, is the direct development associated with carrying a clutch of eggs in a pouch (marsupium) of the females. Although many crustaceans care for their clutches by carrying them around, Peracarida also care for the first larval stages in this way, and the life cycle of Peracarids, therefore, does not include a free-swimming larva. The oldest fossil evidence for the presence of a thoracic sac and larvae developing within it in terrestrial isopods comes from Mexican amber, which is 23 million years old (Broly et al. 2017), although care of offspring and a marsupium in Peracarida arose much earlier. The transition of terrestrial isopods to land was greatly facilitated by this circumstance. Terrestrial isopods mate and breed on land, making them entirely independent of seawater. Broods of some species can contain more than 100 eggs, but only around half of these typically develop into mancas (Hutchins et al. 2004). Following copulation, some of the spermatozoa move from the female's oviduct to the seminal receptacle, which is located at the point where the oviduct joins the ovary (Ziegler and Suzuki 2011). Niemeyer et al. (2009) attempted to examine the impact of repeated mating on brood size. The reproduction of the isopod *Cubaris murina* Brandt, 1833 was examined in a laboratory setting to determine the number of offspring per brood, the number and duration of incubation periods, and the impact of repeated mating on the number of offspring per brood. Two different groups were used in the experiment: female-male pairs and females that were isolated after mating. No significant differences were found between the two groups of females for either the mean number of young per brood or the duration of the incubation period. At least five broods were produced by a single mating, with no decrease in brood size (Niemeyer et al. 2009). In *A. vulgare*, reproduction is stimulated not only by environmental factors (e.g. photoperiod) but also by the behaviour of the male. Males accelerate the onset of female reproduction by stimulating vitellogenesis and then mate with a female before parturial molting. Female-female intermittent crowding can also significantly accelerate vitellogenesis (Caubet et al. 1998). Additionally, laboratory experiments on *P. scaber* from zinc and lead mining areas showed that populations inhabiting metal-polluted sites have probably undergone evolutionary changes. The isopods inhabiting metal-contaminated areas appear to have evolved to reproduce earlier and allocate more resources to reproduction (Donker et al. 1993). Moreover, the size of the male copulatory organ and the female marsupium of *P. laevis* changed significantly as a result of exposure to a static magnetic field (Béjaoui et al. 2019). Thus, the reproductive pattern of terrestrial isopods can be modulated by environmental factors, such as temperature, photoperiod, and moisture, or by biotic factors, such as male effects and population structure. However, our knowledge remains limited.

How to avoid predation

Terrestrial isopods demonstrate lifespans ranging from one to approximately ten years. The highest mortality rate occurs in the first month of their life outside the brood pouch; in this case, the main causes are believed to be climate change, including factors such as high temperatures, drought and floods (Brereton 1957). Cannibalism of different ontogenetic stages (Brereton 1957), which mainly occurs during molting, could also be a possible cause of death. It can lead to the horizontal transmission of parasites such as *Wolbachia* sp. (Le Clec'h et al. 2013). In addition, some small passerines, such as flycatchers or wrens, rely on terrestrial isopods as a main source of calcium (Bureš 1986; Kristín 1992). There are also predators, for example *Scorpio maurus palmatus* (Ehrenberg, 1828), whose diet consists mainly of the desert isopod *H. reaumurii* at some areas (Warburg 1993). Other notable specialists include spiders from the genus *Dysdera* (Řezáč and Pekár 2007), which possess a variety of hunting strategies, employing their specialised chelicerae. The predator-prey relationship resulted in the evolution of various morphological and behavioural adaptations in the Oniscidea lineage. Among them is conglobation, release of highly acidic secretions, jumping, tonic immobility or clinging to the ground, and efficient escape. Terrestrial isopods can also aggregate with other individuals, stridulate or alter their typical activity period. Some have developed spiny tergites, aposematic colouration or deterrent posture (Tuf and Ďurajková 2022, Appendix II). Consequently, predators have had to adapt to these anti-predator adaptations and improve their hunting techniques. These *army races* led to the evolution of numerous adaptations, both on the part of the predators and their prey, i.e., terrestrial isopods.

One possible result of predator-prey competition could be the development of several ecomorphological types among terrestrial isopod species (Schmalfuss 1984). The first type is the "clingers". Isopods in this group have broad, flat bodies with strong, short pereopods. They are relatively slow-moving and are represented by the genera *Porcellio* and *Trachelipus*. In comparison, fast-moving isopods with long pereopods and slender bodies represent the group of "runners" (e. g. families Philosciidae or genus *Ligidium*). Soil-dwelling species often belong to the group of "creepers". These species are smaller, with an elongated body and ribs on the dorsal surface and require high humidity. Another type is "rollers", represented by species with convex bodies, which are able to roll up and use conglobation as their protection against predators. This includes the families Eubelidae or Armadillidae. Isopods that live outside the topsoil, like genera *Panningillo* and *Echinodillo*, with spikes or thorns on their bodies to protect them from predators are called "spiny forms". They can be found mostly on vegetation in tropical and subtropical areas. Some authors also distinguish the ecomorphological type "jumpers" (Cranbrook and Edwards 1994; Hassall et al. 2006). These jumping isopods have been reported from Borneo and are represented by the genus *Burmoniscus*. However, jumpers and runners differ only in their behaviour (Tuf and Ďurajková 2022, Appendix II).

Change of typical activity time

The most elementary strategy for defending oneself against a predator is to maintain a safe distance from it and to avoid any encounter. A useful tactic is to avoid areas where other individuals have been killed. Yao et al. (2009) experimentally confirmed that chemical substances released by smashed terrestrial isopods, mainly linoleic acid, act as a deterrent to other terrestrial isopods, predominantly those of the same species. In their study, four species of terrestrial isopods belonging to four different genera (*O. asellus*, *A. vulgare*, *P. scaber*, and *Porcellionides pruinosus*) were exposed to crushed and spread body parts and blood, intact corpses, or body extracts of conspecifics soluble in alcohol. Woodlice were observed to enter shelters containing crushed conspecifics, yet they subsequently exited the place after conducting a thorough inspection. The isopods demonstrated a clear aversion to intact dead bodies, blood or crushed remains, and alcohol extracts of isopods bodies (Yao et al. 2009). This finding supports the necromone hypothesis, which suggests that avoiding dead and injured conspecifics may help reduce the risks of predation and infection strongly associated with such markers (Wilson-Rich et al. 2009). However, the presence of cannibalism within the Oniscidea suborder contradicts the given claims.

The same effect was observed when an indirect cue from the predator was presented. Castillo and Kight (2005) examined the direct and indirect impacts of the predatory ant species *Tetramorium caespitum* (Linnaeus, 1758) (Hymenoptera: Formicidae) on the behaviour and reproduction of two isopod species, *A. vulgare*, and *P. laevis*. In the experiment, *A. vulgare* and *P. laevis* were kept in a box with ants. The results showed that both species escaped from their shelters and became more active outside of them due to harassment from the ants. An indirect cue from predator ants caused a similar reaction where both species of isopod exhibited a clear tendency to maintain a greater distance from the source of the ant odour (Castillo and Kight 2005).

Terrestrial isopods have been shown to engage in both spatial and temporal predator avoidance. *Porcellio scaber* and *A. vulgare* spent significantly less time walking in trials involving visual cues of predators than in trials without them (Zimmerman and Kight 2016). Isopods can reduce the risk of predation by regulating their temporal activity patterns, thereby ensuring their safety by operating outside the predator's typical active range. Although the majority of terrestrial isopods are nocturnal, the burrowing species *H. reaumurii*, which is endemic to the arid regions of North Africa, the Middle East, and Central Asia, exhibits activity (in the colder season) primarily during daylight hours (Nasri-Ammar and Morgan 2005). In the warmest period of the year, when the maximum temperature can reach 45 °C, *H. reaumurii* become active before sunrise while risking interactions with their main nocturnal scorpion predator, *S. maurus palmatus*. Therefore, *H. reaumurii* can modify its typical time activity from nocturnal to diurnal to reduce the rate of predation by scorpions. However, experiments that confirm this hypothesis remain lacking (Tuf and Ďurajková 2022, Appendix II). Furthermore, the use of artificial light at night has emerged as a significant ecological disruptor, impacting the behaviour and physiology of terrestrial isopods. *Porcellionides pruinosus* showed prolonged freezing durations in response to an approaching stimulus, as well as increased shelter-seeking behaviour and reduced dispersal within the terrarium (Dissegna and Chiandetti 2025).

Isopods can employ considerations to fine-tune their risk assessment. A series of semi-nested field trials showed that wild isopods *H. reaumurii* can detect the scent of *S. maurus palmatus* scorpions. However, isopods only reacted defensively when this odour was linked to excavated soil or other olfactory signals associated with scorpion burrows. Even in the absence of scorpion olfactory cues, a pile of excavated soil was enough to generate a defensive response. Isopods responded to perceived risk in a graded manner, which may indicate an increase in risk estimation (Zaguri et al. 2018). Moreover, isopods exhibit behavioural alterations in response to acoustic pollution. *Porcellio laevis* walked shorter distances without changing their speed after being exposed to noise (Vidal-Franco 2024).

Cryptism

The ability to avoid being seen may be an important escape mechanism from predators. Colouration is often crucial in the reduction of predation risk. Body colour and colour pattern can help avoid, deter, or confuse the predator (Endler 1978). Crustacean chromatophores usually contain a variety of pigments such as ommochromes, carotenoids, or melanin (Fingerman 1965). The cryptic colouration is intended to reduce the possibility of detection by predators as the animal attempts to blend in with its surroundings (Merilaita 1998). Among terrestrial isopods, cryptic colouration is typical of epigeic species with twilight or daytime activity like *P. pruinosus*, *Trachelipus rathkii* (Brandt, 1833), *P. scaber*, or *H. reaumurii*. During the day, cryptic species are typically hidden in shelters inside the soil or on its surface (Davies et al. 2012). Some species are capable of changing colour or pattern on the body. Chromatophore-based colour change is triggered by sensory detection of the environment (Nery and de Lauro Castrucci 1997). The coastal isopod *Ligia oceanica* (Linnaeus, 1767) uses its melanophores to change colour as part of a circadian rhythm (Hultgren and Mittelstaedt 2015).

Cryptic polymorphism (Veselý et al. 2024) is the occurrence of different colour forms within the same species. The predator has a specific image in its memory (the search image) that it uses to look for its prey (Punzalan et al. 2005). This means that if a predator detects one type of morph, other individuals with a different pattern are relatively safe (Hughes and Mather 1986; Veselý et al. 2024). This mechanism protects individuals with rarer colouration, as the predator mainly preys on the more abundant patterns of the prey. Thus, because the preference for a particular pattern is determined by the current frequency of the colouration, polymorphism is maintained in the population by predation. For morphs with a high degree of conspicuousness like albinos (see Achouri and Charfi-Cheikhrouha 2009), however, this principle does not apply. However, a better understanding of the problem can only be achieved through field experiments.

Defensive glands

Once a terrestrial isopod has been spotted and recognised by a predator, it can use several strategies to avoid predation. One common strategy is to use defensive gland secretions to repel predators. Terrestrial isopods are known to possess tegumental glands, and it has been hypothesised that a specific type of these glands, namely the lobed glands, may serve a defensive function. In the majority of the species that have been the subject of study, these glands secrete either through the lateral plates or through the uropods (Gorvett 1951; Ďurajková et al. 2025, Appendix IV). The research carried out so far on their secretory products has shown that the lobed glands that are secreted by the uropods produce a sticky, proteinaceous secretion that can deter some potential predators, such as ants and some spiders (Gorvett 1952; Deslippe et al. 1996). When attacked by a predator, isopods of the species *P. scaber* secrete protein-rich substances from their tegumental glands. Recent analyses of gland secretion extracts using gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry identified four metabolites, three of them have not been identified before (Fischer et al. 2025). In behavioural experiments, *Steatoda grossa* (C. L. Koch, 1838) spiders preyed on *Tenebrio molitor* Linnaeus, 1758 beetles that were chemically undefended, but avoided chemically defended *P. scaber*. Additionally, spiders rejected the beetles as prey when topical applications of either *P. scaber* gland secretion extract or synthetic metabolites identified in these extracts rendered them chemically defended. The hypothesis that the evolutionary transition of crustaceans from aquatic to terrestrial habitats prompted the evolution of defensive metabolites against terrestrial predators is supported by these data. Thus, like many insects, crustaceans are chemically defended against predators (Fischer et al. 2025). A secretion from lobed glands is not associated with the hormonal or nervous system. Rather, it is likely to be the result of the contraction of adjacent muscles, as stimulation needs to be very strong. Simple shaking or squeezing the animal will not stimulate gland secretion (Gorvett 1956). Gorvett noted that after a spider bites an isopod, the proteinaceous secretion leaks from the isopod's gland openings, causing the spider to clean its mouthparts and reject the prey. Food containing traces of isopod body secretions was also not consumed by most spiders (Gorvett 1956). These secretions were equally repellent to ants (Deslippe et al. 1996) and could also be effective against centipedes (Paris 1963), scorpions (Herold 1913) as well as shrews (Churchland 1994). According to Herold (1913), the development of these glands is limited in myrmecophilous species. This may be due to the protective benefits provided by ants. Moreover, reducing the function of these glands may explain why some myrmecophilous species are adopted by ants, as they are chemically insignificant to them (Parmentier 2016) and difficult to detect in an ant nest. Additionally, there may be some correlation between the degree of development of the lobed glands and the level of distastefulness (Gorvett 1956) or predator avoidance strategies (Ďurajková et al. 2025, Appendix IV).

We conducted a study based on the defence mechanisms of *Armadillidium* species with distinctive aposematic colourations, investigating the secretion properties and histological characteristics of their tegumental glands (Ďurajková et al. 2025, Appendix IV). Six species of isopods were examined. *Armadillidium gestroi* Tua, 1900, *Armadillidium klugii* Brandt, 1833, and *Armadillidium versicolor* Stein, 1859 were considered aposematically coloured, while *P. scaber*, *P. laevis*, and *A. vulgare* were cryptically coloured. Subsequently, histological preparations were prepared from all the studied species. We accidentally observed that placing isopods in the freezer for a few minutes to immobilise them resulted in lobed gland secretion. For the examination of the secretory products of the glands, we placed the specimens individually in a freezer at -20 °C in a fresh plastic Petri dish for 5 minutes. After this time, only the uropod glands were discharged in some individuals. If the pereon glands failed to discharge, additional individuals were exposed for 10 min, after which the pereon glands were also expected to discharge. In all species except *P. laevis*, in which all lobed glands discharge rapidly, this approach allowed us to study the secretory products of the uropod and lateral plate glands separately. To assess the odour of the secretory products, the isopods were observed under a stereomicroscope to confirm the discharge of the lobed glands, the Petri dish was then opened and smelled. Our results showed that all six species have lobed glands in the epimera of the pereon and pleon, as well as in the uropods. There are clear differences between them concerning the diversity and abundance of glands. All species discharged sticky, viscous secretions from the uropod glands. However, only strikingly coloured *A. klugii* and *A. gestroi* produced an unpleasant odour from the lateral plate glands when exposed to cold. These results suggest that both chemical and visual cues may be used by aposematic species of *Armadillidium* to honestly signal their unpleasantness. This is consistent with our study of Great Tits, which avoided consuming *A. gestroi* (Ďurajková et al. in rev., Appendix V). However, cryptic species or those with no detectable odour can imitate toxic species without producing chemical defences or can produce substances that make them unpalatable (Ďurajková et al. 2025, Appendix IV). The palatability and predator preference of different isopod species also varies, as was demonstrated in a study involving two shrew species and isopods *P. scaber, A. vulgare, Philoscia muscorum* (Scopoli, 1763) and *O. asellus*. The results of the tests clearly showed that *Philoscia* was the most palatable, *Armadillidium* the least, and that *Porcellio* and *Oniscus* occupied intermediate positions. The shrews usually sniffed at all the animals before selecting *Philoscia*. *Armadillidium* was always eaten last (Crowcroft 1957). In different isopod ecomorphotypes, diversity in gland morphology and secretion properties appears to be related to antipredator strategies such as clinging, rolling, or running (Ďurajková et al. 2025, Appendix IV).

Warning colouration

Rather than using repellent glands to deter a predator from attacking, terrestrial isopods can indicate unpalatability from a distance to avoid the risk of damage. One way of doing this is to use a warning aposematic colouration. Several insects, myriapods (Marek and Bond 2009; Przeczek et al. 2008), and arachnids use this strategy. Conspicuous colours (usually black in combination with yellow, red, or orange) can signal unpalatability and/or dangerousness to predators through hereditary neophobia or learned avoidance of these colours (Vickers et al. 2021). To date, there is only limited evidence for the aposematic function of colouration in terrestrial isopods. Levi (1965) made a comparison between the bright red spots on isopod *A. klugii*, the millipede *Glomeris pulchra* CL Koch, 1847 and the widow spider *Latrodectus tredecimguttatus* (Rossi, 1790). All of these species live syntopically near city of Dubrovnik (Croatia) and use chemical defences to support their Mullerian mimicry system against attack by nocturnal mammals, gallinaceous birds, thrushes, or lizards (Schmalfuss 2013). In the genus *Porcellio*, yellow and white spots are quite common. However, almost black (i. e. dark violet-brown) and white have been reported for several unrelated litter-dwelling species in West Africa (Schmalfuss and Ferrara 1982). It has been suggested that their antipredator function is not only a warning colouration but also a cryptic colouration that dissolves the body outline (Schmalfuss and Ferrara 1982).

Vivid aposematic colouring is easier for a predator to remember. It helps to avoid dangerous and/or inedible prey in the future. We investigated whether conspicuous colouration has an aposematic function in terrestrial isopods. For this purpose, three studies with selected predators and isopod species were conducted (Skočková et al. 2025, Appendix III; Ďurajková et al. in rev., Appendix V; Ďurajková et al. in prep., Appendix VI).

The first study used *Porcellio scaber* as a model species for prey and the Italian Wall Lizard *Podarcis siculus* (Rafinesque-Schmaltz, 1810) as a predator (Skočková et al. 2025, Appendix III). To mimic the distinctive colouration of isopods, we marked their dorsal plates with six yellow dots, which is reminiscent of the colouration of the *Porcellio haasi* Arcangeli, 1925. The control group of woodlice was painted with six grey dots. Four young, immature lizards (2 females and 2 males) were used in the experiments. These lizards were naive and had been kept in captivity since they were born. During the experiment, the lizards were only fed on a trial-by-trial basis to see if there would be any change in feeding behaviour. To test whether the bright colour of the isopods has an aposematic function, we used selection tests in which five aposematically (grey-yellow experimental group) and five cryptically (grey-grey control group) painted isopods were simultaneously released into a box containing one predator. Predator behaviour was classified into three categories: 1) prey consummation, 2) prey manipulation, which consisted of biting the isopod or chewing and then spitting it out, and 3) prey observation, which consisted of turning the head to follow the prey or following the isopod. The most common type of behaviour for all lizard individuals was an observation, with aposematically and also with cryptically coloured prey. Manipulation or direct consumption of the isopod was less common. Our results showed differences in prey observation both between sexes and between prey colours. However, only males observed aposematic prey significantly more often. On the other hand, females were more interested in observing potential prey in general. The consumption rate of unpainted prey decreased from 65% to 5% during the five weeks. The consumption of cryptic prey was higher when it was followed by a longer interval of time (four or more days between the trials). This relationship was less obvious for aposematic prey. We found statistically significant differences in consummation between the sexes of predators. Females ate more prey, both cryptic (significant) and aposematic (not significant). However, we did not find any significant differences in the consumption of aposematically and cryptically coloured prey. From the interaction with the unpainted prey, it was clear that the predators found the isopods unpalatable. The predators chewed isopods for a long time before spitting them out (Skočková et al. 2025, Appendix III).

Our second study was conducted with Great Tits (*Parus major* Linnaeus, 1758) captured in the wild and thus possessing some experience with common native isopod species. We tested the bird's responses to the five isopod species: *P. scaber* (native, inconspicuous), *O. asellus* (native, moderately conspicuous), *Armadillo officinalis* Duméril, 1816 (non-native, moderately conspicuous), *A. versicolor* (native, conspicuous), and *A. gestroi* (non-native, conspicuous). We compared bird responses to isopods with reactions to the Guyana Spotted Roach (*Blaptica dubia* (Serville, 1839)), an edible insect whose third larval instar is similar to isopods in size and appearance. The birds used in the experiments were adults caught in the wild at a winter feeding site near the city of České Budějovice (Czech Republic). We ran two types of experiment. In the single prey treatment, each prey was presented in one (middle) cup of a circular feeding tray. The bird was first given a mealworm to control foraging interest. When the bird had completely swallowed the mealworm, a single isopod was offered. Each bird was given a series of 5 trials to avoid the effect of neophobia (Marples and Kelly 1999). The choice tests were conducted in a similar way, except that the bird was presented with two types of prey simultaneously when Guyana Spotted Roach served us as a second prey. Mealworms were only used as an appetite indicator. For the choice tests, we used the cockroach (preferred prey) and *P. scaber* (cryptically coloured prey) and cockroach and *A. gestroi* (aposematically coloured prey). We recorded bird behaviour using Behavioural Observation Research Interactive Software (BORIS) and scored several bird behaviour: (1) attacking the prey, (2) eating the prey, i. e., consuming at least part of the body of the prey, (3) observing the prey, and (4) discomfort behaviour as cleaning the bill, drinking water, or rinsing the feathers.

Prey species had a significant effect on the frequency of attacks. Cockroach was attacked and eaten significantly more often than isopods, but there was no difference in attack frequency between all isopod forms. The prey type interaction and the occurrence of attacking the prey significantly affected the number of signs of discomfort (beak cleaning, feather rinsing, drinking) performed by the tested bird. Birds showed discomfort most often when attacking woodlouse with aposematical colour pattern.

At first glance, the third instar of the cockroach appears similar, differing from isopods mainly in the shape of the antennae. Karlíková et al. (2016) demonstrated the use of such detailed features in prey recognition. Their study tested whether Great Tits could discriminate between edible cockroaches and inedible firebugs, *Pyrrhocoris apterus* (Linnaeus, 1758), when their colouration was made identical by placing paper stickers on their backs. It was shown that some of the birds were able to recognise edible cockroaches and only attacked them (Karlíková et al. 2016). We failed to demonstrate that conspicuously coloured isopods are better protected against bird attack than inconspicuous species. There was no statistical difference in the occurrence of attacks between the five isopod species. The isopods were attacked equally often when presented alone or with cockroaches. However, when a cockroach was presented with isopods, the cockroaches gained a certain degree of protection. We conducted the experiments during the autumn period, when Great Tits are preparing for winter and some individuals are dispersing (Andreu and Barba 2006). Foraging motivation is generally higher before and during migration, which may also increase their willingness to attack even unprofitable prey.

In conclusion, we have shown that isopods are generally better protected from bird attack than cockroaches, but their colour pattern does not affect the level of protection. Non-native species benefited either from the generalisation of protection provided by native isopods or from neophobia. Strikingly coloured *A. gestroi* caused significantly more post-attack discomfort in birds than any other isopod, suggesting that there are differences in chemical protection between the species tested (Ďurajková et al. in rev., Appendix V).

In our third study, we tested hypotheses related to prey palatability and predator foraging specialisation by investigating the feeding preferences of wild-caught avian predators for the terrestrial isopod *A. vulgare*. As in the previous study, the third larval instar of the Guyana Spotted Roach (*Blaptica dubia*) was used as the control baseline prey. As predators, we used four species of passerine birds: Great Tit (*Parus major*), European Robin (*Erithacus rubecula* (Linnaeus, 1758)), Common Blackbird (*Turdus merula* Linnaeus, 1758), and Eurasian Tree Sparrow (*Passer montanus* (Linnaeus, 1758)). Great Tit can discriminate between aposematic and non-aposematic prey and avoid attacking them (Exnerová et al. 2003). European Robins have been used to test responses to an insect warning signal (Exnerová et al. 2003; Hotová Svádová et al. 2010; Siddall and Marples 2011). They can recognise aposematically coloured prey and are less likely to attack them (Exnerová et al. 2003). Common Blackbirds have been used in behavioural experiments to test birds' responses to aposematic and non-aposematic prey (Schlee 1986; Exnerová et al. 2003). Common Blackbirds show increased tolerance to chemically protected insects such as firebugs and do not show any signs of discomfort after feeding (Exnerová et al. 2003). Finally, Eurasian Tree Sparrows have been used in behavioural experiments to test the efficacy of visual and chemical signals of unpalatable insects (Veselý et al. 2017; Aslam et al. 2019). Although juvenile sparrows avoid chemically protected insects such as ladybugs, adult individuals can tolerate chemicals and include ladybugs in their diet (Veselý et al. 2017). All birds used in the experiments were adult birds captured from October to November 2023 in the city of České Budějovice. The experiments were carried out in the same way as those with Great Tits (see Ďurajková et al. in rev., Appendix V). This time, only single prey trials were conducted, as our previous study on Great Tits showed that a cockroach provides protection when present together with an isopod (Ďurajková et al. in rev.). We scored three possible outcomes of prey presentation to the bird: 1) the bird attacked the prey, 2) the bird attacked the prey and subsequently ate at least part of its body, 3) the bird neither attacked nor ate the prey. The attack rate during the trials was significantly influenced by the interaction between bird species and prey type. Great Tits were the only species that attacked isopods significantly less often than cockroaches. For the other species, there was no significant difference, although isopods were attacked slightly less frequently than cockroach prey. Cockroaches were frequently attacked by all bird species. There was no effect of the interaction between bird species and prey species on the likelihood of eating prey that had already been attacked. All bird species ate both cockroaches and isopods very often once they had decided to attack them.

In conclusion, we tested experimentally whether birds avoid terrestrial isopods as prey depending on the size of the bird and its usual diet. We did not prove that wild birds avoid eating isopods. The results show that all bird species consumed both cockroaches and isopods after deciding to attack them. Only the Great Tit considered the pill bug as an unpalatable prey. Bird species with a higher proportion of insects in their prey were also more likely to accept cockroaches, but this difference was not significant. The willingness to consume terrestrial isopod prey was not related to the size of the avian predator. European Robins, Common Blackbirds, and Eurasian Tree Sparrows also consumed pill bugs less willingly, but this difference was not significant. The greatest willingness to consume all presented prey was shown by the European Robin, whose population at the time of the experiment was just before its seasonal migration to the wintering grounds. We suggest that not only species-specific traits such as body size, diet and foraging habitat, but also individual conditions and motivation may have important effects on the tendency to attack and consume unprofitable or suspect prey (Ďurajková et al. in prep., Appendix VI).

Spiny tergites

The elongated spines of terrestrial isopods may also, in theory, serve as a defence against predators. The spiky yellow isopod *Pseudolaureola atlantica* (Vandel, 1977) inhabits the leaves of tree ferns, and its bright colouration is complemented by long spines (Dutton and Pryce 2018). They can also be found in the *Laureola* and *Calmanesia* genera, which also live on vegetation. The role of these structures has not yet been investigated, but it is thought that the spines protect terrestrial isopods from being swallowed by small predators like birds, frogs, geckos, and other lizards or ants (Schmalfuss 1975). A comparable defensive function could be provided by shorter and stronger thorns, which are more common in isopod species. Strong thorns may also provide protection for larger, soil-dwelling terrestrial isopods like *Hemilepistus aphganicus* Borutzky, 1958. The anterior part of the head and the posterior margin of the first four pereonites of the genus *Hemilepistus* have strong spines, whose function is to block the entrance to the burrow and thus protect the individuals hiding inside (Schmalfuss 1975). Genera such as *Pseudarmadillo*, *Acanthoniscus*, *Cristarmadillidium*, *Echinodillo*, and *Ctenorillo* also have strong thorns and predominantly inhabit the ground.

Turn alternation

While in open space, terrestrial isopods like *A. vulgare* tend to move in a linear path (Iwata and Watanabe 1957). Generally, forward movement in a straight line is the most sensible choice when information about potential danger is lacking (Jander 1975). One way to maintain a linear direction of movement in poorly permeable environment is to alternate right and left turns (Hughes 1967). Turn alternation ensures the highest probability of reaching the greatest distance from a starting point (Hughes 1989; Moriyama and Migita 2004). Turn alternation can be seen as an indicator of stress, as terrestrial isopods use this strategy to escape from unpleasant places (Houghtaling and Kight 2006). The refined method for monitoring the stress of terrestrial isopods is tracking their movements through a T-maze (Ono and Takagi 2006). Carbines et al. (1992) tested the stress level of the *P. scaber* exposed to the predatory spider *Dysdera crocata* C. L. Koch, 1838. In one trial, a woodlouse was placed in the start chamber with a spider. As a control, the woodlouse was placed in a starter chamber with either a ball of cotton or with fly *Musca domestica* Linnaeus, 1758. In the event of a successful test, contact was initiated by either the fly, the spider, or the cotton wool. After this interaction, the thread was tightened, thus drawing the spider, fly, or cotton wool towards the side of the chamber opposite the exit hole. The exit hole was then opened, thus providing the woodlouse with access to the T-maze. Once in the maze, the woodlouse was permitted to move freely, and its final position was recorded. Results showed that *P. scaber* significantly more alternated turns when exposed to a spider in comparison to a harmless fly or flock of cotton (Carbines et al. 1992). Another study with *P. laevis* and *A. vulgare* demonstrated that chronic stress induced by indirect predatory stimuli (e.g., ant odour) can result in increased turn alternation, leading to the desertion of a high-risk location. During the experiment isopods were placed within the entrance of the T-maze while exposed to short-term vs. long-term exposure to indirect cues from predatory ants *Tetramorium caespitum* (Linnaeus, 1758). The behaviour of the isopods was then recorded until they reached one of eight possible ending points of the T-maze. The results demonstrate that neither isopod species exhibited an increase in the number of alternating turns in response to short-term indirect exposure to ant. Both species made significantly more alternating turns after a week of continuous indirect exposure to ants (Hegarty and Kight 2014). However, *P. leavis* showed a significant reduction in turn alternation due to habituation to disturbance (Houghtaling and Kight 2006).

Turn alternation can also be influenced by external stimuli such as increased temperature or vibration. We conducted experiments with the *P. scaber* and *P. pruinosus* exposed to stressors consisting of increased temperature, vibrating surface, or a combination of both (Ďurajková et al. 2022, Appendix I). An increased temperature was considered between 27–31 °C, while room temperature (18–24 °C) was used as a control. Similarly, the presence of artificially created vibrations served as a stress factor, while the absence of vibrations was used as a control. We used the same recording as was applied by Cividini and Montesanto (2018a, b, c), which was played on an MP3 player connected to the speakers to produce microvibrations. The vibrations were monitored with an oscilloscope application from a mobile phone located in a T-maze between the speakers. Isopods were placed in the T-maze, and then we recorded how they alternated turns as they tried to reach one of the eight possible termination points. We also recorded changes in the isopod's decision to make a turn and the time taken to reach one of the endpoints of the maze. Contrary to the study by Moriyama et al. (2016), our results showed that the effect of vibrations on the rate of turn alternation of *P. scaber* was not significant. When the substrate did not vibrate, *P. pruinosus* showed a significant increase in turn alternation. Vibrations did not affect the time spent in the maze or change the pattern of turn alternation for either species. This may be due to the lack of stridulatory or auditory organs to absorb vibrations in the examined species. Although it has been shown that *P. laevis* can sense the stomping of a predator (Zimmerman and Kight 2016), *P. scaber* is not deterred by monotonous noise. Another explanation may be that our isopods were collected in the city and, like *P. laevis* from urban environments (Houghtaling and Kight 2006), were accustomed to vibrations. Higher temperature and the combination of higher temperature and vibrations were stressors only for *P. scaber*. This species went more slowly through the T-maze at higher temperatures, and although they made more turn corrections, they alternated turns less intensely (Ďurajková et al. 2022, Appendix I).

Turn alternation is a result of the isopod’s decision-making, as was shown in a study with *A. vulgare* examined in a T-maze (Moriyama 1999; Moriyama and Migita 2004). This finding indicates that pill bugs possess an inbuilt mechanism that acts to maintain turn-alternating behaviour. In the experiment involving three dozens of *A. vulgare*, each of which completed 130 successive T-maze trials, the usage of directional changes was observed to prevent isopods from turning in the same direction on two subsequent turns. This behaviour, called turn repetition, resulted in an increased number of turn alternations (Moriyama et al. 2016). The similarity in these antipredator mechanisms between distantly related species of terrestrial isopods suggests evolutionary conservation of turn alternation (Hegarty and Kight 2014). Furthermore, data from a study of the terrestrial isopods *Porcellio spinicornis* Say, 1818 and *T. rathkii* suggest that turn alternation may follow a sex-specific selection pattern that differs among species (Caster et al. 2025).

Running, diving and jumping

According to Schmalfuss's (1984) ecomorphological classification, the "runners" group of terrestrial isopods has well-developed eyes and a relatively slender body with long pereiopods, making them suitable for rapid retreat (Schmalfuss 1984). Such a species is, for example, *P. muscorum*, whose narrow body and long legs are well adapted to a fast and surprising escape (Sutton 1972). One example of an interesting antipredator strategy is the diving capability of *Ischioscia hirsuta* Leistikow, 2001, which can hide under the water surface when disturbed (Leistikow 2001). Similar behaviour is known for rocky coasts inhabiting species of genus *Ligia*, these isopods are also runners and can hide under water. A more unpredictable strategy than running is jumping, as has been reported in several species of *Philoscia* (*Ischioscia*). Williams (1941) observed that species of *Philoscia* (*Ischioscia*) jump, similar to springtails

(Collembola). Van Name (1925, 1926) also mentioned jumping or leaping in *Ischioscia variegata* (Dollfus, 1893) and *Ischioscia nitida* (Miers, 1877, but considered a nomen dubium). Leistikow (2001) compared the jumping distance of *I. variegata* and *Ischioscia pariae* Leistikow, 2001. The first species was observed jumping up to 20 cm, while the second only jumped 5 cm. Such distances are sufficient to avoid hunters (such as spiders). A jump of 20 cm was also enough to escape from the human collectors. Jumping terrestrial isopods have also been described from Borneo, a species of *Burmoniscus*, which was later classified as the ecomorphological type "jumper" (Cranbrook and Edwards 1994; Hassall et al. 2006), although jumpers lack any obvious modification of legs for jumping.

Aggregation

One of the simplest strategies for prey is to stay together with neighbouring individuals in one group. The formation of aggregation is considered an effective evolutionary way to deal with water deficits, ambient temperature, or predator pressure. Other benefits of aggregation include reduced oxygen consumption, increased body growth, biotic stimuli for reproduction, improved access to mates, the promotion of coprophagy as a secondary food source, and the uptake of internal symbionts. The size of a group and its population dynamics are the result of a trade-off between costs (e.g., sexual conflict, exploitative and interference competition, and the risk of disease and parasitism) and those benefits (Broly et al. 2013). According to Courchamp et al. (2008), an increase in the number of individuals in the group results in additional costs for each individual, which may eventually outweigh the benefits of group membership. Overall, the tension between intragroup competition (more pronounced for large groups) and intergroup competition along with predation risk (especially significant for small groups) is likely to be a critical factor influencing group size (Markham 2015). Broly et al. (2012) found that stable aggregations under shelters often extended beyond their edges, exceeding the optimal group size, regardless of density. This is probably because the benefit to the individual is greater than the average loss to the individual in the created aggregation since no one expels the newcomer.

Aggregation as a form of antipredatory behaviour leads to predator confusion as well as oversaturation. Even a skilled predator can't attack all individuals in a group. Thus, as the size of the group grows, the probability that an individual within the group will be targeted by a predator decreases despite a higher visibility of large groups (Krause and Ruxton 2002). In aggregations, information about an upcoming danger can be spread from individuals who have observed the danger to those who have not yet noticed it. Such behaviour has been documented in *A. officinalis*, which can produce vibrations in the substrate to warn neighbouring individuals (Cividini et al. 2020). Furthermore, aggregation can enhance the effect of individual defence mechanisms such as repellent secretions or necromones (Yao et al. 2009), acting as a collective defence behaviour (Broly et al. 2013). Aggregation pheromone is deposited in isopod feces (Kuenen and Nooteboom 1963;). Yao et al. (2009) studied intra- and interspecific responses of four isopod species to fecal material to quantify the attractiveness of aggregation pheromone. It was shown that the aggregation pheromone may even act with necromones to convey that "the dead are conspecifics" (Yao et al. 2009). The study by Cividini and Montesanto (2018a) demonstrated that the response of the isopod to microvibrations leads to a greater number of aggregates, considering that microvibrations can warn of an upcoming danger.

In our study (Ďurajková et al. 2022, Appendix I), we decided to test the hypotheses that aggregating behaviour can be influenced by external stimuli such as increased temperature or vibration. *Porcellio scaber* and *P. pruinosus* were exposed to vibrating surface, increased temperature, or their combination (for further description of stress factors see Ďurajková et al. 2022, Appendix I). For the observation of aggregation behaviour, thirty individuals of the same species were placed in a box and filmed for two hours. The analysis of the videos was conducted by the calculation of the number of isopods touching each other, i.e., the presence of thigmotaxis, for a total of twelve images (one every ten minutes) for each video. *Porcellio scaber* showed a statistically significant difference in aggregation dynamics after being exposed to vibrations. Additionally, its aggregation behaviour was also influenced by temperature. At control (room) temperatures, relatively stable aggregations of ~23–26 individuals were observed. At higher temperatures, the number of aggregated isopods reached ~28 individuals within half an hour and then stabilized. This confirms that the aggregation behaviour of *P. scaber* is indeed an indicator of stress. The aggregation of *P. pruinosus* was significantly influenced by vibrations, along with increased temperatures. Isopods aggregated more when they were exposed to control (room) temperatures. Compared to this, the lowest numbers of aggregated isopods were found at higher temperatures in the absence of vibrations. However, we were unable to confirm that *P. pruinosus* was stressed by vibration or temperature. Thus, comparing aggregation behaviour and stress levels was not possible. *Porcellionides pruinosus* is often found in composters in the Czech Republic, where the temperature rises due to the microbial decomposition of organic matter. Therefore, the elevated temperature likely wasn't stressful for it. Our results suggest that factors that have been used as stressors for certain species in some studies cannot be automatically applied as stressors for all terrestrial isopods (Ďurajková et al. 2022, Appendix I).

The aggregation behaviour of terrestrial isopods is being studied in detail (Broly et al. 2012, 2013, 2014, 2016; Broly and Deneubourg 2015; Pogson 2016); a complete study of the effect of aggregation on predation risk in terrestrial isopods, however, has yet to be conducted.

Tonic imobility

Tonic immobility, or the tendency not to attract predators by movement, is another anti-predator strategy commonly used by terrestrial isopods (Quadros et al. 2012; Tuf et al. 2015). The most common response is to stop and don´t move. *Armadillidium vulgare* and *P. laevis* showed a strong response to vibrational stimuli produced by predatory wolf spiders (Araneae: Lycosidae) (Zimmerman and Kight 2016). The ability to recognize and respond to substrate-transmitted vibration, rises with age (Cividini and Montesanto 2018b).

On the other hand, tonic immobility is a reversible state of physical immobility and muscle hypertonicity where animals are unresponsive to external stimuli (Gallup 1974). It cannot be synonymized with thanatosis (i.e., death feigning), which is not necessarily tonic (Francq 1969, Holmes 1916). It also differs in the typical posture (cf. Holmes 1916). Typical tonic immobility posture does not resemble the posture of a genuinely dead individual and differs between Schmalfuss´s (1984) ecomorphological groups (for exact description see Quadros et al. 2012 and cf. Verhoeff 1930). According to Tuf et al. (2015), adopting a posture increases an animal's protection against being swallowed by a predator, while thanathosis reduces the likelihood of being seen. Tonic immobility is therefore used to defend against visually oriented predators (Tuf and Ďurajková 2022, Appendix II). The duration of tonic immobility may also depend on the daily light regime, how it was found in freshwater crabs (Zimmermann et al. 2009) or the coleopteran (Miyatake 2001). Temperature can also affect responsiveness to tonic immobility (Miyatake et al. 2008). However, more research is needed to prove these theories. Quadros et al. (2012) observed intraspecific variation in the duration of tonic immobility in three isopod species (*Balloniscus glaber* Araujo & Zardo, 1995, *Balloniscus sellowii* (Brandt, 1833), and *Porcellio dilatatus* Brandt, 1833). Their study showed a correlation between the duration of tonic immobility and prey survival. Additionally, response can be influenced by the type of stimulus or by the animal´s age. Juveniles of *B. sellowii* use tonic immobility more often compared with older and larger individuals who use more active escape strategies, such as running (Quadros et al. 2012). Size may also play a critical role in the effectiveness of tonic immobility, as smaller animals are more likely to be ignored by a predator (Tuf and Ďurajková 2022, Appendix II). The choice of escape strategy may also be influenced by the individual's personality. *Porcellio scaber* exhibited a notable individual pattern in the duration of tonic immobility, remaining stable not only across treatments but also over time (Tuf et al. 2015).

The simple act of falling from a tree and getting lost in the leaf litter when attacked by birds is another example of the effectiveness of tonic immobility supported by cryptic colouration (cf. Butler 1889). This behaviour is most common in the ecomorphological group of "clingers" (Schmalfuss 1984), which use their short and strong legs to cling tightly to the substratum, leading to tonic immobility to enhance the effect of cryptic colouration (Achouri and Charfi-Cheikhrouha 2009). Thus, an increase in survival rate through tonic immobility can occur due to reduced visibility of the animal (Bergey and Weis 2006) or by protecting the vulnerable ventral body part (Quadros et al. 2012).

Conglobation

The ventral muscles, together with the shape of tergites, enable certain species of isopods to bend inward and thereby protect their soft, vulnerable ventral surface. This tonically immobile posture (typical for families Scleropactidae, Armadillidae, Armadillidiidae, Tylidae, Cylisticidae, or Trichoniscidae) allows them to persist in conditions that may be fatal to other species (White and Zar 1968; Smigel and Gibbs 2008). The smooth surface of the conglobate isopod denies predators find suitable place for attack (Řezáč et al. 2008). Species with the ability to roll up have arched body shapes, often disposed of by additional body part adaptations (see Sutton 1972; Vittori 2025). Of particular interest are the physiological adaptations related to the female internal organs, which are compressed and displaced in ovigerous females, with the eggs condensed in the anterior part of the marsupium (a brood pouch). Despite females possessing oostegites that enable them to bend enough to conglobate (Csonka et al. 2015), in advanced stages of gravidity, females may stop feeding themselves (Appel et al. 2011). Additionally, predator pressure can cause a shortening of the length of the breeding period (Castillo and Kight 2005), supporting the theory that conglobation serves as an antipredatory strategy (Tuf and Ďurajková 2022, Appendix II). Volvation can be a successful antipredatory strategy even in the case of larger or visually oriented predators. A smooth surface can cause prey to drop and roll away with the help of its spherical body. This is specifically important for non-perfect conglobation (typical of the genus *Cylisticus*), with the uropods and antennae sticking out of the protective ball (Tuf and Ďurajková 2022, Appendix II).

Conglobation is tuned to predator cues in a manner consistent with balancing costs and benefits, which may vary between the sexes (Zamora-Camacho 2023). A behavioural study of *A. vulgare* revealed significant differences among individuals in mean risk-taking behaviour (time spent immobile in conglobation), as well as in environmental and time-induced behavioural plasticity and residual variation (Horváth et al. 2019). Conglobation is mostly induced by external stimuli, like strong vibrations or pressure (Horváth et al. 2019), as was observed in the case of *A. officinalis* (Cividini and Montesanto 2018a). Another stimulus may be the smell of a predator. When *A. vulgare* was exposed to chemical cues from predators (toad feces), the animals engaged in conglobation for a longer duration in the presence of the perceived threat. However, this result applied only to males. This sexual dimorphism in anti-predator behaviour may be due to the fact that males are at greater risk of predation than females and thus have developed more sophisticated anti-predator strategies (Zamora-Camacho 2023).

On the other hand, males showed a trend toward higher average risk-taking than females when conglobated following mechanical stimuli (Horváth et al. 2019, 2025).

Additionally, conglobation can be affected by magnetic waves. A study of three terrestrial isopod species (*Armadillidium granulatum* Brandt, 1833, *A. vulgare*, and *P. laevis*) exposed to a static magnetic field showed that *A. vulgare* switched from euspheric to mesospheric volvation, and *A. granulatum* didn't conglobate under the influence of the magnetic field (Béjaoui et al. 2019). In addition to that, the behaviour of wild and captive isopods differs in terms of volvation frequency and the duration of tonic immobility. A study of three isopod species (*A. vulgare*, *A. granulatum* and *A. officinalis*) showed that the frequency of volvation was highly species- and stimulus-specific, and that the duration of tonic immobility, as well as the response delay to each stimulus, was always higher in wild groups than in captive ones. The evolutionary and ecological characteristics of each species, as well as the importance of environmental pressures in shaping the behaviour of terrestrial isopods to optimise their life strategies, may be reflected in these substantial differences (Cazzolla Gatti et al. 2020).

Stridulation

Only one species of isopod, *A. officinalis*, is known to be capable of producing sounds audible to the human ear. This sound is produced by the stridulation of a ridge of scales that is located on the propodus of the fourth and fifth pereopod (Caruso and Costa 1976). This trait is present in both sexes from early developmental stages (Taiti et al. 1998; Montesanto 2018) and occurs in all *Armadillo* species (Schmalfuss 1996). Terrestrial isopods do not have a sense of hearing. However, they can register the vibrations of the ground produced by their stridulation (Cividini and Montesanto 2018a, b). While juveniles usually conglobate, adults react to substrate vibrations by conglobating or moving away from the vibration source (Cividini and Montesanto 2018c). Such a reaction may be caused by associating the vibration with danger (Zimmerman and Kight 2016). *Armadillidium vulgare* and *P. leavis* also showed escape behaviour in response to vibration (Moriyama 2004; Houghtaling and Kight 2006). *Porcellio laevis* systematically alternated its turns in a T-maze in response to those stimuli, if not previously exposed to the vibration (Houghtaling and Kight 2006). Although a sensory receptor has not yet been reported in terrestrial isopods, the high sensitivity of *A. officinalis*, *A. vulgare*, as well as *P. laevis* to vibrations is an indication of its presence (Tuf and Ďurajková 2022, Appendix II). One explanation for why some species of isopods are sensitive to vibrations is intraspecific communication. Stridulating *A. officinalis* can create substrate-borne vibrations and thus alert other individuals to potential danger or adverse conditions. Responding to microvibrations causes aggregation behaviour (Cividini and Montesanto 2018a), which results in higher survival of individuals (Cividini et al. 2020).

Males of *A. officinalis* can also use stridulation to persuade rolled females to unroll and mate with them, as can be seen in deaf giant pill millipedes (Sphaerotheriida) (Wesener et al. 2011). However, studies that provide information on these kinds of behaviours in pill bugs are lacking.

Similar to giant pill millipedes (Sphaerotheriida) (Gravely 1915), terrestrial isopods could also use stridulation to deter predators (Tuf and Ďurajková 2022, Appendix II). Defensive sounds could be said to resemble aposematic signals, differing only in the time of their functionality, which encompasses both day and night. Despite the absence of evidence supporting a mimicry model for *A. officinalis*, which would enable predators to avoid dangerous vibrating prey, stridulation can be an effective defensive behaviour without causing pain to the predator. The generated vibrations can startle predator and cause prey to become dislodged and subsequently dropped, resulting in their loss to the ground. With its spherical shape, the conglobated stridulating *Armadillo* is especially well-adapted to roll away. However, further research in the form of experiments involving experienced and naïve predators of *A. officinalis* is necessary to support this theory of stridulation as the deimatic behaviour (Tuf and Ďurajková 2022, Appendix II).

A threatening posture

Another successful defence against predators can be a threatening posture, as seen in centipedes, solifuges, tarantulas, and scorpions (Kronmüller and Lewis 2015). Several large Iberian *Porcellio* species can bend the rear part of their body upwards, with their long uropods pointing forward and their antennae wide open when disturbed. Orange coloured *Porcellio magnificus* Dollfus, 1892 also likes to adopt this posture. This position is similar to that of a scorpion (Tuf and Ďurajková 2022, Appendix II). There are no less than dozen species of the scorpion genus *Buthus* on the Iberian Peninsula (Teruel and Turiel 2020), all of which have a pale orange-brown colour and thin chelae. It is known that some lizards from southern America (Brandão and Motta 2005) and Asia (Autumn and Han 1989) are also scorpion mimics, although there is no experimental evidence that this behaviour can avert predation. Due to its likely nocturnal activity, it is reasonable to assume that the *Porcellio* can adopt this posture to defend against surface predators during moonlit nights. However, no research has yet been carried out on this subject.

How to explore a new environment

To colonise a new habitat, isopods must become sensitive to environmental novelty, which involves adapting to new conditions in their surroundings. When placed in an unfamiliar experimental enclosure, isopods typically run around and climb up the walls, demonstrating both horizontal and vertical exploration (Anselme 2019). In experiments examining the sensitivity of *P. scaber* to tactile novelty, it was shown that woodlice exhibited increased locomotion time and distance traveled, as well as increased speed, in the novel environment compared to the familiar one (Anselme 2013a). The presence of a survival-related stimulus, such as shelter, in an otherwise unappealing location, like a smooth floor, can attract isopods to that location, reducing the time they spend in a more appealing, though survival-unrelated, location, such as a rough floor (Anselme 2013b). Additionally, random visuo-tactile patterns have an activating effect on the exploratory behaviour of *P. scaber*, and this effect develops more quickly if the woodlice are pre-exposed to these patterns initially. This behaviour is probably related to searching for better habitat conditions, as the presence of a shelter abolishes it (Anselme 2015). Moreover, studies of *A. vulgare* indicate that this species is social, but that this behaviour can decline when exposed to a novel environment. This reduction in social behaviour may serve to decrease the urge to join the larger of the two groups in areas where predation is not a threat, enabling experienced animals to explore new terrain (MacPhail and Thomson 2024).

The behaviour of isopods is influenced by their environment, as was demonstrated in a study involving *A. vulgare* exposed to spherical polystyrene microplastics. Prolonged contact with microplastics in food affected the way individuals took risks. Risk-taking was estimated by the amount of time spent immobile in conglobation following a simulated predator attack. The results showed that exposure to microplastics may make *A. vulgare* more susceptible to predation, which could contribute to population decline (Horváth et al. 2025). Conversely, a study involving car tyre microplastic particles and *Armadillidium pallasii* Brandt, 1833 revealed that animals exposed to microplastics exhibited increased vigilance. While no differences in survival or immune responses were observed, isopods exposed to microplastics exhibited significant weight gain at lower concentrations, but none at higher levels (Torreggiani et al. 2024). The presence of metals also has a significant impact on the behaviour of isopods (Pynnönen 1996; Loureiro et al. 2006; Zidar et al. 2012, 2019; Ghemari et al. 2018), making them relevant models in soil ecotoxicology (Van Gestel and Loureiro 2018).

Conclusion

Terrestrial isopods are an interesting group of specialised crustaceans whose many behavioural and morphological adaptations to the terrestrial environment have not been sufficiently investigated. Although we have some indications of how they cope with predation pressure, experimental verification of their antipredatory strategies is largely lacking. According to current knowledge supplemented by our experiments, it seems that efficiency of the isopods' antipredator strategies is influenced by the predator's food motivation and size, as well as other factors that need to be investigated further. The five studies and the overall review of the antipredatory behaviour of terrestrial isopods provided in the appendix can be used to gain a better understanding of this interesting topic.

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Ďurajková, B., Hladký, R. & Tuf, I. H. (2022): Higher temperature and substrate vibrations as stress factors for terrestrial isopods – model species matter. *ZooKeys*, 1101, 71–85.

Appendix II

Tuf, I. H. & **Ďurajková, B.** (2022): Antipredatory strategies of terrestrial isopods. *ZooKeys*, 1101, 109–129.

Appendix III

Skočková, L., **Ďurajková, B.** & Tuf, I. H. (2025): Predator responses to artificial aposematic and cryptic colouration in terrestrial isopods (Isopoda, Oniscidea). *ZooKeys*, 1225, 141–153.

Appendix IV

Ďurajková, B., Tuf, I. H. & Vittori, M. (2025): Repugnatorial glands and aposematism in terrestrial isopods (Isopoda: Oniscidea: Armadillidiidae, Porcellionidae). *Journal of Crustacean Biology*, 45, ruaf014.

Appendix V

Ďurajková, B., Veselý, P. & Tuf, I. H.: Function of vivid coloration of terrestrial isopods from the point of view of an avian predator. *Insects*. (in rev.)

Appendix VI

Ďurajková, B., Veselý, P. & Tuf, I. H.: Different responses of insectivorous birds to terrestrial isopods. (in prep.)

Appendix I

Ďurajková, B., Hladký, R. & Tuf, I. H. (2022): Higher temperature and substrate vibrations as stress factors for terrestrial isopods – model species matter. *ZooKeys*, 1101, 71–85.

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Higher temperature and substrate vibrations as stress factors for terrestrial isopods – model species matter

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Abstract

This study was focused on behaviour of the Common Rough Woodlouse (*Porcellio scaber*) and the Plum Woodlouse (*Porcellionides pruinosus*) under the influence of stressors in the form of increased temperature, the vibrating surface, or their combination. Two types of experiments were performed. First, woodlice placed in a labyrinth were observed, to determine the degree of turn alternation, the speed of passing through the labyrinth, and the corrections of turn alternation, when exposed to stressors. In the second experiment how woodlice aggregate in the aforementioned potential stressors was recorded and whether the change in aggregation behaviour can be an indicator of the degree of stress. Increased temperature and the combination of increased temperature and vibrations were stressors only for *P. scaber*. The results show that vibrations are not a stress factor for *P. scaber* or *P. pruinosus*. *Porcellio scaber* passed through the labyrinth more slowly at increased temperatures, and although they made more turn-corrections, they alternated turns less intensely. Its aggregation behaviour was mainly influenced by temperature, which confirms that the aggregation behaviour of *P. scaber* actually indicates a degree of stress.

Keywords

Aggregation, Isopoda, Oniscidea, stress factor, turn alternation

Introduction

Humans are not the only ones to face stress. Although this may seem trivial from today's perspective, soil invertebrates such as isopods may also suffer from stress (Elwood et al. 2009). They have to solve existential problems to fulfil their necessities, such as the need for water or moisture (Cloudsley-Thompson 1956), food (Brody and Lawlor 1984), shelter (Allee 1926), and mating (Sutton 1972). This all takes place at constant risk of predation, either by one's own kind (cannibalism) (Sutton 1972) or by other species such as spiders (Gorvett 1956), ants, birds, amphibians, and mammals (Hegarty and Kight 2014). If isopods suffer from a deficiency or excess of any of these factors or the risk of predation, their behaviour may be affected (Sutton 1972). Just like vertebrates, invertebrates respond to stress caused by changes in the environment with a stress reaction. Isopods secrete substances into the haemolymph which are similar to glucocorticoids that can be found in vertebrates (Elwood et al. 2009). Stress can also shorten the maternal care period of the clutch (Kight and Nevo 2004). Isopod response to specific stimuli in nature is influenced by the mutual effect of distinctly intense individual stimuli and also by the current physiological state of isopods (Sutton 1972).

In the present study, we examined two types of defence behaviour of isopods, namely systematic turn alternation and the formation of an aggregation. A tendency to alternate turns is a behaviour known for different organisms including humans. Turn alternations are characterised by two types of reactions. A spontaneous reaction (Richman et al. 1986) is based on environmental stimuli, such as rodents' responses to a new maze (Montgomery 1952), or the reaction of cockroaches to a change in luminosity in a maze (Wilson and Fowler 1976). The second type of turn alternations is caused by the body's internal physiological response (Hughes 1989). Isopods are the most researched group for turn alternation patterns (Hughes 1967, 1978). When facing obstacles, isopods turn in opposite directions to create a deviation from linear motion (Hughes 1989) without being forced to do so by other external factors (Dingle 1965). Turn alternation patterns are probably caused by internal reactions to foot movements (Beal and Webster 1971). Several studies regarding this topic have been carried out (Kupfermann 1966; Hughes 1967, 1978, 1985, 1987, 1989, 2008; Moriyama 1999). Turn alternation pattern enables an escape from places with unfavourable conditions, such as lack of food (Hughes 1978), vibrations (Houghtaling and Kight 2006), the presence of predators (Carbines et al. 1992), and dehydration (Hughes 1967). Thus, turn alternations can serve as an indicator of stress. It is known that terrestrial isopods increase turn alternations in unfavourable environmental conditions to escape effectively, but previous habituation to disturbance can significantly reduce the stressor's effect (Houghtaling and Kight 2006). The extent of stress impact on turn alternation patterns depends on how long the isopods will be exposed to stress factors, i.e., how isopods will accustom or acclimate to a given source of stress (Warburg 1964; Cloudsley-Thompson 1969). Cividini and Montesanto (2018a) investigated the effect of vibrations on the alternate turns of isopods. They observed the increase of turn alternations rate in adult individuals of *Armadillo officinalis* Dumeril, 1816 with the presence of vibrations when compared to *Armadillidium vulgare* (Latreille, 1804). The ability to perceive and respond to substrate-transmitted vibrations, in conjunction with alternate turns, increases with age (Cividini and Montesanto 2018b). Animals are likely to interpret species-specific and non-specific substrate-borne stridulations as a source of potential danger (Cividini et al. 2020). Turn alternation as an antipredatory strategy of woodlice has been examined by many authors such as Carbines et al. (1992), Houghtaling and Kight (2006), Hegarty and Kight (2014), and Cividini and Montesanto (2018b).

The formation of aggregations can be considered as an evolutionary successful reaction to unfavourable temperature, water loss, or predator pressure (Broly et al. 2013). One of the main reasons is that the isopods forming the aggregation makes individuals lose less water and are thus much less affected by the lack of humidity of the environment. Another reason is the reduction of CO_2 production (Allee 1926). Aggregation is mainly affected by thigmotaxis, attraction by individuals of the same species (Devigne et al. 2011), or by negative phototaxis. According to Allee (1926), there are two main types of grouping. True aggregation represents the stacking of individuals' bodies on top of each other with strong cohesion. More diffuse aggregations are typical by the lower number of individuals, higher mobility, and shorter length of contacts. Cividini and Montesanto (2018c) investigated the effect of vibrations on aggregation rates in *A. officinalis* and *A. vulgare*. Consistent with their previous work (Cividini and Montesanto 2018a), they found that *A. officinalis* responded to vibrations significantly and avoided zones of higher vibration intensity. Their ability to form large aggregations was lowered probably due to a reduced ability to find other individuals. In comparison to a sample of individuals with the absence of vibrations, they formed a large number of small aggregations. Even though aggregation behaviour in woodlice was examined in many works (Broly et al. 2013, 2014; Broly and Deneubourg 2015; Pogson 2016), understanding about the impact of aggregation on predation in terrestrial isopods is still relatively weak.

Despite turn alternations, Cividini and Montesanto (2018c) found that stressing conditions can alternate the aggregation behaviour of some terrestrial isopods too. Thus, the goal of this study was to test this claim on different species of terrestrial isopods and environmental stimuli. We evaluated the level of stress of two species exposed to substrate microvibrations, increased temperature, or their combination using turn-alternation in a T-maze. We assumed that both factors are stressful for isopods. Next, we tried to analyse the level of stress on aggregation behaviour under the same conditions.

Materials and methods

Porcellio scaber Latreille, 1804 (9–14 mm length) were hand-picked in an urban area of the village Bučovice, while *Porcellionides pruinosus* (Brandt, 1833) (3–8 mm length) were collected from a garden compost in the town of Hodonín. Woodlice were placed in 17 × 17 × 8 cm plastic boxes with a thin layer of plaster to maintain humidity, with egg cartons used as an underlay. The plaster was kept moist, and isopods were fed on carrots ad libitum. Animals were kept in constant darkness with a temperature of 18–20 °C.

The experiment

The behavioural reaction to two stress factors or their combination was observed in both species. The first factor was increased temperature (27–31 °C, treatment coded as T+) while normal temperature (18–24 °C, called lower temperature and coded at T-) was used as a control. The second factor was the presence of microvibrations (coded as V+) and the absence of vibrations served as a control (V-). The experiments were carried out from August to October 2020. Before the beginning of each experiment, woodlice were exposed to a specific combination of conditions (T+V+, T+V-, T-V+, or T-V-, respectively) for two hours.

Two speakers with a power of 5 W were used to test the responses of the isopods to non-specific vibrations. There was a chipboard plate on the top of the speakers. The vibrations were produced by an artificially created recording, the same as the recording used by Cividini and Montesanto (2018a, b, c). The sound was played on an MP3 player connected to the speakers. Vibrations were measured with an oscilloscope application from a mobile phone placed of a T-maze between speakers, using the VibSensor application. RMS values of vibrations during minute measurement were X: (0.03); Y: (0.04); Z: (0.055) m/s^2.

To measure alternating turn behaviour, we used a plastic T-maze of size 15 × 11 cm consisting of two parts, i.e., the bottom labyrinth part and the cover with a small hole that served as an entry spot for subjects. Isopods were placed into the maze with entomological forceps, and then we observed how they alternate turns when trying to reach one of the six possible ending points. To prevent that woodlouse follow conspecific cues left by the previous woodlouse, the plastic labyrinth was placed on white A4 paper which was replaced after each trial. We also recorded changes in isopod´s turn decision (isopod made U-turn and used opposite corridor), and the time needed for reaching one of the end points of the labyrinth (Fig. 1). If woodlice did not start to move within 3 min, trial was terminated. To simplify the calculation of turn alternation pattern, a specific value was assigned to each end point of the labyrinth (depending on how

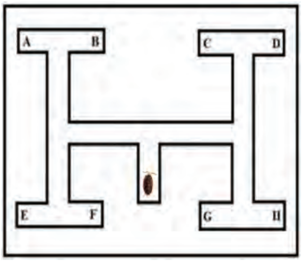

Figure 1. Scheme of T-maze (labyrinth) used for evaluation of turn alternations.

many alternating turns isopod made). Reaching the points A or D with assigned value 1 meant that turn alternation pattern was systematic, i.e., turn left-right-left (L-R-L) or turn right-left-right (R-L-R, see Fig. 1). Points B and C had a value of 1.6 because animals started turn alternation pattern but did not complete it (turns L-R-R, or R-L-L). Ends E and H with a value of 2.3 indicated that isopods did not start with a turn alternation pattern from the beginning but only after the second turn (turns L-L-R, or R-L-L). Reaching the points F and G with the value of 3 was considered the result of not systematic turn alternation (R-R-R or L-L-L).

To observe aggregation behaviour, 30 individuals of the same species were placed into a box, and recorded on camera for two hours. Before the experiment, the plaster inside each box was thoroughly moistened to provide enough humidity. For filming, a small Niceboy outdoor camera installed on a tripod was used. We analysed 12 images (one every 10 min) of each video and calculated the number of isopods touching each other, i.e., the presence of thigmotaxis. After filming, isopods were returned to the breeding boxes. The results are expressed as the average aggregation dynamics for all four variants of observation. In total, 46 aggregation dynamics of *P. scaber* and 49 aggregation dynamics of *P. pruinosus* were analysed.

Data analysis

For T-maze experiments, we analysed the level of turn alternations according to the end point, the time needed for reaching the end point, and the number of changes in turn alternation. For aggregation experiments, the number of aggregated animals (individuals in contact) every ten min were analysed. All results were evaluated using a one-way ANOVA with a significance level of $\alpha = 0.05$. The presence of vibrations with the increased temperature was coded by the number 1, while the absence of both factors was marked as 0. Pearson's correlation test was used to evaluate the dependence of the turn alternation and the speed of passage through a maze.

Results

Turn alternation

Three behavioural characteristics of movements were examined in the maze. The first was the rate of a random ramble (negatively correlated with turn alternation). The second variable was the time spent in the labyrinth, measured from the entry of an isopod into the maze until it reached one of the possible end points. The third variable was the extent of changes in turn alternation pattern, i.e., the number of returns and changes in the turn alternation in the labyrinth. A total of 280 individuals of *P. scaber* and 301 individuals of *P. pruinosus* were tested in this type of experiment.

Our results did not show a statistically significant association between the rate of a random/unspecific ramble (reversed value of systematic turn alternation) and the presence of microvibrations (F = 0.09; p = 0.761) for *P. scaber*. The average rate of a random ramble for the presence of vibrations was 1.74 and for the absence of vibrations was 1.71. There was no significant effect of vibrations on the time spent in the labyrinth (F = 1.45; p = 0.229), although the individuals of *P. scaber* exposed to vibrations ran through the labyrinth with an average time of 38 sec vs. 45 sec with the absence of vibrations. Also, there was no significant association between the presence of vibrations and changing of the turn alternation pattern for this species (F = 0.20; p = 0.657). An average number of changes during the presence of vibrations was 0.87 in contrast with 0.77 during their absence.

In contrast, for *P. pruinosus*, the association between the rate of a random ramble and the presence of microvibrations was statistically significant (F = 5.01; p = 0.026). The average rate of a random ramble during the presence of vibrations was 1.86 and during their absence was 1.67. Isopods made more systematic turn alternation with the absence of vibrations. There was no significant effect of vibrations on the length of the time spent in the labyrinth (F = 0.03; p = 0.862). The average time spent in the labyrinth with the presence of vibrations was 37 sec while with the absence of vibrations it was 38 sec. There was no significant association between the presence of vibrations and change of turn alternation (F = 2.67; p = 0.103). An average number of changes in turn alternation with the presence of vibrations was 1.13 and 0.74 when vibrations were absent.

In the case of *P. scaber*, we found out the significant associations between increased temperature and the rate of a random ramble (F = 21.84; p < 0.001). The average rate of random ramble during exposure to increased temperatures was 1.92, while at lower temperatures it was 1.52. Thus, this species made less alternating turns in an increased temperature environment. Results also showed a statistically significant association between the time spent in the labyrinth and increased temperature (F = 30.65; p < 0.001). Individuals exposed to increased temperatures ran through the labyrinth with an average time of 58 seconds while in lower temperatures it was 25 seconds. Thus, isopods spent more time in a maze when temperatures were increased. We also found a significant association between temperatures and changes in turn alternation pattern (F = 25.56; p < 0.001). An average number of changes of turn alternation during exposure to increased temperature was 1.33 in comparison to 0.30 at lower temperature. An increasing number of changes in turn alternation was observed in woodlice behaviour when the temperature was increased.

For *P. pruinosus*, the associations between increased temperature and the rate of a random ramble were not significant (F = 0.02; p = 0.891). The average rate of random ramble during exposure to increased temperatures was 1.76, while at lower temperatures it was 1.77. There was no significant association between the time spent in the labyrinth and increased temperature (F = 0.79; p = 0.375) for this species. The average time spent in the labyrinth was 35 seconds per individuals exposed to increased temperatures and 40 seconds per those exposed to lower temperatures. We prove the existence of a significant association between temperatures and change of turn alternation pattern (F = 4.44; p = 0.036). An average number of changes in turn alternation during exposure to increased temperature was 1.2; for lower temperatures it was 0.7. For *P. pruinosus*, the increased temperature significantly increased the number of changes in turn alternation pattern.

Combination of increased temperatures and the presence of vibrations has significant effect on rate of random ramble (F = 8.99; p < 0.001; Fig. 2a) for *P. scaber*. The highest average rate of the random ramble was at increased temperatures with the presence of vibrations (2.04) in opposite to lower temperatures with the presence of vibrations (1.47). Also, the effect of a combination of temperatures and vibrations on time spent in the labyrinth was statistically significant (F = 15.80; p < 0.001; Fig. 2c). *Porcellio scaber* ran through the labyrinth the fastest at lower temperatures with vibrations (13 sec) and the slowest at increased temperatures associated by vibrations (66 sec). The vibrations thus increased the isopod´s speed while the increased temperature slowed them down. The effect of the combination of increased temperature and vibrations on changes in turn alternation was significant (F = 9.38; p < 0.001; Fig. 2e) for this species. In average, highest numbers of changes in the turn alternation were made at increased temperatures associated by vibrations (1.55 changes) compared to lower temperatures with presence of vibrations (0.24 changes). The presence of vibrations thus reduced the number of changes in turn alternation, while the higher temperature, on the contrary, increased them.

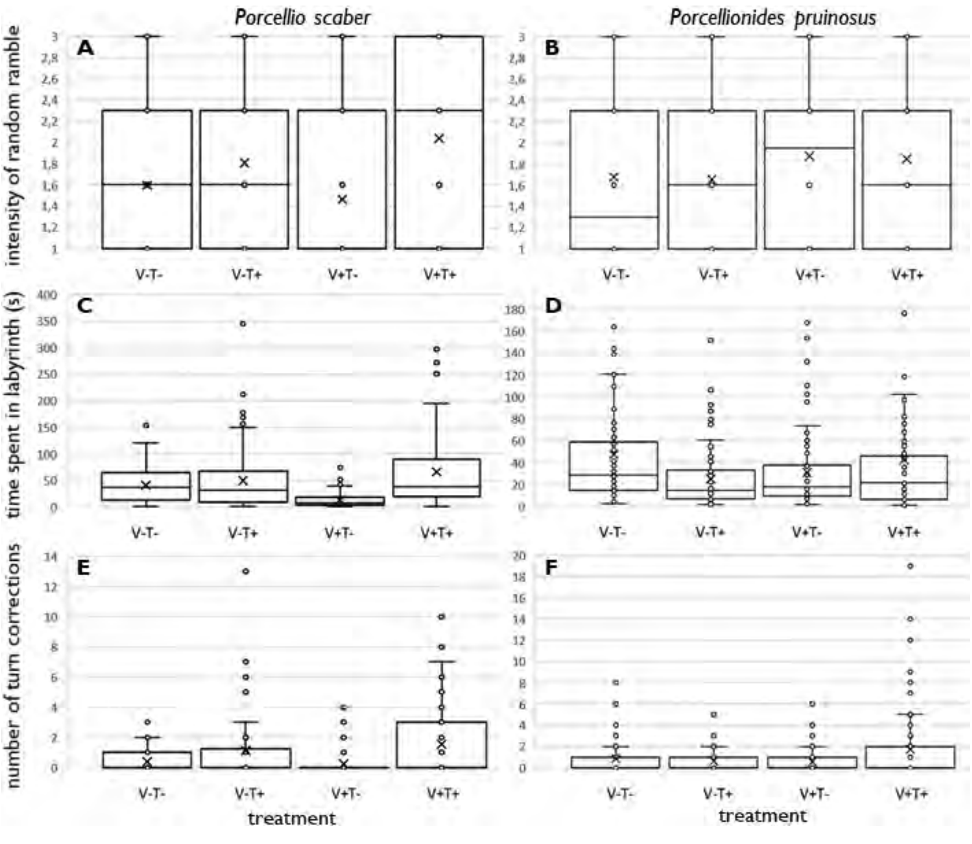

Figure 2. Characteristics of movement in T-maze of *P. scaber* (**a, c, e**) and *P. pruinosus* (**b, d, f**) at different treatments: **a, b** intensity of random/unspecific ramble, i.e., reversed value of systematic turn alternation **c, d** speed of passing through the labyrinth **e, f** number of self-corrective turns. Treatments V-/V+ mean absence/presence of substrate vibrations and T-/T+ mean low/high temperature.

For case of *P. pruinosus*, combination of increased temperatures together with presence of vibrations has no significant effect on rate of random ramble (F = 1.69; p = 0.170; Fig. 2b). The highest average rate of the random ramble was at low temperatures along with the presence of vibrations (1.87) and the lowest at increased temperatures with the absence of vibrations (1.65). The effect of a combination of temperatures and vibrations on time spent in the labyrinth was statistically significant (F = 3.34; p = 0.020; Fig. 2d). Isopods ran through the labyrinth with the highest speed at increased temperatures with the absence of vibrations (25 sec) and the slowest at lower temperatures with the absence of vibrations (47 sec). We proved the existence of statistically significant effect of the combination of increased temperature, vibrations on the change of turn alternation (F = 5.38; p = 0.001, Fig. 2f). On average, highest numbers of changes in the turn alternation were made at increased temperatures combined with the presence of vibrations (1.71) and the lowest numbers when isopods were exposed to lower temperatures combined with the presence of vibrations (0.54).

We also found out a significant weak positive correlation (R = 0.32, p < 0.00001.) between the intensity of the turn alternation of *P. scaber* (i.e., the probability of running to one of the ends, indicating systematic turn alternation like A or D) and the speed of passage through the maze. When *P. scaber* ran slowly, there was higher probability that it will reach one of the "wrong" ends, which indicates unsystematic alternating turns. Results for *P. pruinosus* show no correlation (R = 0.06, p = 0.264619) between the intensity of the turn alternation and the speed rate of passage through a maze.

Aggregation

Group of two or more woodlice in contact were considered to be an aggregate. The distributions of the individuals were determined by counting the number of aggregated individuals in each box every 10 min during the 120-min experiment.

For *P. scaber* the results showed that there is a statistically significant difference in aggregation dynamics of isopods exposed to vibrations (F = 5.71; p = 0.003). Fig. 3 shows that the isopods presented different aggregation dynamics depending on different temperatures. At a lower temperature, ~ 23–26 individuals (out of a total number of 30) were aggregated during the whole two hours of observation (Fig. 3). In comparison, at increased temperatures the aggregations were initially smaller (20 individuals) but increased within half an hour, and stabilised at the number ~ 25–28 individuals (Fig. 3). Aggregations on a vibrating surface were usually smaller than aggregations on a stable substrate.

In *P. pruinosus*, vibrations together with increased temperature had a significant effect on the dynamic and size of aggregation (F = 83.52; p < 0.001). A higher number of aggregated individuals was observed among isopods exposed to lower temperatures combined with the presence of vibrations (after an hour, half of the total of 30 individuals were in aggregations). In comparison, numbers of aggregated isopods were the lowest at increased temperature with the absence of vibrations. In all of the observed variants it can be seen a slight increase in the number of aggregated individuals over time (Fig. 3). Aggregations tended to repeatedly appear and disappear.

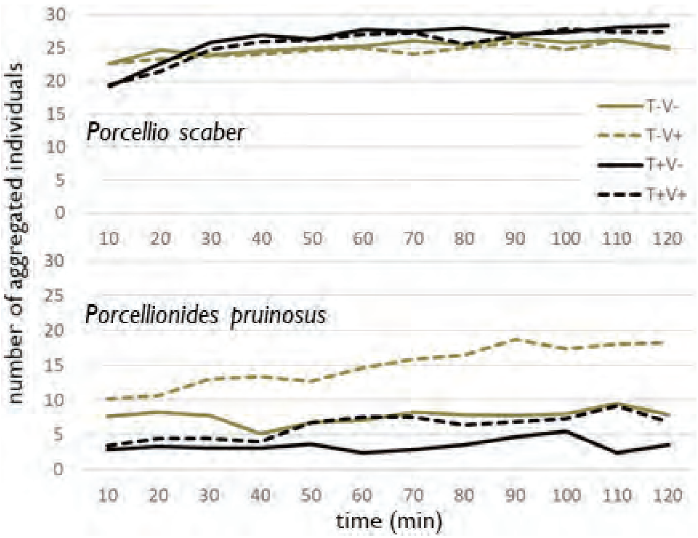

Figure 3. Dynamics of aggregations of *P. scaber* and *P. pruinosus* during 2-hr observations at different treatments. V-/V+ mean absence/presence of substrate vibrations and T-/T+ mean low/high temperature.

Discussion

Turn alternation

Our results showed that the effect of vibrations on the rate of turn alternation of *P. scaber* was not significant. When the substrate did not vibrate, *P. pruinosus* significantly increased turn alternations. For both species, the vibrations did not affect the time spent in the labyrinth or changed turn alternation pattern. This is probably because neither *P. scaber* nor *P. pruinosus* has any stridulatory or auditory organs to absorb vibrations. Those can be found in species like *A. officinalis* that is probably able to generate and receive vibrations (Cividini and Montesanto 2018a, 2020). The presence of mechanoreceptors that detect substrate vibrations in isopods is well documented (Zimmerman and Kight 2016), but the monotonous vibrations probably do not resemble an oncoming predator. The effect of monotonous and interrupted substrate vibrations on isopod behaviour should be studied in future studies.

A different effect of vibrations to turn alternations was reported by Moriyama et al. (2016), who observed woodlice at room temperature. In their study, approximately a quarter of the woodlice exposed to vibrations alternated their directions. Houghtaling and Kight (2006) proved that habituation to disturbance can significantly reduce turn alternations. Insignificant reaction to vibrations could be caused by the fact that before each experiment, woodlice were exposed to vibrations for a period of two hours. The animals could be used to the vibrations, thus the weak response during the experiment.

An increased temperature did not stress *P. scaber*, because isopods spent more time in the maze and alternated their turns less systematically. This result is unexpected because it is contrary to the expectation based on the findings of Warburg (1964) and Schuler et al. (2011), who reported that at increased temperatures, *P. scaber* ran faster. It is also contradictory to the findings of Cloudsley-Thompson (1956), who mentioned that increased temperatures represent a stressful factor for woodlice, mainly due to its impact on water loss (causing their gradual drying and therefore triggering more movement). In contrast, there was no significant effect of increased temperature on the rate of a random ramble or on the time needed to complete the labyrinth in *P. pruinosus*. This species can be commonly found in compost or stacked bales of hay (Frankenberger 1959), which means they could be used to higher temperatures that compost goes through during intense microbial decomposition. *Porcellionides pruinosus* is more tolerant to increased temperatures showing a stable feeding rate for 20 °C as well as 28 °C than *P. scaber* is (Römbke et al. 2011). Meanwhile, *P. scaber* collected in Central Europe has a temperature optimum of 21 °C (Antol et al. 2019), indicating that increased temperature should be considered a stress factor for this species. After exposure to increased temperatures, both species made a higher number of returns or turn alternations. Hughes (1967) reported, that alternation of turns can be caused by the effort to escape from adverse conditions. Moriyama et al. (2016) found that ca. a quarter of the total number of tested woodlice made more returns or changes in the turn alternation pattern. The effect of increased temperatures was also mentioned by Warburg (1964), who found out that the *Oniscus asellus* Linnaeus, 1758 as well as *A. vulgare*, made more alternating turns after the exposure to increased temperatures. In our experiment, *P. scaber* ran slower and alternated turns less systematically at increased temperatures. This could be caused by the fact that before each experiment, isopods were exposed to the tested temperature for two hours. Khan and Khan (2008) reported loss of body mass of the water flea *Daphnia magna* Straus, 1820 in increased temperature resulting from previous hyperactivity. In similar fashion, *P. scaber* could be exhausted and therefore did not show a stronger activity. Also, Ferreira et al. (2016) stated that *P. pruinosus* shows signs of stress when exposed to temperatures above 30 °C, while to the temperatures below 20 °C it does not react at all. Refinetti (1984) and Nair et al. (1989) reported that *A. vulgare* and *Porcellio laevis* Latreille, 1804 can quickly acclimatise to increased temperature. Nevertheless, this may not apply for *P. scaber* from Central Europe, as both mentioned papers deal with (sub)tropical populations.

Vibrations, together with increased temperature, have a significant effect on the rate of random ramble, time spent in the labyrinth, as well as the change of turn alternation in *P. scaber*. These results have the same pattern as those with increased temperature alone. Apparently, vibrations were not stressful for *P. scaber*, probably due to its origin in the city environment. Houghtaling and Kight (2006) reported that urban isopods were adapted to microvibrations. Our results showed that the combination of temperature and vibrations, similar to increased temperature alone, did not affect turn alternation of *P. pruinosus*, potentially due to characteristics of the places they typically occur at. The increased temperature together with vibrations significantly affected changes in turn alternation. Changes in turn alternation were significantly affected by the higher temperature together with vibrations, as well as by the higher temperature alone.

Aggregations

Porcellio scaber showed a statistically significant difference in aggregation dynamics after their exposure to vibrations. At lower temperatures, somewhat stable aggregations of ~ 23–26 individuals were formed. At increased temperatures, within half an hour, the number of aggregated isopods increased to approximately 28 individuals and then stabilised. This is probably because the optimal temperature for *P. scaber* is 21 °C (Antol et al. 2019) (i.e., our experimental "lower temperature"), and because at colder conditions water loss does not occur as quickly as at increased ones (Cloudsley-Thomspon 1956). Woodlice exposed to increased temperatures tend to lose water faster, so they start to aggregate to prevent water loss. Isopods that are in the upper layer of crowded individuals leave the group more often to look for a more suitable place due to the quicker water loss (Allee 1926).

The aggregation of *P. pruinosus* was significantly affected by vibrations along with increased temperatures. Isopods aggregated more when exposed to lower temperatures. In an hour after the exposure more than half of the individuals were aggregated. This is in agreement with results of Cividini and Montesanto (2018c) regarding *A. officinalis*. This is probably related to the finding of Allee (1926), who mentioned that woodlice form two types of aggregations, namely the bunching or true aggregation that is seen in *P. scaber* and crowding or a more diffuse grouping observed in *P. pruinosus*, depending on their mutual contact and interactions. The isopods aggregated less at increased temperatures than at the lower one. According to the findings of Allee (1926), when isopods were under conditions unfavourable to aggregation such as the exposure to low temperatures, the tendency to aggregate increased. However, the increased temperature was not a stressful factor for *P. pruinosus*, probably because this species is used to the increased temperatures. Numbers of aggregated isopods were slightly higher due to vibrations.

Porcellionides pruinosus aggregated in greater numbers during the presence of vibrations than during non-vibration treatment. The same results showed an experiment by Cividini and Montesanto (2018c), who tested the effect of vibrations on *A. officinalis* at room temperature (20 °C). In the non-vibration treatment, isopods quickly began to aggregate and formed a single stable aggregation. However, *A. officinalis* aggregated less with the absence of vibrations. Cividini and Montesanto (2018c) mentioned that isopods could create a sound by rubbing different parts of the body against each other when conglobated, which could evoke other individuals to stay inactive and do not aggregate. Although *P. pruinosus* is not able to stridulate, its antipredatory response is not volvation, only running away and looking for hiding place.

Based on our findings, the pairing model species – stressor can be further refined for more significant results. Further research should be aimed at how stressful various temperature ranges for different isopod species are. Due to the ability of woodlice to acclimate to substrate vibrations, the future use of vibrations in experimental studies is very problematic. Perhaps shorter experiments with low air humidity as a stressor can be less difficult as our knowledge about the demands of different species is sufficient. The ability of different isopod species to habituate to stress factors could also play a certain role in this matter and future studies of this topic are encouraged.

Conclusions

Our results showed that for *P. scaber* and *P. pruinosus*, vibrations are not a stressful factor. This may be related to the fact that tested individuals have been collected in an urban environment where road and rail transport is a permanent source of substrate microvibrations, and the isopods are used to it. The increased temperature was a stressor only for *P. scaber*. This species did not show any major response to increased temperature; it went through the labyrinth more slowly at increased temperatures, and although they made more changes, they alternated turns less intensely. This behaviour could be caused by previous too long exposition to experimental conditions. *Porcellionides pruinosus* was not stressed by the increased temperature, which is probably caused by their occurrence in composts, where the temperature is often increased due to intense microbial decomposition. The aggregation dynamics of *P. scaber* was affected by the increased temperature. Initially, at increased temperatures isopods aggregated less or formed more unstable aggregations than the control group, but later the aggregations were stable and slightly larger than in the control group. Thus, the aggregation behaviour of *P. scaber* shows certain degree of stress, but its interpretation is relatively complicated. We were not able to confirm that *P. pruinosus* was stressed by vibrations or temperature, so it was not possible to make the comparison of the aggregation behaviour and the degree of stress. These results suggest that factors that have been used as stressors for specific species in some studies cannot be automatically used as stressors for other terrestrial isopod species.

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Appendix II

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Antipredatory strategies of terrestrial isopods

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Abstract

Terrestrial isopods (Oniscidea) represent a widespread group of land Crustacea that have been able to successfully adapt to the terrestrial environment and occupy newly formed ecological niches. During the colonisation of land, they faced numerous challenges, including finding an effective way to avoid their new terrestrial predators. In response to predation pressure, they have developed various behavioural and morphological adaptations. These include tonic immobility, conglobation, clinging to the ground, releasing strongly acidic secretions, jumping, and efficient running away. Furthermore, terrestrial isopods can aggregate with other individuals, use stridulation, or change their typical activity time. Some of them also developed spiny tergites and aposematic colouration or posture. The majority of these strategies have not yet been studied.

Keywords

Aggregation, aposematism, behavioural traits, death feigning, defence, mimicry, Oniscidea

Introduction

Oniscidea, commonly called terrestrial isopods, woodlice or pill bugs, represent one of the eleven suborders belonging to the order Isopoda (Pericarida, Crustacea) that first appeared on land during the lower Carboniferous (Schram 1982; Fu et al. 2010). According to Broly et al. (2013), the oldest fossils of Oniscidea come from the Early Cretaceous, but some indications suggest that they could have already appeared in the pre-Pangaean period, most likely in the Carboniferous interval of the Late Palaeozoic. They were able to colonise various terrestrial habitats ranging from sea level to high mountains (Hornung 2011), and are represented by ~ 4,000 species distributed in more than 500 genera and 38 families (Javidkar et al. 2015; Sfenthourakis and Taiti 2015; Dimitriou et al. 2019; WoRMS 2021). Thus, Oniscidea represents the most flourishing crustacean group that has ever colonised the land. As mentioned in many previous studies, oniscid isopods must evolve various adaptations for their terrestrial life. They had to solve ecological and physiological problems, such as respiration, feeding, locomotion, reproduction, and at the same time protect themselves against desiccation in their new terrestrial habitats (Hornung 2011).

As terrestrial isopods colonised land some 300 MA, they faced several predators, such as centipedes, spiders, amphibians, and reptiles. Predator pressures caused Oniscidea to develop various morphological and behavioural adaptations. The lifespan of terrestrial isopods ranges from 1 to ~ 10 years. The highest mortality rate is within the first month of their life outside the brood pouch; this is most frequently due to climate factors, such as high temperatures, drought, and floods, as well as due to cannibalism of different ontogenetic stages (Brereton 1957). As previously mentioned, terrestrial isopods are the prey of a wide variety of predators. Additionally, for some of them (e.g., small passerines such as flycatchers or wrens), terrestrial isopods are a main source of calcium (Bureš 1986; Krištín 1992). Also, there are specialists, such as *Scorpio maurus palmatus* (Ehrenberg, 1828), whose major prey consists of the desert isopod *Hemilepistus reaumurii* (Milne Edwards, 1840) at some localities (Warburg 1993). Other well-known specialists are spiders of the genus *Dysdera* (Řezáč and Pekár 2007), with numerous species using at least three different hunting strategies by means of their specifically adapted chelicerae.

Predators keep up with antipredator adaptations of prey and improve their own hunting techniques accordingly. This never-ending struggle causes the creation of numerous adaptations of both predators and prey, i.e., terrestrial isopods. Although the various types of antipredation strategies among animals are well known, a comprehensive overview on the subject concerning terrestrial isopods is lacking. Therefore, this text provides a systematic review of currently known information regarding the antipredation strategies of terrestrial isopods. The known methods of terrestrial isopod defence against predators, both behavioural as well as morphological, are summarised below, including tentatively proposed strategies.

Not to be there

The simplest strategy to defend oneself against a predator is to keep one's distance from it, and to avoid an encounter with it. A useful strategy is to avoid staying in places where other individuals were killed. Supporting a necromone hypothesis, Yao et al. (2009) experimentally confirmed that terrestrial isopods are deterred by chemical substances (mainly linoleic acid) released by smashed terrestrial isopods (mainly their conspecifics).

In general, movement in a linear path represents the most efficient adaptive escape strategy when precise information about environmental risks for an animal is lacking (Jander 1975). In an open space, terrestrial isopods like *Armadillidium vulgare* (Latreille, 1804) move forward in a straight line (Iwata and Watanabe 1957). When *A. vulgare* and *Porcellio laevis* (Latreille, 1804) were kept in a box with the ants *Tetramorium caespitum* (Linnaeus, 1758), both species escaped the shelters and were more active outside of them as a result of ant harassment. The same effect was caused by an indirect cue from the predatory ants: both species of isopod kept themselves further away from the source of the ant odour (Castillo and Kight 2005).

As terrestrial isopods usually live on the soil surface, it is difficult for them to walk straight and maintain their direction while walking around numerous obstacles (e.g., stones, pebbles, and vegetation stems). The most effective way for them to keep a straight-forward direction is through systematic turn alternations (Hughes 1967). This is the way to reach the greatest distance from a starting point with the highest level of probability (Hughes 1989; Moriyama and Migita 2004). This behavioural pattern is shared by a diverse range of animals, including humans (Lepley and Rice 1952; Grosslight and Harrison 1961; Pate and Bell 1971).

A sophisticated method for testing the stress of terrestrial isopods is by keeping track of their movements through a T-maze (Ono and Takagi 2006). Terrestrial isopods use turn alternation as their strategy to escape from unpleasant places, and the intensity of the turn alternation is used to evaluate their level of stress (Houghtaling and Kight 2006). *Porcellio scaber* Latreille, 1804 more intensively alternated turns when they met a spider (e.g., the dangerous *Dysdera crocata* CL Koch, 1838) than when they met a harmless fly or cotton flock (Carbines et al. 1992). Chronic stress induced by indirect predatory cues (e.g., ant odour) can lead to increased turn alternation in *P. laevis* and *A. vulgare* (Hegarty and Kight 2014), behaviour that leads to the desertion of a dangerous place. Also, these isopods can use small chronical substrate vibrations for the detection of a predator (Zimmerman and Kight 2016). Habituation to the disturbance can significantly reduce turn alternation (Houghtaling and Kight 2006). The similarity in turn alternation of distantly related species of terrestrial isopods suggests evolutionary conservation of these antipredator mechanisms (Hegarty and Kight 2014). It is also known that this escape behaviour is a result of the isopod's own decision-making (Moriyama 1999; Moriyama and Migita 2004) and that *A. vulgare* can correct its turns to increase its level of alternation (Moriyama et al. 2016).

Terrestrial isopods not only engage in spatial predator avoidance, but they also engage in temporal predator avoidance. Terrestrial isopods can avoid encounters with a predator by changing the time range in which they are active. For example, while most terrestrial isopods are nocturnal, the burrowing isopods *H. reaumurii*, from the arid regions of North Africa, the Middle East, and Central Asia, are active in the colder season during full daylight (Nasri-Ammar and Morgan 2005). During the warmest months when the temperature can increase to 45 °C, *H. reaumurii* becomes active before sunrise, thereby exposing themselves to *Scorpio maurus*, their main nocturnal predator. Therefore *H. reaumurii* can switch its typical terrestrial isopod activity from night to day for the sake of reducing the rate of its predation by scorpions. Although no study confirming this claim has been published yet, similar behaviour has been observed in other animals. For example, European rabbits have switched from nocturnal to diurnal activity after the appearance of European polecats, a typical night-time predator, in an area where rabbits were already present (Bakker et al. 2005).

Not to be seen

The tendency "not to be seen" can be an escape mechanism from predators. Some terrestrial isopod species, the so-called "runners" group according to the ecomorphological classification of Schmalfuss (1984), have well-developed eyes and a relatively narrow body with long pereiopods; these traits make them suitable for a quick escape (Schmalfuss 1984). For example, *Philoscia muscorum* (Scopoli, 1763) is well adapted for a fast and surprising retreat thanks to its slim body and long legs (Sutton 1972). An astonishing antipredatory strategy related to "not to be seen" is diving (Leistikow 2001). An example is *Ischioscia hirsuta* Leistikow, 2001, which can hide under the water surface of small streams if disturbed.

A jumping strategy is more unpredictable than a running strategy for a hunter. For several species of *Philoscia* (*Ischioscia*), jumping, akin to springtails (Collembola), was reported (Williams 1941). Van Name (1925, 1926) mentioned leaping or jumping for *Ischioscia nitida* (Miers, 1877, but considered as nomen dubium) and *Ischioscia variegata* (Dollfus, 1893). Leistikow (2001) compared the jumping distance of *I. variegata* and *Ischioscia pariae* Leistikow, 2001 with each other. He described how the first species was recorded jumping up to 20 cm, while the second was recorded only jumping ~ 5 cm. Such distances should be far enough to avoid hunters (e.g., a spider). Leistikow (2001) also mentioned that a 20 cm-long jump was sufficient to escape human collectors. Jumping terrestrial isopods were reported also from Borneo, a species of *Burmoniscus*, and were subsequently assigned to the ecomorphological type "jumper" (Cranbrook and Edwards 1994; Hassall et al. 2006); this, despite the fact that jumpers and runners differ only in behaviour.

Visually oriented larger predators, such as amphibians, lizards, or birds, are attracted by the movement of prey. A very simple strategy related to "to not be seen", not only used by terrestrial isopods, is to stay inactive when disturbed. When *A. vulgare* and *P. laevis* detect a predatory spider, they reduce their activity as a response (Zimmerman and Kight 2016). This behavioural strategy is typical for species of the ecomorphological group "clingers" (Schmalfuss 1984), which have short strong legs, and can cling firmly to the substratum. Their dorsal part is protected by a strong exoskeleton that has broad tergites that expand their body shape, making it impossible to catch them or to turn them when clinging onto the substrate. Another strategy which is, for instance, characteristic for species living on tree bark is to fall down. When a bird tries to detach a terrestrial isopod from a bark, there is a high probability that the isopod will fall off the tree and become lost in the leaf litter around the base of the tree trunk (cf. Butler

1889). The subsequent immobility causes invisibility by the cryptic colouration of the terrestrial isopods, stained by different shades of grey, brown, and beige (Achouri and Charfi-Cheikhrouha 2009).

The tendency of terrestrial isopods to not instigate predators by their movement can also be related to "tonic immobility". This is the state of reversible physical immobility and muscle hypertonicity during which the animals do not respond to external stimuli (Gallup 1974). Immobility is an often-used form of passive anti-predator behaviour adopted by a wide scale of animals, including terrestrial isopods (Quadros et al. 2012; Tuf et al. 2015). Tonic immobility is not a simple synonym of death feigning, i.e., thanatosis. Thanatosis is not necessarily tonic, such as in invertebrates or opossums (Francq 1969); it can also be in a relaxed state, such in some birds, mammals, or snakes (Holmes 1916). Moreover, the typical posture of a tonically immobile individual is usually unlike the posture of a genuinely dead individual, as mentioned by Darwin (cf. Holmes 1916). Typical tonic immobility posture of the clinger ecomorphological group of terrestrial isopods was described by Quadros et al. (2012) as follows: "The contraction of the body to form a comma-like shape and the contraction and folding of the legs towards the ventral side while holding the antennae folded or extended backward and pressed against the dorsal contour of the first pereonites". Terrestrial isopods of the ecomorphological group "rollers" (Schmalfuss 1984) adopt specific ball-like postures, this behaviour is called conglobation (or volvation, cf. Verhoeff 1930).

While adopting a posture increases the protection of an animal against being swallowed by a predator (Tuf et al. 2015), feigning death reduces the probability of being seen by predators. Thus, tonic immobility is a defence strategy against visually oriented predators. There are also some indications that the duration of thanatosis depends on the daily light regime. This was recorded in the freshwater crab *Trichodactylus panoplus* (von Martens, 1869) (Zimmermann et al. 2009) and the coleopteran *Cylas formicarius* (Fabricius, 1798) (Miyatake 2001). Another factor that can influence responsiveness to tonic immobility is temperature (Miyatake et al. 2008). Additionally, the type of stimulus can influence responsiveness, as was proved by Quadros et al. (2012) in their study of three terrestrial isopods *Balloniscus glaber* Araujo & Zardo, 1995, *Balloniscus sellowii* (Brandt, 1833), and *Porcellio dilatatus* Brandt, 1833. The duration of tonic immobility varies intraspecifically, and is related to the survival probability of prey. The antipredator behaviour of terrestrial isopods can be age-dependent, and may change during their life course. For example, *B. sellowii* uses tonic immobility more often when young and small when compared with older and larger individuals that employ more active escape strategies, such as running (Quadros et al. 2012). Body size can also play a crucial role in the effectiveness of tonic immobility because smaller animals are more likely to be disregarded by a predator. The discrepancy between escape and tonic immobility, both effective strategies, can lead to distinguishable personalities of terrestrial isopods, as shown for *P. scaber* (Tuf et al. 2015). Thus, terrestrial isopods can increase their survivorship using tonic immobility in one of two ways: they can either increase their resemblance with the surrounding environment and be less visible (Bergey and Weis 2006), or they can protect their vulnerable ventral surface (Quadros et al. 2012).

Not to be bitten

The soft vulnerable ventral surface of any terrestrial isopod is best protected during conglobation, which allows them to survive in conditions that may be lethal to other species (White and Zar 1968). This behaviour can be found among mammals, such as pangolins, hedgehogs, echidnas (Sigwart et al. 2019), tenrecs, and armadillos. It is also typical for arthropods such as pill millipedes, giant pill millipedes, soil mites, cuckoo wasps (Tuf et al. 2015), multi-shelled chitons (Eernisse et al. 2007), beetles (Ballerio and Grebennikov 2016), cockroaches (Perry and Nalepa 2003), trilobites, and some larvae of other groups (Haug and Haug 2014). This tonically immobile posture is typical among members of the families Armadillidae, Armadillidiidae, Cylisticidae, Tylidae, Helleriidae, Buddelundiellidae, Scleropactidae, or Eubelidae.

The ability to conglobate depends on several body characteristics. The bend of the tergites and the ventral muscles are the most important features that enable conglobation. Aside from an animal's arched shape, there are a wide range of additional body part adaptations common for species capable of conglobation. These include the shape of the head, the shape and length of the antennae, the shape of the epimers of the pereonites, and the shape of the pleon, telson, and uropods. Species with the ability to roll up have often developed head grooves in which the antennae can fit (Sutton 1972). Also, conglobation leads to some adaptations of the female´s marsupium (a brood pouch in peracarid crustaceans). In ovigerous females, the oostegites allow them to bend enough to conglobate (Csonka et al. 2015). As consequence of conglobation, the female's internal organs are compressed and displaced, with the eggs are condensed into the anterior part of the marsupium. As a result, females may stop feeding themselves in advanced stages of gravidity (Appel et al. 2011). Although the marsupium of conglobating species does not protrude as in non-conglobating species, in the last days of the incubation of mancas it can prevent perfect conglobation. The length of the breeding period can be shortened due to the presence of predators (Castillo and Kight 2005), which is indirect support that conglobation is an antipredatory strategy. Additionally, due to the smooth surface of the conglobated isopod, it is more difficult for predators to find a suitable place for attack (Řezáč et al. 2008).

Conglobation is usually triggered by external stimuli, such as strong vibrations or pressure (Horváth et al. 2019). Cividini and Montesanto (2018a) documented that *Armadillo officinalis* Duméril, 1816 responded to substrate vibrations by conglobation. Even against larger, visually oriented predators, conglobation can be a useful adaptation since a ball-like body can roll away and disappear into leaves or debris. This is more important for non-perfect conglobation (typical of the genus *Cylisticus*), because the uropods and antennae are not well protected. Anecdotally, it is cruelly ironic that the typical ball-like shape of a defending *A. vulgare* was the only reason that humans ate them (giving the name of "pill-bug" to all conglobating terrestrial isopods) – its antipredatory strategy was, in this case, a reason for higher predatory pressure (Duméril 1816).

Although intraspecific variability in the use of tonic immobility in the "clinger" species *P. scaber* is high (Tuf et al. 2015), conglobation used by rollers is more constant

(Matsuno and Moriyama 2012; Cazzolla Gatti et al. 2019). In addition to its function for protection against predators, terrestrial isopods can use conglobation to limit water loss (Smigel and Gibbs 2008).

Not to be alone

If a prey species is distributed homogenously, it is easier for a predator to encounter prey frequently and eat ad libitum; therefore, a very simple antipredatory strategy is for prey to be grouped together. Aggregation into groups is considered an evolutionarily successful response to predator pressure, ambient temperature, and water deficits (Broly et al. 2013). The advantages of living in aggregations were described by Allee (1926), and positive density dependency, or the positive correlation between population density and individual fitness, is called the "Allee effect" (Krause and Ruxton 2002). As Krause and Ruxton (2002) pointed out, aggregative defensive behaviour is a common response to the risk of predation, and is widespread among a diverse range of animal groups.

One of the basic characteristics of this type of defensive behaviour is the predator confusion. People who have tried to collect aggregated isopods can confirm that individually handling them can give the majority of isopods enough time to disappear. Even a skilled predator is not capable of eating all individuals in a group. The size of the group has an inverse correlation with the probability that a particular individual in the group will be attacked; i.e., the larger the group, the less likely it is for an individual to be attacked. Actually, a higher visibility of large groups of prey, i.e., a higher attack rate per group, is less important for each individual than is the much lower probability of being eaten while "hiding" in large groups (Krause and Ruxton 2002).

In aggregations, information about an approaching attack can be transmitted from individuals who did observe the danger to those who have not yet noticed it. Such behaviour was documented in *A. officinalis*, which can produce substrate vibrations to warn neighbouring individuals (Cividini et al. 2020). In addition, aggregation can intensify the effect of individual defence mechanisms, such as repulsive secretions or necromones (chemical compounds released by dead terrestrial isopods, cf. Yao et al. 2009), and thus functions as a shared defence behaviour (Broly et al. 2013). The study of Cividini and Montesanto (2018a) proved that the isopod's response to micro-vibrations leads to a greater number of aggregates, considering that micro-vibrations can warn for an impending danger. Aggregation behaviour in terrestrial isopods is thoroughly studied (Broly et al. 2012, 2013, 2014, 2016; Broly and Deneubourg 2015; Pogson 2016); however, a comprehensive study of the impact of aggregation on predation risk in terrestrial isopods is yet to be done.

Only one terrestrial isopod, *A. officinalis*, is known to produce sounds that are audible to humans. This sound is produced by stridulation through a ledge of scales situated on the propodus of the fourth and fifth pereopod (Caruso and Costa 1976). This feature is present in both sexes (Taiti et al. 1998) from the early stages of development onwards (Montesanto 2018) and occurs in all species of *Armadillo* (Schmalfuss 1996).

Although terrestrial isopods do not have a sense of hearing, they can register substrate-borne vibrations caused by their stridulation (Cividini and Montesanto 2018a, b). *Armadillo officinalis* responds to substrate vibrations by conglobation or by deviating from the source of vibration, although juveniles usually conglobate (Cividini and Montesanto 2018c). This response may be caused by the perception that these vibrations are a sign of danger (Zimmerman and Kight 2016). Escape behaviour in response to vibrations was also noted in *A. vulgare* by Moriyama (2004), and *P. laevis* systematically alternates its turns in a T-maze in response to vibrations when not habituated to the vibrations beforehand (Houghtaling and Kight 2006). Although no sensory receptor in terrestrial isopods has been reported yet, the high sensitivity of *A. vulgare*, *P. laevis* as well as *A. officinalis* to vibrations suggests its presence.

Additionally, substrate-borne vibrations induced by stridulations can be a strategy of intraspecific communication. The pill bug *A. officinalis* can probably warn other individuals of imminent danger and adverse conditions, and thus ensures a higher survival rate of neighbouring individuals (Cividini et al. 2020). Indeed, its response to micro-vibrations is an intensification of aggregation behaviour (Cividini and Montesanto 2018a). Perhaps *A. officinalis* can also use stridulation during mating to convince females to uncoil and mate, as the morphologically similar deaf giant pill millipedes (Sphaerotheriida) do (Wesener et al. 2011); however, this topic has not been studied yet.

Stridulation could also work as an antipredatory strategy. In giant pill millipedes (Sphaerotheriida) this function of stridulation was reported more than a hundred years ago (Gravely 1915) as protecting *Arthrosphaera aurocincta* Pocock, 1899 against reduviid bugs of the genus *Physorhynchus*, but without a description of the mechanism. As in the case of *A. officinalis* (Cividini et al. 2020), these millipedes are only able to stridulate during the conglobation (the antipredator behaviour mentioned above). Thus, stridulation, as a secondary form of defence, can be used to discourage predators more effectively. These millipedes stridulate in response to handling (Gravely 1915) in a similar way to *A. officinalis*.

Defensive sounds could similarly be aposematic; that is, they could be the acoustic counterparts of visual aposematic signals, differing only in the way that they can fulfil their function both in daylight and in darkness. Defensive stridulation is known from many species of arachnids, myriapods, insects, as well as crustaceans. Usually, these species use stridulation to warn predators against inflicting an attack on poisonous scorpions, spiders, harvestmen, centipedes, or mutilid wasps (Iorio 2003; Pomini et al. 2010; Esposito et al. 2018; Gall et al. 2018; Stidham 2019). The sounds could therefore fulfil the same role as gaudy colouration (Rowe 2002). Stridulation also can be mimicked e.g., harmless spiders which mimic mutilid wasps in size, colour, and stridulation (Pekár et al. 2020), though this is rarely used as part of the behaviour of a mimicry model. Although we do not have any evidence about a possible mimicry model for *A. officinalis*, which can teach predators to avoid dangerous vibrating prey, stridulation can be effective as a defensive behaviour without a painful experience for the predator. Vibrating can cause prey to be dropped, resulting in its loss on the soil surface. The ball-like shape of conglobated stridulating *Armadillo* makes it predestined to roll away. However, experiments with experienced and naïve predators of *A. officinalis* should be done to confirm this theory.

Not to be edible

When a terrestrial isopod is found and recognised by a predator, there are some other possible strategies it can use to avoid being consumed. A widespread strategy to repulse predators is the use of excretions from the defensive glands. A detailed description of the glands of terrestrial isopods, which are diverse and numerous, was done by Gorvett (1946, 1951, 1952, 1956). There are several different kinds of glands, occurring in almost every part of the body. As pointed out by Gorvett (1951), lobed glands are the largest, as well as the most interesting, of the tegumental glands. They have numerous long ducts that end in separate external openings along the lateral plates and uropods. After strong stimulation, visible droplets of a viscous, smelly secretions appear from these openings. The distribution and size of these glands were studied by Herold (1913), who explained the reduction of their function in some myrmecophilous species, that are defended by tenant ants. On the other hand, the reduction of the function of these glands can be a reason for ant adoption of some myrmecophilous species, as they are chemically insignificant for them (Parmentier 2016), and difficult to recognise in an ant nest.

The substance of lobed glands has a proteinaceous composition with a secretion that is not associated with the hormonal or nervous system; it is instead probably caused by the contraction of adjacent muscles. The stimulation must be very violent: simple shaking or squeezing of the animal does not affect gland secretion, in general (Fig. 1). Gorvett (1956) experimentally confirmed that droplets of secretion appeared

Figure 1. A male of *Porcellio bolivari* Dollfus, 1892 releasing proteinaceous secretion from the uropods (string with droplets) (photograph Adrián Purkart).

after a spider bit a woodlouse on the leg, or after an experimental pinprick. It is apparent, then, that the function of these glands is to produce defensive secretions against spiders that belong to the most significant predators of terrestrial isopods. The pores of the lobed glands are in an optimal position for maximal effect against attacking spiders (Gorvett 1956), centipedes (Paris 1963), or ants (Deslippe et al. 1996). After a spider bites, using its chelicerae, lobed glands begin to secrete defensive secretions, causing the predator to retreat in order to clean its mouth parts. This observation is supported by the fact that terrestrial isopods with missing parts of the uropods or lateral parts are frequently found in nature. This may be caused by shrews' (Brereton 1957) or scorpions' (Herold 1913) incomplete attacks (Gorvett 1956).

Instead of discouraging a predator from an attack using repellent glands, terrestrial isopods may display unpalatability from a distance in order to avoid risking damage. One way to do this is by using a warning aposematic colouration. This strategy is widely adopted by different insects, millipedes (Marek and Bond 2009), and arachnids. Warning colours (usually black combined with red, orange, or yellow) can warn predators of their unpalatability through inherited neophobia or learned avoidance for these colours (Vickers et al. 2021). So far, we have only anecdotal evidence for the aposematic function of colouration in terrestrial isopods. Levi (1965) compared bright red spots on *Armadillidium klugii* Brandt, 1833, the millipede *Glomeris pulchra* CL Koch, 1847, and the widow spider *Latrodectus tredecimguttatus* (Rossi, 1790), all living syntopically near Dubrovnik. All of these species defend themselves chemically, that support their Mullerian mimicry system against attacks from thrushes, gallinaceous birds, nocturnal mammals, and geckos (Schmalfuss 2013).

There are dozens of species with an ostentatious colouration (Fig. 2). Yellow and white spots are quite common in the genus *Porcellio*, but "black" and white patterns (more precisely dark violet-brown and white patterns) were reported for several non-related litter-dwelling species in western Africa (Schmalfuss and Ferrara 1982). Its antipredation function was suggested to not only be a warning colouration, but also a cryptic colouration that dissolves the body outline (Schmalfuss and Ferrara 1982). Some species are capable of polychromatism (Achouri and Charfi-Cheikhrouha 2009), in which individuals with different colour patterns coexist in the same population. Such variability in colour pattern can be useful for individuals having less frequent variations since predators are generally attracted to the most frequent prey type due to perceptual learning ("search image", see Punzalan et al. 2005). Thus, polychromatism is a mechanism of negative frequency-dependent selection, where a rare morph prey experiences a higher survival rate than those of more common types (Hughes and Mather 1986).

Warning colouration works only against visual predators, but there could be another possible method to warn other predators: spines on the dorsal surface of terrestrial isopods. Extravagant pin-like or blade-like spinulation is typical for several species of the families Armadillidae (species of the genera *Pseudolaureola*, *Calmanesia*, *Echinodillo*, *Tridentodillo*, *Globarmadillo*, *Polyacanthus*), Eubelidae (*Panningillo*), and Delatorreidae (*Pseudarmadillo*, *Acanthoniscus*). All of these species are able to conglobate, and are of small size (~ 1 cm at maximum).

Figure 2. Colourful species of terrestrial isopods: **A** *Armadillidium werneri* Strouhal, 1927 **B** *Porcellio duboscqui* Paulian de Félice, 1941 **C** *Armadillidium gestroi* Tua, 1900 **D** *Porcellio haasi* Arcangeli, 1925 **E** "*Merulanella*" sp. 1 from Thailand **F** "*Merulanella*" sp. 2 from Thailand (photographs Adrián Purkart).

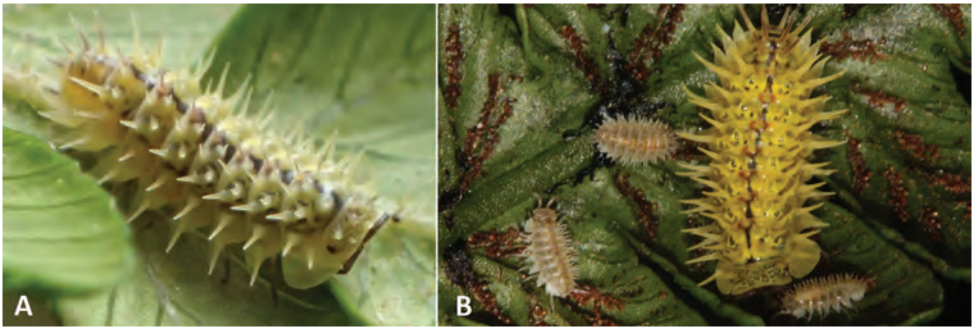

Figure 3. The spiky yellow woodlouse *Pseudolaureola atlantica* (Vandel, 1977), endemic to St. Helena Island **A** its bright coloration and spines can serve as protection from potential predators (photograph Amy-Jayne Dutton, St Helena National Trust) **B** juveniles of *P. atlantica* are well protected as soon as they leave the marsupium, and remain close to their mother (photograph Phil Lambdon).

Figure 4. Threatening posture of a male of *Porcellio magnificus* Dollfus, 1892 resembling the threatening posture of a syntopic *Buthus* scorpion (photographs Adrián Purkart).

Long spines on terrestrial isopods can also, theoretically, be useful against predators (Fig. 3). The spiky yellow isopod, *Pseudolaureola atlantica* (Vandel, 1977), lives on tree fern leaves, and, despite its vivid colour, softens its body outline with its long spines (Dutton and Pryce 2018). The function of long spines has not been studied to date, but it is assumed that spines protect terrestrial isopods against swallowing by geckos and other lizards, frogs, and birds, as well as from ants and other smaller predators (Schmalfuss 1975). Among millipedes, soft-bodied bristly millipedes (Polyxenida) without defence glands wear lateral tufts of setae and use them against ants (Eisner et al. 1996). Perhaps long spines on some small conglobating terrestrial isopods can prevent them against grasping by ant mandibles. A similar function can be ascribed to shorter and stronger thorns, which are more frequent among terrestrial isopods. Strong thorns can also protect larger soil-dwelling terrestrial isopods. The genus *Hemilepistus* (e.g., *Hemilepistus aphganicus* Borutzky, 1958) has strong thorns on the anterior part of the head and posterior margin of the first four pereonites. The function of this armature is to plug the entrance of its burrow, and to protect the individuals inside against small predators and other intruders (Schmalfuss 1975).

A threatening posture, as seen in scorpions, bird-spiders, or centipedes, is another warning signal that protects prey from predators (Kronmüller and Lewis 2015). Several large species of *Porcellio* from the Iberian peninsula are capable of bending the posterior part of their body upward, with their long uropods targeted forward, and with widely outspread antennae (Fig. 4) when they are disturbed. This posture resembles the posture of a scorpion. The Iberian peninsula is inhabited by at least a dozen species of the scorpion genus *Buthus* (Teruel and Turiel 2020), all of which have pale orange-brown colour and thin chelae. *Porcellio magnificus* Dollfus, 1892 is of orange colour and readily takes this posture. Although we lack supporting experimental evidence about whether this behaviour can avert predator attacks, it is known that even some lizards from Southern America (Brandão and Motta 2005) and Asia (Autumn and Han 1989) are known to be scorpion mimics. A scorpion-like threatening posture is not possible to use in a tight shelter or a burrow but only at the surface. Due to probable nocturnal activity, as well as its only superficial resemblance, it is plausible to suppose that *Porcellio* can use this posture during moonlit nights against their predators. However, this topic has not been studied yet.

Conclusions

We have summarised what is known about the antipredatory strategies of terrestrial isopods. Some anatomical and behavioural traits should be classified as pre-adaptations because they help to solve other challenges of woodlouse life, such as the reduction of water loss. Examples include conglobation and aggregation, as well as clinging (Warburg 1993). Additionally, some proposed strategies can execute different functions, e.g., long spines can be used as tactile sensors or for collecting water from fog as well as defence mechanism. On the other hand, the significance of the jumping or scorpion-like threatening posture is difficult to understand without considering the behaviour of its predators. Some of the strategies mentioned above, such as escape by turn alternation, tonic immobility, production of repelling secretions, or conglobation were studied with their respect to predation pressure, but most of them were only suggested to have this protective function against predators. These knowledge gaps deserve attention in future research.

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Appendix III

Skočková, L., **Ďurajková, B.** & Tuf, I. H. (2025): Predator responses to artificial aposematic and cryptic colouration in terrestrial isopods (Isopoda, Oniscidea). *ZooKeys*, 1225, 141–153.

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Research Article

Predator responses to artificial aposematic and cryptic colouration in terrestrial isopods (Isopoda, Oniscidea)

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Abstract

Aposematism is a distinctive or warning signal that provides the animal with protection against a potential predator. Aposematic colouration is easier for a predator to remember and to avoid a dangerous and/or unpalatable prey in the future. We investigated whether distinctive colouration has an aposematic function in terrestrial isopods. The common rough woodlice (*Porcellio scaber*) were used as a model species of terrestrial isopods and the Italian wall lizard (*Podarcis siculus*) as a predatory species. To imitate the distinctive colouration on isopods we marked their dorsal plates with yellow dots. The control group of the woodlice were marked with grey spots. Differences in behaviour (observation, manipulation and consummation) and the lizards' behaviour changes towards aposematically and cryptically coloured prey were analysed. Differences were found in prey observation both between sexes and between prey colours.

Key words: *Podarcis siculus*, *Porcellio scaber*, terrestrial isopods

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Introduction

To leave the sea and fully transition to a terrestrial way of life, terrestrial isopods had to develop many adaptations (Hornung 2011). Different ecomorphological types can be distinguished in this group (Schmalfuss 1984). The first type are the "clingers", which have strong, short pereopods and their body is broad and flat, so they move relatively slowly (genera *Trachelipus*, *Porcellio*). Another type is the "runners", which, on the other hand, move very fast because they have long pereopods and slender bodies (families Ligiidae, Philosciidae). The species that inhabit the soil belong frequently to the group of "creepers", they are smaller, have an elongated body and ribs on the dorsal surface. These species need high ambient humidity. The fourth group are the "rollers", which use conglobation, have a convex body and can roll up (families Armadillidae, Eubelidae). In tropical and subtropical areas, "spiny forms" of isopods are found. They have spikes or thorns on their bodies to protect them from predators and live outside the topsoil (genera *Panningillo*, *Echinodillo*).

Terrestrial isopods have a large number of natural predators. The main predators include centipedes, spiders, various insects, but also insectivorous vertebrates. Predation pressure has helped the isopods to develop various defence and protection mechanisms, whether it be morphological adaptations or behavioural changes (Tuf and Ďurajková 2022). Increased consumption by birds and reptiles is due to an increased need for calcium necessary for eggshell formation (Bureš 1986; Krištín 1992).

Colouration of terrestrial isopods

The body colouration of animals has three main purposes; these are thermoregulation, intraspecific communication, and reduction of predation risk. Body colour and colour pattern play a major role in intraspecific communication, e.g., mate recognition or courtship. Colouration is also important in relation to predation; the animal wants to avoid, deter, or confuse the predator (Endler 1978).

Crustacean chromatophores usually contain a variety of pigments. There are different forms of chromatophores (polychromatic, monochromatic, bichromatic) (Knowles and Carlisle 1956). The brown-black pigment in the melanophores of crabs is melanin, but the dark chromatophore pigments in other crustaceans are ommochromes (Fingerman 1965). The pigments in erythrophores and xanthophores are carotenoids, but there are some exceptions; the most common carotenoid in chromatophores is astaxanthin (Fingerman 1965). Pigments may be distributed freely in the exoskeleton or in chromatophores (Chang and Thiel 2015). Chromatophores are often clustered into larger groups, these multicellular clusters are called chromatosomes. Crustacean chromatophores are asymmetric and mononuclear (Chang and Thiel 2015).

The purpose of cryptic colouration in animals is to reduce the possible detection by predators. In many cases, this colouration can indicate that the animal is trying to blend in with its surroundings (Merilaita 1998). Within terrestrial isopods, such colouration is typical for epigeic species with dusk or daytime activity (e.g., *Porcellio scaber*, *Porcellionides pruinosus*, *Trachelipus rathkei*, *Hemilepistus reaumurii*). During the day, cryptic species usually hide in shelters in or on the soil surface (Davies et al. 2012). Some species are capable of colour-change or pattern transformation on the body. Colour-change, which is based on chromatophores (melanophores and leucophores), is triggered by sensory detection from the surrounding environment (Nery and de Lauro Castrucci 1997). The littoral isopod *Ligia oceanica* uses melanophores to change colour within a circadian rhythm (Hultgren and Mittelstaedt 2015).

In cryptic polymorphism, different colour forms occur within the same species (Veselý et al. 2024). This is because the predator has a certain image in memory (the search image) by which it searches for its prey (Punzalan et al. 2005). Thus, if a predator detects one morph, other individuals with a different pattern are in relative safety (Hughes and Mather 1986; Veselý et al. 2024). This principle protects individuals with rarer colouration, as the predator mainly targets the more numerous colouration pattern of prey. Thus, polymorphism is maintained in the population by predation, as the preference for a particular pattern is determined by the current commonness or rarity of the colouration. However, this principle does not apply to morphs with a high degree of divergence in conspicuousness. Albinos are always conspicuous, even if they are rarer. Within crustaceans, albinism is a relatively rare phenomenon (Geiser 1932). In the isopods, this phenomenon is common in species that live in caves or deeper in the soil. In some species, albinos are also rarely found in natural conditions, but they are regulated by a higher level of predation (Achauri 2009).

Aposematism is a conspicuous warning colouration or other type of warning signal by which an individual alerts potential predator to its (real or perceived) inedibility or toxicity. There are colour combinations that are typical: black or dark brown in combination with yellow, red or orange, or sometimes even white. Stripes or spots on the bodies of aposematically coloured individuals are also common (Davies et al. 2012). Aposematic colouration uses colour contrast, where we can observe as differences in shades or saturations of colour between a given organism and the environment in which it lives. It can also make use of luminance contrast, where the amount of light reflected from the organism and its surroundings varies. Colour contrast is considered important for the effectiveness of aposematism, especially in the case of avian predators and diurnal lizards (e.g., Lacertidae), which have tetrachromatic vision. Conversely, for colour-blind predators, luminance contrast is an important factor that enables them to detect aposematic prey (Prudic et al. 2007). Aposematic colouration is very conspicuous to predators, they can easily spot and recognise it, but also remember it very easily (Prudic et al. 2007). Predators avoid such distinctive individuals, either through innate neophobia (individual avoids things it does not already know) or learned avoidance of a particular pattern or colour (Vickers et al. 2021).

The light spots on the bodies of woodlice are typical for some species. They are often found on species that are active on vertical surfaces, such as rocks or tree trunks, and are typical of the morphotype “clingers” (*Oniscus asellus*, *Porcellio spinicornis*), but also “rollers” (*Armadillidium pictum*, *A. opacum*). Vividly coloured are also the vegetation-dwelling “spiny forms” (e.g. *Pseudolaureola atlantica*).

One theory of the origin of aposematic colouration is that the cryptically coloured toxic species was accidentally consumed by predators who mistook it for a harmless species. They preferentially avoided the more conspicuous specimens that they could readily identify. Through this selection, the conspicuous pattern gradually dominated the prey population (Davies et al. 2012). This theory appears to be applicable to Mediterranean terrestrial isopods, where a similar colour pattern also occurs in unrelated species. The shared colour pattern in syntopic species of millipede, pillbug, and spider was pointed out by Levi (1965) as Müllerian mimicry. If several species have the same predators, therefore the more similar species there are, the more likely the predator will learn to recognize them and not attack them (Davies et al. 2012).

Evidence that distinctive colouration has an aposematic function in terrestrial isopods is still lacking (Tuf and Ďurajková 2022). Yellow and white patches are relatively common in the genera *Armadillidium* and *Porcellio*, but a pattern of a combination of black (dark purple-brown) and white has been observed in related species in West Africa (Schmalfuss and Ferrara 1982) too.

Cognitive abilities of vertebrate predators

The cognition encompasses a set of mental processes that include perception, learning, long-term memory, working memory, attention and, last but not least, decision making (Dukas 2004; Shettleworth 2010). By learning and then remembering aposematic prey, predators can avoid it in the future (Shettleworth 2001).

Birds and diurnal lizards are primarily visual creatures. Both groups have tetrachromatic vision, with four types of cones (Chen and Goldsmith 1986; Pérez i de Lanuza and Font 2016). Taste receptors are relatively poorly developed in birds, with taste buds located at the root of the tongue, in the posterior palate, and in the pharyngeal mucosa (Bill 2007). Olfaction in reptiles is provided by the vomeronasal organ (via the forked tongue) and the olfactory mucosa in the paired nasal cavity (Vitt and Caldwell 2014).

The main aim of this work is to find out whether the colour of the woodlice has an aposematic meaning. We wanted to find out how reptilian predators would react to presented prey and whether colouration would play a role in prey selection. We also investigated whether predatory behaviour and foraging motivation would change in a model predator species over the course of the experiment. We tested the following hypotheses: 1) there is difference in lizards' behaviour towards aposematic and cryptic prey, 2) isopods with aposematic colouration are less consumed than isopods with cryptic colouration, 3) there is no difference in prey consumption between males and females of lizards, and 4) predatory behaviour and foraging motivation of lizards can change throughout the experiment.

Material and methods

Model species

The model prey species was the common rough woodlouse (*Porcellio scaber*). This species has a cosmopolitan distribution (Capinera 2001). It is coloured in shades of brown and grey but may show small patches of colour (black, red, orange, yellow) (Capinera 2001). A number of colour forms have been bred in hobby breeding. Birds, lizards, newts, spiders, beetles, centipedes, and shrews are considered to be the main predators of woodlice.

The collection of woodlice took place in the autumn of 2022 in Olomouc. They were subsequently kept in plastic boxes with lids, inside there was soil, leaves, and shelters (bark, stones), the substrate was kept moist in places, with a constant temperature (18–22 °C). Individuals of 8–10 mm in length were used in the experiments to make them attractive to the predators of interest.

The model reptile predator species was the Italian wall lizard (*Podarcis siculus*). Adults can reach lengths of up to 25 cm and weights of 15 g. The original distribution area is thought to be the Apennine Peninsula (Rivera et al. 2011), but it is now also widespread in the Iberian Peninsula and North Africa. This species is popular among breeders, and the lizards are fed mainly by mealworms and crickets.

Experiments were conducted with four young immature lizards (2 males and 2 females). These lizards were naïve and kept in captivity from their birth. Animals were housed in a terrarium (120 cm × 50 cm) with an 8:16 light regime at the time of the experiment, and the ambient room temperature, but the terrarium also contained a heating pad. Inside the terrarium was a sandy lignocel mixture as a substrate, a water bowl, and bark pieces as a shelter. Before the start of the experiment, the animals were fed by crickets. During the experiment, the lizards were fed only during individual trials to see if their feeding behaviour would change.

Experiment

The experiments were conducted from 24 October to 30 November 2022 according to standard procedures (Exnerová et al. 2006; Veselý et al. 2006; Dolenská et al. 2009). Prior to the experiment, predators had to be placed in empty transparent plastic boxes (57 × 39 × 28 cm) at least two hours before the experiment to ensure habituation and that the experiment was not influenced by fear of the unfamiliar environment (Exnerová et al. 2006). The predators had access to water throughout the two hours. Furthermore, it was necessary to paint the woodlice. Six yellow dots were made on the dorsal plates with nail polish (reminiscent of the colouration of *Porcellio haasi*). The control group consisted of isopods with the same number of spots, painted with grey nail polish (Fig. 1). Thus, both groups were equally altered and differed only in vivid (aposematic) and cryptic colouration. After drawing dots on the isopods, the polish was allowed to dry for at least an hour so that its odour would not interfere with the experiment. As polish nail can affect activity of isopods for more than week (Drahokoupilová and Tuf 2012), fresh new isopods were dotted before each experiment. Its activity one hour after dotting resembled that one of unpainted isopods.

We used selection tests to test whether the isopods' vivid colour has an aposematic function. The selection tests consisted of releasing five aposematically (grey–yellow experimental group) simultaneously with five cryptically (grey–grey control group) painted isopods into the box where one predator was placed, and then observing and recording the predator's behaviour. Before the insertion of the painted prey, the predator was presented with a mealworm to control feeding motivation. In one experiment each lizard experienced a series of ten tests in the following order: mealworm – painted (grey and yellow) isopods – mealworm – painted isopods, etc. Unpainted prey (*P. scaber* without drawn dots) was also presented to predators, always at the beginning and end

Figure 1. Individuals of common rough woodlouse (*Porcellio scaber*) grey painted as cryptic (**A**) and yellow painted as aposematic prey (**B**).

of the experiment. One test lasted for seven minutes. Each predator was tested in this way twice a week for five weeks. Altogether 10 experiments consisting of 10 tests were performed with each lizard giving a total of four hundred observations for each behaviour. Experiments were done during day hours in laboratory with ambient light and temperature. All four lizards were tested on the same days with a time interval of 1.5 hours. Entire experiments were recorded on camera (Niceboy VEGA X PRO) for control possibility.

Predator behaviour was divided into three categories: 1) prey consummation, which involved the direct eating of prey 2) prey manipulation, which involved biting the isopod or devouring it and then spitting it out, and 3) prey observation, which involved turning the head to follow the prey or chasing the isopod. Unless consummation was preceded by a prolonged examination of the prey, this behaviour was not considered as observation. The trials with mealworms were not analysed, they were just used to control for foraging motivation during the whole session.

Data analysis

Graphical representations of behavioural changes (consummation, manipulation, observation) in predators over the course of the experiment were made using MS Excel.

The aim of the two-factor analysis of variance (ANOVA) was to determine whether there were differences between the behavioural factors (consummation and observation). The significance level for the analysis of variance was set at 5%. Predator sex (♂, ♀) and prey colour (A, C) were designated as factors. A separate ANOVA was conducted for each behavioural type. A normality check was performed using graphical display and Levene's test to verify that the assumptions of the ANOVA were met. Due to the failure of the data to follow a normal distribution for one type of behaviour (manipulation), a Kruskal–Wallis test (non-parametric one-factor ANOVA) was performed. The Kruskal–Wallis test tests one factor at a time and compares whether there are differences in variances between the selected groups. The factors tested were predator sex (M, F) and prey colour (A-aposematic, C-cryptic). Two such tests were performed on each factor, and the significance level was set at 5%.

Tukey's test (multiple comparisons test) was performed to determine which factors (and combinations of factors) may influence predator behaviour change. R-Studio software was used to analyses of variance and Kruskal–Wallis tests, followed by graphical representations.

Results

We presented the naïve lizards with yellow painted (aposematic colouring), grey painted (cryptic colouring) as well as with unpainted (*P. scaber* without drawn markings) isopods. During the observed period (i.e., five weeks), the consumption rate of unpainted prey decreased from 65% to 5%.

The most frequent type of lizards' behaviour was the observation of prey regardless of its colouration (Fig. 2), while manipulation of the presented isopods was the least frequent. Consumption of cryptic prey was higher if it

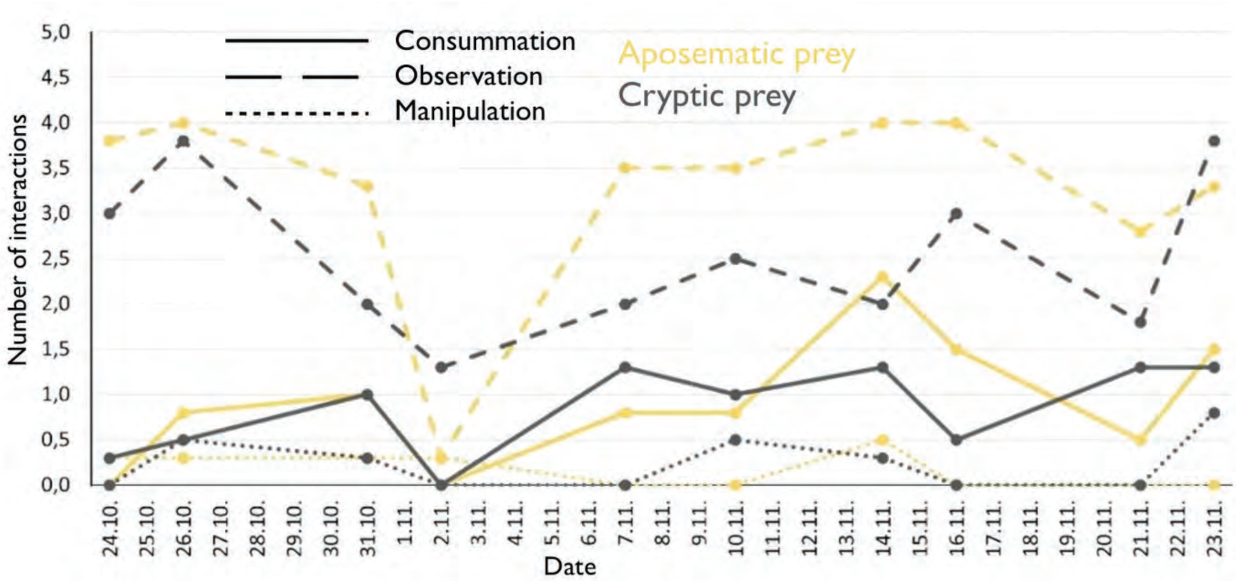

Figure 2. Average number of measured interactions of all tested predatory behaviours of Italian wall lizards (*Podarcis siculus*) towards aposematically or cryptically coloured common rough woodlice (*Porcellio scaber*).

followed a longer time interval (four or more days between experiments) (Fig. 2). For aposematic prey, this relationship was not as apparent. Due to an injury to one female, two of her last trials (24.11. and 30.11.) were not included in the behavioural display.

Differences in lizard behaviour

Two hundred of tests for both males and females (two individuals of each sex, 10 experiments of 10 tests) presented to both types of prey simultaneously were compared. Normality of the data was not confirmed by graphical display, which is not a major obstacle in ANOVA. The analysis of variance is robust to a small failure to meet this assumption, especially if the samples have a size of at least 20, which samples met with exception of data about prey manipulations.

There were statistically significant differences between predator sexes in consummation (ANOVA, $F = 15.72$, $p < 0.001$) but no differences between aposematic and cryptic prey. Significant differences in behaviour are only within predator sex (Fig. 3). Females consumed more prey, both cryptic (significantly) and aposematic (unsignificantly).

The manipulation was relatively rare category thus frequency was tested by the Kruskal–Wallis test, nevertheless, there were no differences between males and females manipulating prey, as well as between aposematic and cryptic prey manipulated ($p > 0.05$).

On the other hand, observation of prey differs significantly between male and female lizards (ANOVA, $F = 5.65$, $p = 0.020$), when females were more interested in observing potential prey as well as between cryptic and aposematic prey ($F = 7.10$, $p = 0.009$), when aposematic prey was more focused. Males observed less cryptic prey than aposematic prey (Fig. 4, $p = 0.047$). Furthermore, there was also a significant difference between females more observed aposematic prey than males observed cryptically coloured prey ($p = 0.004$).

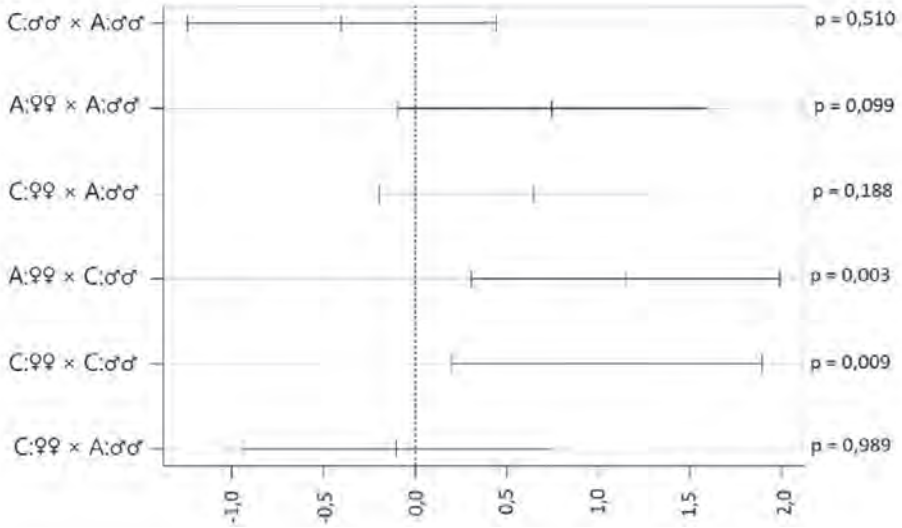

Figure 3. Visualisation Tukey's post hoc tests of consummation of painted common rough woodlice (*Porcellio scaber*) by males and females of Italian wall lizard (*Podarcis siculus*). The mean difference between means of both categories with 95% CI and p-values are presented. Treatment of prey: A – aposematic, C – cryptic.

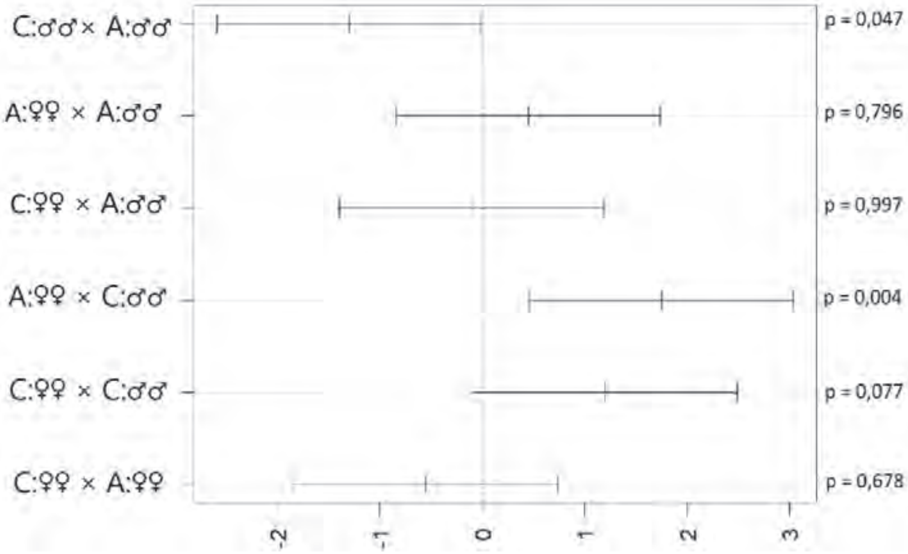

Figure 4. Visualisation Tukey's post hoc tests of observation of painted common rough woodlice (*Porcellio scaber*) by males and females of Italian wall lizard (*Podarcis siculus*). The mean difference between means of both categories with 95% CI and p-values are presented. Treatment of prey: A – aposematic, C – cryptic.

Discussion

We tested the effect of prey colouration on predator behaviour by using choice tests in which lizards were presented with yellow painted woodlice to determine whether it would have an aposematic function.

Already during the interaction with the unpainted prey, it was evident that the predators found the isopods unpalatable; the lizards spat out the prey and chewed it for a long time. This behaviour was also observed in the anole (*Anolis carolinensis*) after consuming the bugs *Neacoryphus bicrucis* and *Lopidea instabilis* (McLain 1984). Woodlice are themselves disgusting, as they produce ammonia, which they excrete from the body via vapours (Sutton 1972) and also a smelly sticky secretion from repugnatory glands. Many animals that exhibit aposematic signals also aid in deterring predators with chemical compounds such as alkaloids (Holloway et al. 1991) or cardenolides (Reichstein et al. 1968). When presented with unpainted prey at the beginning of an experiment, predators consumed more of it than at the end of the experiment. This could have been due to being hungry or curious at the beginning, they certainly did not show the neophobia typical of avian predators (Veselý et al. 2024). In contrast, by the end of the experiment, they had experienced large amounts of presented isopods as distateful prey, and although their foraging motivation (tested on mealworms) was often proven, they were mostly no longer willing to consume the isopods. In experiments with reptilian predators, they orient themselves not only by sight but also by smell, so they may perceive different aposematic signals than birds (Reznick et al. 1981; Hasegawa and Taniguchi 1994; Bonacci et al. 2008; Lee et al. 2018).

For all lizard individuals, the most common behavioural type was observation, with aposematically coloured and also cryptically coloured prey. Less frequently, manipulation or direct consummation of isopod occurred. Prey must move to get their attention, hence the high number of sightings (Sexton 1964). During actual consumption, the odour of the prey plays a large role. Prey odour may be one of the components of aposematic signals in some species (Tseng et al. 2014).

Throughout the experiment, there was no increase in food motivation with the intensity we expected. McLain (1984) conducted experiments with anoles (*Anolis carolinensis*) that he starved for three days before each subexperiment to induce increased appetite. In this condition, the lizards could disregard the odour of prey and attend more to its colouration. Although the rate of interaction with prey increased slightly over the course of the experiments, we did not observe any major changes in their behaviour. On average, males showed less interest in prey than females, which may be due to the different metabolic energy requirements of males and females. Namely, the size of females during the breeding season may affect the number of clutches per year (Galan 1997).

One female lizard had a hind limb injury, so she missed two partial trials (31.10. and 2.11.). At that time the female was fed mealworms to give her enough energy to recover. In subsequent trials she showed a marked increase in interest in the presented isopods, which was probably due to the increased energy required for full recovery.

In experiments with Taiwan japalure (*Diploderma swinhonis*), researchers found that there was a difference in prey generalization between males and females (Ko et al. 2020). The individuals used in the experiments were from the wild. As part of the experiments, they were presented with crickets that were dyed red and green, and were infused with chemicals that ensured that the prey was unpalatable. They were also presented with control prey (black). The females were more cautious after exposure to the toxic prey, and avoided the control group of crickets as well. Males, on the other hand, took more risks and tried the prey even assuming that it might be unpalatable. The researchers explain these differences in behaviour between males and females by the fact that males living in the wild have to defend their territory, which is energetically demanding for them; they are also restricted to hunting only in their territory, so they have to take more risks when choosing food because of limited resources. Females may move across territories of different males, so may be more conservative in their food choices (Ko et al. 2020). In contrast, we observed greater activity by females within as well as outside of sub-trials. Females observed and consumed presented prey to a greater extent than males. The feeding motivation of our female lizards seemed to be more influenced by their young age. These young males had yet to defend any territory. Although the difference in the observation of cryptic and aposematic prey was significant, it was significant only for males.

The predator's size influences feeding behaviour too. Exnerová et al. (2003) found in experiments with *Pyrhocoris apterus* bugs that smaller insectivorous birds (*Parus major*, *Cyanistes caeruleus*, *Erithacus rubecula*, *Sylvia atricapilla*) discriminate well between aposematically and cryptically coloured prey, in contrast to larger (*Turdus merula*) or granivorous birds (*Fringilla coelebs*, *Chloris chloris*, *Emberiza citrinella*). The smaller birds showed nausea when consuming large quantities of unpalatable prey. Perhaps even lizards, after reaching a larger size, will stop being picky and will consume or even discriminate more readily.

In conclusion, the results show that there are differences in prey consumption between the sexes of lizards. Differences were also found in prey observation both between sexes and between prey colour.

In future experiments on this topic, it would be useful to conduct more experiments with different sized lizards. It would also be useful to combine painted prey and preferred prey, such as cockroach nymphs, in selection tests. It would be appropriate to use a different type of prey colouring, such as mixtures of tapioca starch and food colouring, which are free of distinctive taste and odour (Ko et al. 2020).

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: IHT, BĎ. Data curation: BĎ, LS. Formal analysis: LS. Methodology: BĎ, IHT. Resources: BĎ. Supervision: IHT. Validation: LS. Writing – original draft: LS. Writing – review and editing: IHT.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Appendix IV

Ďurajková, B., Tuf, I. H. & Vittori, M. (2025): Repugnatorial glands and aposematism in terrestrial isopods (Isopoda: Oniscidea: Armadillidiidae, Porcellionidae). *Journal of Crustacean Biology*, 45, ruaf014.

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OXFORD

Repugnatorial glands and aposematism in terrestrial isopods (Isopoda: Oniscidea: Armadillidiidae, Porcellionidae)

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ABSTRACT

Invertebrates exhibit a wide diversity in defensive secretions used to deter predators. Terrestrial isopods possess lobed glands, thought to secrete defensive substances, particularly through uropods or lateral plates. Our study explores the defensive mechanisms of species of *Armadillidium* Brandt, 1833 with distinctive aposematic colourations, investigating the secretion properties and histological characteristics of their tegumental glands. Histological analysis revealed the presence of lobed glands across all examined species, with notable interspecific variation in abundance and morphology. While all species produced sticky, viscous secretions from uropod glands, only *Armadillidium gestroi* Tua, 1900 and *Armadillidium klugii* Brandt, 1833 discharged an unpleasant odour from lateral plate glands when exposed to cold. These findings suggest that aposematic species of *Armadillidium* may signal their distastefulness honestly through both chemical and visual cues. Cryptic species or those lacking detectable odour, however, may mimic toxic species without producing chemical defences or can produce substances with an unpleasant taste. The diversity of gland morphology and secretion properties appears to be linked to predator avoidance strategies, such as clinging, running, or rolling, across different isopod ecomorphotypes.

KEYWORDS: *Armadillidium*, Crustacea, distasteful prey, *Porcellio*, chemical signals, warning display

INTRODUCTION

There is great variability in the production of defensive secretions in invertebrates. Such diversity might have been evoked by numerous invertebrate predators, which mainly include arachnids, centipedes, and a variety of insects such as Hymenoptera, Coleoptera, Diptera, and Hemiptera. Many insects, such as beetles, ants, and termites, use toxic substances to discourage predators (Schildknecht, 1970; Pasteels & Grégoire, 1983; Eisner *et al.*, 2005). The larvae of some phytophagous butterflies and moths can store chemicals from their host plants in their bodies and use them for their own defence (Nishida, 2002). Aside from the clade Pancrustacea (Regier *et al.*, 2005), exocrine glands producing defensive substances have been found in several groups of arachnids (Pekar & Raspotnig, 2022). Adult opilionids use scent gland secretion to deter fire ants (Blum & Edgar, 1971). Acetic acid in the whip scorpion *Mastigoproctus giganteus* (Lucas, 1835) appears to be highly repellent to invertebrate predators such as ants and camel spiders (Eisner *et al.*, 1961). Millipedes also effectively defend themselves against ants, beetles, spiders, and slugs (Herbert, 2000) by secreting toxic substances as documented in many studies (Shear, 2015). Defence glands of invertebrates are also widely used against vertebrate predators such as fishes, reptiles, and amphibians. Numerous publications have covered this topic (Eisner, 1970; Burghardt, 1973; Halpern, 1992; Garrett & Card, 1993; Cooper 1994a, b; Eisner *et al.*, 2005; Sugiura, 2020).

Terrestrial isopods are known to have tegumental glands and it has been proposed that a certain type of these glands, the lobed glands, has a defensive function. In most studied species, these glands secrete either through the lateral plates or through the uropods. Some authors distinguish between lateral plate glands, present in the epimera of the pereon, and the uropod glands, present in the pleon and the uropods, but secreting predominantly through the uropods (Gorvett, 1951). Research on their secretory products undertaken so far has shown that the lobed glands that secrete through the uropods produce a sticky proteinaceous secretion that may deter certain potential predators, such as ants and some spiders (Gorvett, 1952; Deslippe *et al.*, 1996). Gorvett observed that after a spider bites an isopod, the proteinaceous secretion leaks out of the isopod´s glands openings, leading the spider to clean its mouthparts and reject the prey. Food with traces of isopod body fluids was also rejected by spiders. Moreover, most spiders

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found isopods unpalatable and avoided their consumption (Gorvett, 1956). The secretion of lobed glands had an equally repulsive effect on ants (Deslippe *et al.*, 1996) and could also affect centipedes (Paris, 1963), scorpions (Herold, 1913) as well as shrews (Churchland, 1994). As suggested by Gorvett (1956), there might be some correlation between the level of development of the lobed glands and the degree of distastefulness. The size and localization of lobed glands were studied by Herold (1913), who suggested a low development of their function in myrmecophilous species protected by ants. A detailed description of tegumental glands was provided by Gorvett (1946, 1951, 1952, 1956) and Weirich & Ziegler (1997).

The distastefulness of invertebrates is often accompanied by a distinctive colour (aposematism), which reduces the likelihood of being attacked by visually oriented predators. While many terrestrial isopods are cryptically coloured, some have conspicuous colour patterns (Schmalfuss & Ferrara, 1982; Tuf & Ďurajková, 2022). Among European terrestrial isopods, brightly coloured species are found particularly in *Armadillidium* Brandt, 1833 although bright spots on the tergites occur in other genera as well (Tuf & Ďurajková, 2022). A well-known example is *A. klugii*, which is popular in pet trade and is known as the "clown isopod" due to its vivid colouration. While there is variability in colouration, these isopods are generally black, with red epimera and three series of yellow or whitish spots on the tergites (Fig. 1A). This species is also the only terrestrial isopod with an aposematic function attributed to its colouration (Levi, 1965; Schmalfuss, 2013).

Should the aposematic colouration of pill bugs be an honest signal of distastefulness, it might be related to the secretion of their lobed glands. Alternatively, the colouration of pill bugs could be a case of Batesian mimicry, in which an edible animal is protected by its resemblance to a noxious one that is avoided by predators, with the isopods producing no chemical deterrents, but simply resembling other unpleasant arthropods. In this work, we aimed to explore whether vividly coloured species of *Armadillidium* produce a detectable odour when disturbed, and whether this odour is linked to the secretion of tegumental glands. We conducted a comparison of vividly and dull-coloured species within two genera of terrestrial isopods. We also aimed to assess the histological properties of lobed glands in these species in order to establish whether specialized tegumental glands are present that might produce distasteful secretory products.

MATERIAL AND METHODS

Examined isopod species

Specimens of *Porcellio scaber* Latreille, 1804 (Fig. 1F), *P. laevis* Latreille, 1804 (Figure 1E), *Armadillidium versicolor* Stein, 1859 (Fig. 1C), *A. klugii* Brandt, 1833 (Fig. 1A), and *A. gestroi* Tua, 1900 (Fig. 1B) were maintained in culture at the Department of Biology, University of Ljubljana, Slovenia at 21°C in separate containers with soil and *Fagus sylvatica* leaf litter, supplemented with Premium Gold fish flakes (Vitakraft, Třanovice, Czech Republic). Specimens of *A. vulgare* (Latreille, 1804) (Fig. 1D) were collected and used immediately. The description and origin of the isopod material are presented in Table 1.

Among the examined species, *A. gestroi*, *A. klugii*, and *A. versicolor* can be considered aposematically coloured. The remaining isopods used in this study were considered cryptically coloured.

Histology

Isopods were killed by decapitation and fixed in 96% ethanol. Prior to embedding, specimens were decalcified in 5% aqueous acetic acid for 2 h. Samples were then dehydrated in an ascending series of ethanol, cleared in xylene, and embedded in paraffin. Sections (7 μm) were cut using an RM2265 rotary microtome (Leica Biosystems, Wetzlar, Germany), transferred to glass slides, stained with haematoxylin and eosin and covered with CV mount (Leica). Micrographs were obtained using an Axioscope microscope (Opton, Oberkochen, Germany) equipped with a DFC290 HD digital camera (Leica). Gland diameter was measured using Fiji software (Schindelin *et al.*, 2012).

Properties of lobed-gland secretions

We observed by chance that placing isopods in the freezer for a few minutes in order to immobilize them resulted in the discharge of lobed glands. To examine the glands' secretory products, we individually placed specimens in a freezer at −20°C in a fresh plastic Petri dish for 5 min. Only the uropod glands in some individuals discharged after this time. If no discharge of the glands in the pereon was observed, additional individuals were exposed for 10 min, after which time, the glands in the pereon would discharge as well. This approach allowed us to examine the secretory products of the uropod and the lateral plate glands separately in all species except for *P. laevis*, in which all lobed glands discharged rapidly.

To assess the smell of the secretory products, the isopods were examined under a stereomicroscope to confirm the discharge of lobed glands, after which the Petri dish was opened and smelled by two researchers. Isopods and the secretory products of their lobed glands were imaged using the SMZ25 stereomicroscope equipped with a Digital Sight 10 camera (both from Nikon, Tokyo, Japan).

RESULTS

Histology

All examined species possess lobed glands in the epimera of the pereon and pleon as well as in the uropods. There are clear differences between them in terms of gland diversity and abundance.

Lobed glands in *Porcellio* Latreille, 1804

There are numerous lobed glands very similar in appearance in the epimera of the pereon of *P. laevis*, varying in diameter between approximately 30 and 130 μm (Fig. 2A). Glands in the pleon, averaging approximately 120 μm in diameter were present, with narrower spaces between lobes, but similar in other respects to the larger glands of the pereon (Fig. 2B).

The lobed glands were less numerous in *P. scaber* than in *P. laevis* in the lateral plates of the pereon, but also very numerous in the pleon and the uropods. Unlike *P. laevis*, the lobed glands in the epimera of the pereon in *P. scaber* can be differentiated into several types. The more abundant type are large glands (about 130 μm in diameter) that stain more faintly, whereas fewer small glands averaging about 50 μm in

Figure 1. Aposematically (A–C) and cryptically (D–F) coloured isopods used in this study. *Armadillidium klugii* (**A**), *A. gestroi* (**B**), *A. versicolor* (**C**), *A. vulgare* (**D**), *Porcellio laevis* (**E**), *P. scaber* (**F**).

diameter that stain more intensely with eosin are located further towards the edges of the epimera (Fig. 2C). A third type of gland that is large (about 150 μm in diameter), stains faintly, and has very thick and poorly defined lobes (Fig. 2C). Glands are large (averaging 140 μm in diameter) in the pleon and stain intensely (Fig. 2D).

Lobed glands in *Armadillidium*

The results were similar for all examined *Armadillidium* species. Only a few lobed glands are present in each epimeron of the pereon, and these glands are of two clearly distinct types (Fig. 3). Large glands average 130–150 μm in diameter in the examined species. Their secretory cells have thick lobes that stain faintly. Small glands average 70 μm in diameter in *A. klugii* and 50 μm in diameter in the other species stain more intensely with eosin. Glands in the pleon and in the uropods are abundant, large (averaging 90 μm in diameter in *A. versicolor* and 120 μm in the other species), and resemble those of *P. scaber* in staining properties, but generally have thicker lobes (Fig. 3).

Table 1. Description and origin of the examined isopod material.

Species	**Size**	**Colour**	**Distribution**	**Origin of specimens**
Armadillidium klugii Brandt, 1833	21 mm	greyish brown with red epimera and white or yellowish dots	Western Balkans	Skradin, Croatia
Armadillidium gestroi Tua, 1900	20 mm	dark to light brown with vivid yellow spots	Northwestern Italy	Purchased from Insectenliebe.de
Armadillidium vulgare (Latreille, 1804)	18 mm	completely black or with small to large yellow spots	introduced globally	Pivka, Slovenia
Armadillidium versicolor Stein, 1859	10 mm	black-brown to olive-brown with pale yellow margins and light spots	Estern and Central Europe	Ljubljana, Slovenia
Porcellio laevis Latreille, 1804	20 mm	dark brown	introduced globally	Purchased from Insectenliebe.de
Porcellio scaber Latreille, 1804	20 mm	dark slaty grey or brightly mottled, cream, brown, orange, and red forms	introduced globally	Črnomelj, Slovenia

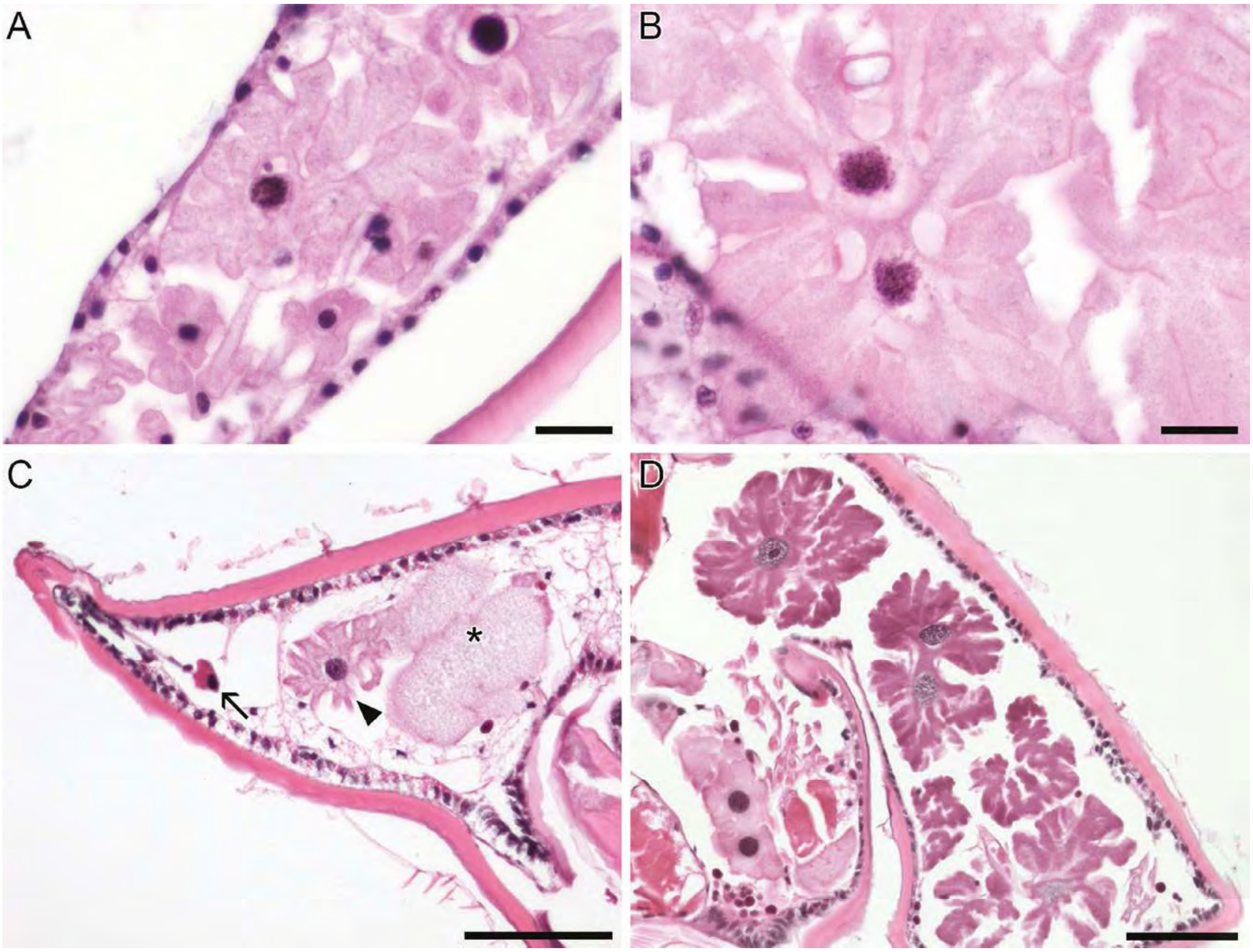

Figure 2. Lobed glands in *Porcellio*. Lateral plate glands in the pereon, with numerous glands of different sizes (**A**) and uropod glands (**B**) in *P. laevis*. Lateral plates in the pereon, with small (arrow), large (arrowhead), and blobby (asterisk) lobed glands (**C**) and uropod glands in the epimera of the pleon (**D**) in *P. scaber*. Scale bars: 20 μm (A, B), 100 μm (C, D).

Properties of the secretory products

All examined species discharged glands on the pereon as well as from the uropods when exposed to cold (Fig. 4, Table 2).

Both the uropod and the lateral plate glands of *P. laevis* secrete large, clear droplets of viscous, sticky secretory products without a detectable odour (Fig. 4A, B). The uropod glands of *P. scaber* secrete products that resemble those observed in *P. laevis* (Fig. 4C). The secretory products of the lateral plate glands, however, are secreted as small, round droplets (Fig. 4D) that are opaque and much less viscous, as they cannot be pulled out into threads.

The secretory products of the uropod glands in all species of *Armadillidium* were secreted as two small, round droplets at

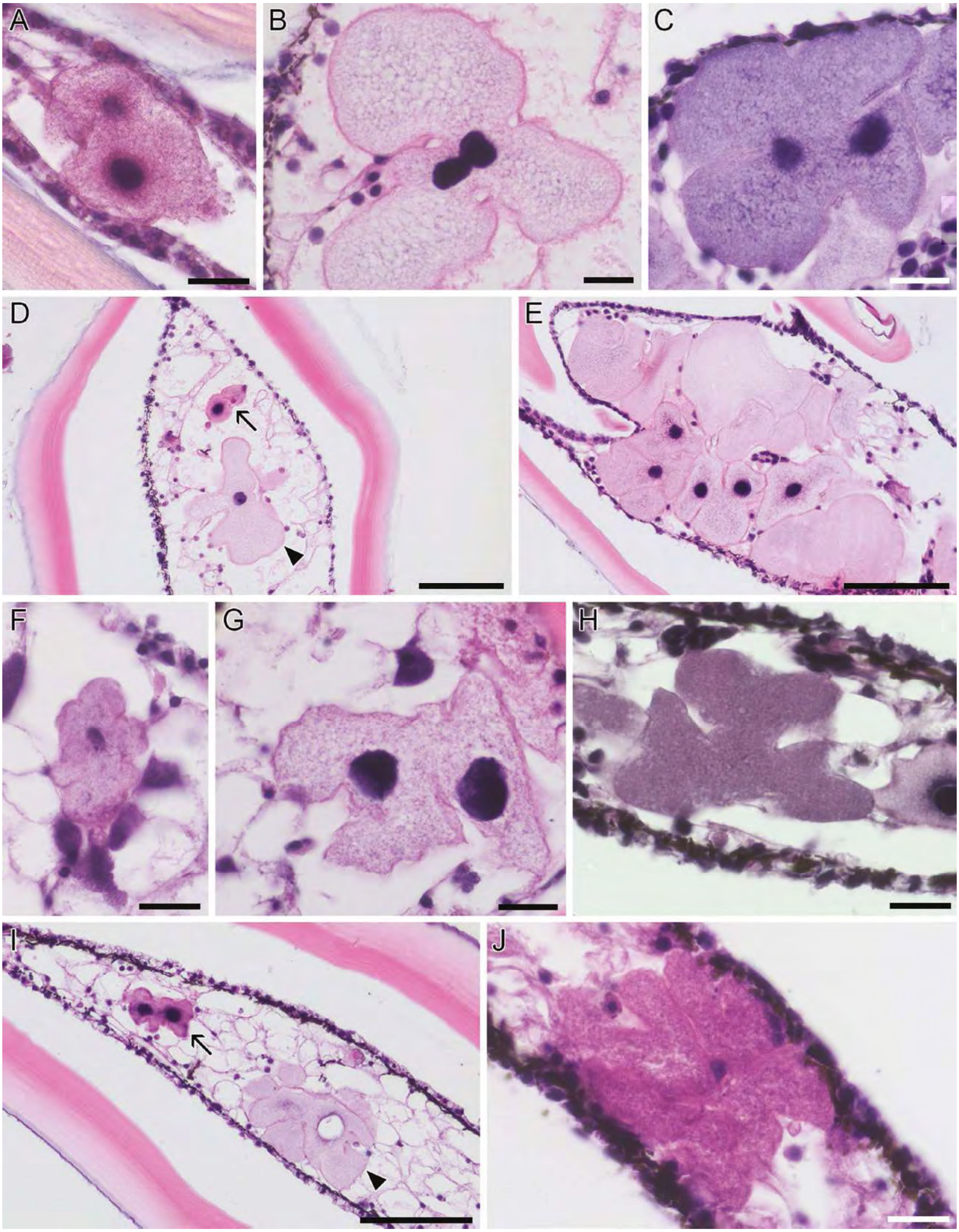

Figure 3. Lobed glands in *Armadillidium*. A small gland in a pereon epimeron (**A**), large gland in a pereon epimeron (**B**) and uropod glands (**C**) of *A. klugii*. A pereon epimeron with small (arrow) and large (arrowhead) glands (**D**) and uropod glands (**E**) of *A. gestroi*. A small (**F**) and large (**G**) glands in a pereon epimeron and uropod glands (**H**) of *A. versicolor*. Small (arrow) and large (arrowhead) glands in a pereon epimeron (**I**) and uropod gland (**J**) of *A. vulgare*. Scale bars: 20 µm (A–C, F–H, J), 100 µm (D, E, I).

Figure 4: Secretory products of lobed glands in *Porcellio* and *Armadillidium*. The viscous uropod gland secretion of *P. laevis* can be drawn out in threads (arrow) (**A**). The similarly viscous lateral plate gland secretion (arrow) of *P. laevis* (**B**). The uropod gland secretion of *P. scaber* (**C**). The watery, opaque lateral plate secretion of *P. scaber* (circled) (**D**). The lobed gland secretion (circled) in *A. versicolor*. The uropod gland secretion (**U**) protrudes from the bases of uropod exopodites (**E**). The lobed gland secretion (circled) in *A. gestroi*. While the uropod gland secretion (U) is odourless, the watery lateral plate gland secretion in this species gives off an unpleasant odour (F).

Table 2. Properties of the uropod gland and lateral plate gland secretion in examined species of isopods. The secretion of the two types of glands differs in all species apart from *Porcellio laevis*. Only the lateral plate gland secretion has a detectable odour in *Armadillidium gestroi* and *A. klugii*.

Species	Uropod gland secretion	Lateral plate gland secretion	Body colouration
Armadillidium gestroi	viscous, odourless	watery, smelly	aposematic
Armadillidium klugii	viscous, odourless	watery, smelly	aposematic
Armadillidium versicolor	viscous, odourless	watery, odourless	aposematic
Armadillidium vulgare	viscous, odourless	watery, odourless	cryptic
Porcellio laevis	viscous, odourless	viscous, odourless	cryptic
Porcellio scaber	viscous, odourless	watery, odourless	cryptic

the base of the uropod exopodites (Fig. 4E, F). These secretory products were slightly opaque, viscous, could be pulled out into threads just like the uropod secretory products of *P. laevis* and *P. scaber*, and had no detectable odour. The secretory products of the lateral plate glands also appeared in small quantity in the form of round droplets. These were, however, clear (Fig. 4E, F), watery, and could not be pulled into threads. In two of the examined species, *A. gestroi* and *A. klugii*, the discharge of epimeral glands resulted in a strong unpleasant odour, despite the minute quantities of the secretory product. The gland discharge of other species did not have a detectable odour, nor was any odour detectable when only the uropod glands discharged in *A. gestroi* and *A. klugii*.

DISCUSSION

Our results demonstrated that all examined species possess lobed glands that differ in morphology, abundance, and diversity among the examined genera.

The histological characteristics of lobed glands in the pereon differ between species, while in the pleon, the glands secreting through the uropods are similar in all species and also produce a similar viscous and sticky secretion without a detectable odour. Glands appear histologically similar in all examined species of *Armadillidium*. As far as gland diversity and histological characteristics are concerned, our results show differences between species in terms of lateral plate gland diversity and histological features, but there are no apparent differences between species with aposematic versus cryptic colouration.

While we found no obvious differences in gland morphology between aposematically coloured and cryptically coloured species, there are differences in the properties of the secretory products of lobed glands. The secretion of the uropod glands is viscous, sticky, and has no detectable odour in all examined species, but that of lateral plate glands differs between species. Most importantly, two examined species of *Armadillidium*, *A. gestroi* and *A. klugii*, give off a repugnant odour as the glands on the pereon discharge, while the other species do not give off a detectable odour.

Certain aposematically coloured species are thus likely distasteful to predators while, among the examined species, cryptic species might not be. The exception to this is *A. versicolor*, which is aposematically coloured, yet it is not demonstrably malodorous. Batesian mimicry is a possible explanation, but one in which different *Armadillidium* species mimic one another, and not spiders, as previously suggested (Levi, 1965).

This shows that certain vividly coloured isopods are indeed signalling honestly, being truly unpleasant to potential predators. Humans as predators almost certainly did not play a role in the evolution of isopod chemical defences, so we must consider these to be directed against other species. In general, olfactory abilities vary not only between predator groups (such as small mammals, insectivorous birds, lizards, amphibians) but also within these groups, with differences both the breadth of the spectrum of detectable substances and the sensitivity to the amount of a particular substance in the air. The sense of smell of insectivores, for example, is very sensitive, but only to a strongly limited spectrum of odours (Marriott *et al.*, 2013). Moreover, olfaction of humans as primates is limited (Matsui *et al.*, 2010). As a result, repugnatorial glands may secrete substances distasteful or poisonous to specific predators, which we are not necessarily able to detect.

While the secretory products of epimeral and uropod glands differed in almost all examined species, they were not noticeably different in *P. laevis*, in which the secretion of epimeral glands resembled that of uropod glands in the other species. *Porcellio laevis* possesses large glands in the pleon and in the pereon that cannot be differentiated into different types histologically. By contrast, diverse glands are present in other species we examined. This simplicity of the types of glands present and the secretions they produce in *P. laevis* may be linked to its mode of defence. Terrestrial isopods can be categorized into several ecomorphotypes in relation to their mode of defence against predators (Schmalfuss, 1984). Among examined species, *P. laevis* is a runner, predominantly escaping predators by moving fast. *Porcellio scaber*, by contrast, is a clinger, with a tough dorsal surface and a broad body and clings tightly (using seven pairs of legs) to the solid substrate (rock, bark) when threatened, protecting its vulnerable ventral side. The examined species of *Armadillidium* belong to a third ecomorphotype, the rollers. They have a thick exoskeleton and roll into a ball when disturbed. It is possible that the uniformly sticky secretion of *P. laevis* is linked to its running mode of defence and the fact that it has little time to deter a predator chemically once it has caught up with it. The clinger *P. scaber* and the rollers in *Armadillidium*, by contrast, may utilize their diverse glands as their thicker tergites allow them to not immediately be squished or pierced by an attacking predator.

While the appearance of the secretory products of the two types of glands have previously been reported for *P. scaber* and *A. vulgare*, and they were reported to differ (Gorvett, 1952), very little is known about the secretory products of epimeral glands in other terrestrial isopods. Only the secretion of the uropod glands has been observed in *Oniscus asellus* Linnaeus, 1758, and it resembled that of the species we studied (Deslippe *et al.*, 1996). The same is true for *Platyarthrus hoffmannseggii* Brandt, 1833 (Gorvett & Taylor, 1960).

We can therefore conclude that gland morphology alone does not necessarily reflect the properties of the secretory products, as similar glands in different *Armadillidium* species produce secretory products that evidently differ in chemical composition, as we were able to detect the odour of some and not others.

The issue left to be considered is that while the gland secretion of brightly coloured *Armadillidium* species certainly smelled intensely, we, as potential predators, were not able to provoke the animals to discharge their lateral plate glands through rough handling alone. This was the case in the examined *Armadillidium* species, which did not discharge any of their lobed glands in response to harassment, and in *P. scaber*, which discharged its uropod glands only. By contrast, *P. laevis* discharged its lateral plate glands as well as its uropod glands readily when grabbed with tweezers. Moreover, only the secretion of the lateral plate glands in the pereon was odorous. It is therefore unclear what causes the lateral plate glands to discharge in real life situations, but it is likely that species with specialized lateral plate glands secrete in response to a specific stimulus, which evidently is not human handling.

As mentioned by Gorvett (1956), the bite of spiders was the only natural stimulus leading to the secretion of lobed glands. No other manipulation by spiders was enough to trigger the chemical defence of isopods. The reluctance of *Armadillidium* to extrude the lobed gland secretion resembles the behaviour of pill millipedes, order Glomerida, which are protected by a secretion that is also sticky and odourless to humans. The synthesis of the secretion of glomerids is very time consuming (it takes weeks to replenish supplies) and it is therefore believed that the secretion can only be used a few times a year (Shear, 2015). This is also why a significant stimulus is needed to expel the secretion; simply handling the millipede is not enough. If an intense stimulus is required to elicit chemical defences in terrestrial isopods, it can be assumed that the inedibility of this prey is not conveyed by smell but by taste. These two senses are primarily neuroanatomically distinct (Derby & Caprio, 2024), but it is nevertheless significant that the defence chemical produced is perceived by the potential predator as it attempts to consume the terrestrial isopod.

Weber (1908) proposed that terrestrial isopods and glomerids are a case of mimicry in which both groups give of a repugnant odour and questioned which of these groups was the mimic. It is impossible to identify the isopods Weber was referring to, but his hypothesis can be interpreted as a suggestion of Müllerian mimicry, in which two or more noxious animals independently evolve similar appearances as a shared protection. Herold (1913) guessed that Weber was referring to *Armadillidium*, as these isopods most closely resemble Glomerida. As Weber maintained *Armadillidium* in culture and never noticed the isopods to give off any specific odour, he dismissed the idea that isopods smelled unpleasantly. Unfortunately, he did not reveal which species of *Armadillidium* he kept.

Levi (1965) interpreted the conspicuous colour pattern of *A. klugii* as a case of mimicry, but his interpretation of what the pill bug mimicked was different. He suggested that rolled up individuals of *A. klugii* resembled the spider *Latrodectus tredecimguttatus* (Rossi, 1790) that lived in the same area in Dalmatia (Croatia) and was known to be unpleasant to vertebrates. His conclusion was based on the claim that *A. klugii* was decorated with rows of red spots on a black background and as it rolled up, these spots would form a pattern closely resembling the pattern of red spots on the black opistosoma of *L. tredecimguttatus*. Furthermore, glomerids in the area also possessed rows of red spots and might have mimicked the same spider. Nonetheless, millipedes are also poisonous. The tergites of glomerids have unpaired ducts of repugnatory defence glands that produce the quinazoline alkaloids glomerin and homoglomerin (Shear, 2015). Their high toxicity to mice was demonstrated by Schildknecht & Wenneis (1967), who also registered strong negative reactions from mice, birds, and amphibians fed with *Glomeris*. Furthermore, wolf spiders are sedated by this secretion (Carrel & Eisner 1984).

Levi (1965) attributed the yellow or whitish appearance of the spots of *A. klugii*, reported by others, to the discolouration in ethanol during preservation. As Levi supposed that isopods are not distasteful, he suggested that *A. klugii* was a harmless mimic of the venomous spider, an example of Batesian mimicry. This idea was later also summarized by Graham (1986). While the shape and pattern of the spots in *A. klugii* truly resembles that of *L. tredecimguttatus*, these markings are white or yellow also in living individuals and we have not yet seen convincing evidence of the existence of *A. klugii* with red markings (Fig. 1A). Nevertheless, *L. tredecimguttatus* is variable in colouration and there are spiders with yellowish markings. Taking this into account, Schmalfuss (2013), in his detailed systematic study of *A. klugii* and related species of *Armadillidium*, found the idea of mimicry plausible, but with yellow-spotted isopods mimicking yellow-spotted spiders. Schmalfuss, however, rightly pointed out that the idea of Batesian mimicry in this case had never been tested. It is not known whether or not potential predators that have had experience with *L. tredecimguttatus* avoid *A. klugii* as a consequence of its colour pattern. Nevertheless, the sympatric presence of a venomous spider, toxic millipedes and foul-smelling pillbugs resembles Müllerian rather than Batesian mimicry, with all participants profiting from the presence of other similarly coloured species. Based on its toxicity and unpalatability, Shear (2015) also suggested *Glomeris pulchra* Koch, 1847 as a co-model for the aposematically coloured black widow spider.

There were others who argued that isopods give off an unpleasant odour from their lobed glands. Verhoeff (1907) believed that such glands protect against predators by secreting a malodorous secretion. His argument in favour of this hypothesis was that isopod *Trachelipus arcuatus* (Budde-Lund, 1885), when placed in ethanol, stained the ethanol light green and gave it a distinctive odour. Verhoeff believed this to be the result of the secretion of lobed glands, but this has not been clearly demonstrated. Collinge (1921) further stated that *Porcellio dilatatus* Brandt, 1833, when disturbed, produced a secretion that could be drawn out in threads and had a distinctive unpleasant odour. Gorvett (1952) claimed that the secretion of lobed glands in *P. scaber* smelled of butyric acid, and that the ethanol the animals were fixed in smelled similarly as a result. He reported that the secretion of the lateral plate glands of *P. scaber* was watery and malodorous, while the secretion of the uropod glands was much more viscous, sticky, and lacked a detectable odour. Our findings are in agreement with this in all respects except for the smell of the lateral plate gland products, which we were not able to detect.

The malodourous discharge of the studied species of *Armadillium* and *Porcellio* comes from the discharge of lobed glands in the pereon, as it is only detected when these glands discharge, but further verification is required. No clearly specialized glands have evolved for the secretion of such substances. Instead, existing lobed glands secrete different substances in different species.

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Appendix V

Ďurajková, B., Veselý, P. & Tuf, I. H.: Function of vivid coloration of terrestrial isopods from the point of view of an avian predator. *Insects*. (in rev.)

insects

MDPI

Article

Function of vivid coloration of terrestrial isopods from the point of view of an avian predator

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Simple Summary: The ability of terrestrial isopods to protect themselves effectively from predation by birds has never been tested. Some species show conspicuous coloration, which might suffice as an aposematic signal. We tested the responses of Great Tits captured in the wild to five isopod species prey, we compared bird responses to isopods with reactions to the Guyana spotted roach, very similar to isopods in size and appearance. Isopods were generally better protected from bird attacks than roaches; however, their color pattern did not affect the level of protection. Birds were able to differentiate isopods from the roach, very likely using detailed features like antennae shape or manner of locomotion as a cue. In experiments, where we presented isopod and roach individuals together, the birds hesitated longer in attacking and observed both prey items for a longer time.

Abstract: (1) The ability of terrestrial isopods (Crustacea: Isopoda: Oniscidea) to protect themselves effectively from predation by birds has never been tested. They are equipped with glands producing chemical substances, moreover, some species show conspicuous coloration, which might suffice as an aposematic signal. We evaluated palatability of isopods by bird. (2) We tested the responses of *Parus major* captured in the wild (and thus possessing some experience with common native isopod species) to the following isopod species: *Porcellio scaber* (native, inconspicuous), *Oniscus asellus* (native, moderately conspicuous), *Armadillo officinalis* (non-native, moderately conspicuous), *Armadillidium versicolor* (native, conspicuous), and *Armadillidium gestroi* (non-native, conspicuous). We compared bird responses to isopods with reactions to the *Blaptica dubia*, an edible roach very similar to isopods in size and appearance. (3) Isopods were better protected from bird attacks than roaches; however, their color pattern did not affect the level of protection. Birds were able to differentiate isopods from the roach; in experiments, where we presented isopod and roach individuals together, the birds hesitated longer in attacking and observed both prey items for a longer time. (4) Non-native species either profited from generalization of the protection of native isopods or from neophobia. Some isopods elicited significantly more discomfort behavior in birds, suggesting differences in the chemical protection among tested species.

Keywords: antipredatory strategy; aposematism; warning signal;

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1. Introduction

Arthropods represent a group commonly armed with specialized chemical substances that serve both as a defense against predators but also in capturing prey, as can be seen in the case of the Hymenoptera´s sting apparatus or in the poison glands of centipedes [1]. Nevertheless, the use of chemicals for purely defensive purposes appears to be frequent across many arthropod species [1]. Apart from venom glands, chemical defense includes a broad spectrum of repugnatorial or odoriferous integumentary glands, the secretions of which are not injected, but applied topically in the form of liquid or as a vapor [2]. Such glands have been found in centipedes [3], millipedes, arachnids [1,4], and many species of insects [5] as well as crustaceans [6]. A detailed description of arthropod defensive secretion was summarized in Roth and Eisner [2]. Additionally, the bodies of some moth and butterfly larvae store secondary plant compounds to enable the manufacture of their own toxins [7]. Predators can detect toxins from their potential prey and avoid attack or consequently spit out or regurgitate the unpalatable prey. Deterrent chemicals thus may protect arthropods from being swallowed when attacked [8].

Nevertheless, the prey usually minimizes the risk of being attacked or even swallowed and regurgitated and thus species that are unprofitable prey often display their unpalatability for predators through aposematic signals [9]. Respectively, natural selection, via predation, favors warning signals helping prey to inform a potential predator about its protection. The toxicity or unprofitability to a potential predator is mostly signaled through conspicuous coloration or patterning [10], but also specific behavior or acoustic signals can be used [11]. This type of coloration is easy to learn [12], recognize and memorize by predators [13]. Aposematic color patterns consist of two types of contrast [14]. While luminance contrast describes dissimilarity between the light reflection of the object and its background, chromatic contrast indicates differences in hue or intensity of color between an object and the background on which it occurs [15,16]. Experiments with avian predators have indicated that color contrast is crucial to the effectiveness of aposematic signals. Nevertheless, the visual ability of predators differs according to their color discrimination capabilities [14], whereas bird and reptile sight is tetrachromatic [17] The luminance contrast component of the aposematic coloration of prey can also function as an effective warning signal, even for color-blind predators [14].

Aposematic patterns provide advantages to both prey and predators, which may have facilitated their evolution from ancestrally cryptic coloration [18,19]. The selection of the warning coloration could also be affected by prey profitability. Highly profitable prey is often consumed by predators who prioritize nutritional benefits despite the negative effect of toxins [20,21]. Thus, the benefits of toxicity signaling through ostentatious coloration may be decreased in larger prey [22], nevertheless this relationship also depends on the size of the predator. Meta-analyses of different taxa have shown a positive relationship between the strength of chemical defenses (i. e. toxicity level) and the overall conspicuousness of the animal. This supports the suggestion that aposematism is a quantitatively honest signal [23]. Warning coloration is widely utilized by various insects [10,24], arachnids [4] as well as millipedes [25].

In the case of terrestrial isopods, there is little evidence of the use of chemical defense. Tegumental glands were described in detail by Gorvett [26–29]. Although numerous and varied glands can be found in almost every part of the isopod body, the defense function was suggested mostly for deeply lobed glands, which occur in the abdominal segments, uropods, and lateral plates. Their proteinaceous stinking secretion is induced only by a violent stimulus (cf. types of violence in [29]). Gorvett [29] stated that the secretions of the lobed glands are primarily directed against spiders as the main predators of isopods. He proved that most spiders considered isopods as distasteful and avoided their consumption. The spider's bite triggered the release of a secretion from the openings of the isopods' glands. Thus, the localization of lobed glands ensures the maximum effect against the attacking spider. Spiders also rejected food material marked by the fluids of woodlice [29]. Those glands can also serve as a defense against scorpions [30], centipedes [31], ants [32], or shrews [33,34]. Herold [30] suggested that the weak development of the lobed glands in myrmecophilous genera such as *Platyarthrus* Brandt, 1833 and *Lucasius* Kinahan, 1859 is caused by their coexistence with ants, providing them protection. This supports Gorvett' s claim about the correlation between the degree of distastefulness and the level of development of such lobed glands [29].

Although aposematic coloration is widespread among many arthropods, there is little evidence for its function in terrestrial isopods. Schmalfuss and Ferrara [35] reported that a dark background color contrasting with whitish spots and stripes in several African isopod species have no intra-specific function and serve as a defence against visually orienting predators by optically dissolving the body silhouette. Levi [36] compared three unrelated species with bright red spots on a glistening black body (the pillbug *Armadillidium klugii* Brandt, 1833, the widow spider *Latrodectus tredecimguttatus* (Rossi, 1790), and the pill millipede *Glomeris pulchra* CL Koch, 1847), all living near Dubrovnik, Republic of Croatia. Levi suggests that these species use Müllerian mimicry, and their chemical defense and conspicuous coloration are directed against attacks from nocturnal mammals and reptiles (during cloudless Mediterranean summer nights), and avian predators such as thrushes and gallinaceous birds [37].

Birds possess among the most advanced color vision abilities in the animal kingdom [38]. Avian retinas are among the most complex of all regarding vertebrates, allowing most birds to obtain information from the environment primarily via their visual sense [39,40]. Their tetrachromatic color vision is based on four visual pigments and has been described in detail [38,41–45]. Avian predators can learn how to avoid unpalatable prey based on their conspicuous coloration [13,20,46–48] or contrasting patterns [49,50]. Additionally, some studies have reported that aposematic prey remain relatively unharmed after bird attacks [51,52].

Moreover, birds have a remarkable ability to respond to chemicals found in their environment during activities such as foraging, navigation, or nest building [e. g. 53–57]. Avian species have also developed chemosenses such as olfaction [58,59], taste [60,61], and a trigeminal system [58,62], which all help them process chemical sensations. One function of those chemosenses is to detect and obtain suitable prey [63]. Birds can differentiate between palatable and unpalatable prey and use taste to control their chemical intake depending on the defense levels of the distasteful prey [64]. This corresponds with the finding that domestic chicks taste and reject aposematically colored prey [12,65].

The coloration of many isopods varies from grey to dark brown or even dark grey, thus we can consider them cryptic-colored species. On the other hand, brightly colored species are also well known. Some isopods have bright yellow dots with unknown function on the dorsal part of the pereon. Our research aim was to examine whether the conspicuous coloration of terrestrial isopods could serve as a warning signal for a visually oriented predator, the Great Tit. In the present study, several species of isopods, with different colorations, and different probabilities of the Czech Great Tits having encountered them in the wild, were tested.

We tested the following hypotheses:

- All isopod species are protected against attack by birds better than edible roach.
- Isopod species with conspicuous coloration are protected against attack by birds better than inconspicuous species.
- Birds do not show any disgust after eating isopods.
- Birds prefer attacking roaches rather than isopods when encountering both simultaneously.

2. Materials and Methods

2.1. Prey

We used five species of terrestrial isopods (Fig. 1a–e):

- The Common Rough Woodlouse *Porcellio scaber* Latreille, 1804 (coded as scaber throughout the text): Body size up to 17 mm, nearly twice as long as wide. The body is strongly irregularly tuberculate. Variable in color. Usually, dark slate grey, sometimes brightly mottled cream, brown, orange, or red forms are found [66]. Distributed across Central and Western Europe but introduced to most countries [67]. Common in the Czech Republic.
- The Common Shiny Woodlouse *Oniscus asellus* Linnaeus, 1758 (coded as asellus throughout the text): Body size up to 18 mm, about twice as long as wide. Usually, shiny slate grey with irregular lighter patterns and two rows of yellow patches [66]. Distributed in Northern and Western Europe east to Finland, Poland, and Ukraine. Also found in Madeira and Azores. Introduced to America [68]. Common in the Czech Republic.
- *Armadillo officinalis* Duméril, 1816 (coded as officinalis throughout the text): Size of body up to 20 mm slate grey in color [69]. Distributed in the Mediterranean and western Black Sea coasts [68]. Not occurring in the Czech Republic.
- *Armadillidium versicolor* Stein, 1859 (coded as versicolor throughout the text): Body size 10 mm long. Dark coloring varies from black-brown to olive-brown, with light spots forming 5 regular rows on the dorsal side and pale-yellow margins of epimeres [70]. Found in Eastern Europe [68]. Rare in the Czech Republic, but locally abundant.
- *Armadillidium gestroi* Tua, 1900 (coded as gestroi throughout the text): Body size up to 20 mm. Color dark or light brown with vivid yellow spots forming 4 regular rows. Found in northwest Italy [68]. Not occurring in the Czech Republic.

Porcellio scaber, *O. asellus*, and *A. versicolor* were hand-picked in an urban area of the city of Olomouc, Czech Republic. *Armadillidium officinalis* and *A. gestroi* were obtained from a local breeder. Isopods were kept under natural light in laboratory conditions (ca 19–

22 °C) in 17 × 17 × 8 cm plastic boxes with a thin layer of plaster and leaf litter to maintain humidity and fed on carrot ad libitum.

As a control baseline prey, we used the Guyana Spotted Roach (*Blaptica dubia* (Serville, 1839); coded as roach throughout the text, Fig. 1f). This species originates from Argentina, it is therefore novel for the European wild caught Great Tits. We used the third larval instar, which is very similar in appearance to isopods, brown-grey with light and dark spots, and a length of 10–18 mm. The Guyana Spotted Roach may use the smell of volatiles derived from their food as a defensive secretion. The experimental individuals were fed with carrot. Despite nothing being known about their ability to produce defensive chemicals, or how they spread through the air, this species is palatable to, and commonly eaten by, Great Tits in laboratory experiments [71–73].

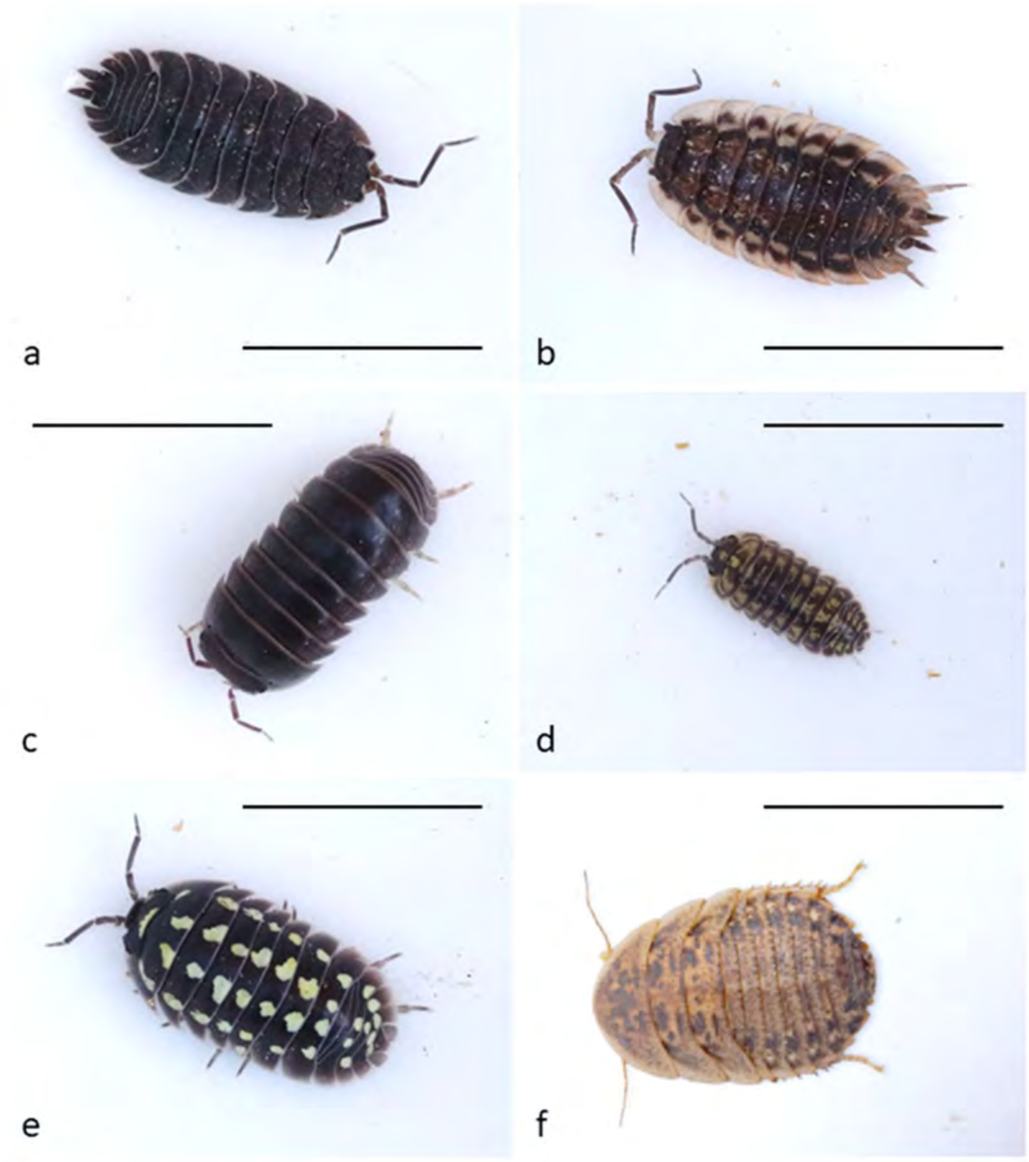

Figure 1. Tested prey types: a *Porcellio scaber* (scaber), b *Oniscus asellus* (asellus), c *Armadillo officinalis* (officinalis), d *Armadillidium versicolor* (versicolor), e *Armadillidium gestroi* (gestroi), f *Blaptica dubia* (roach); scale is 1 cm.

2.2. Predator

We used the Great Tit (*Parus major* Linnaeus, 1758) as a predator as it is a common insectivorous passerine with a broad array of invertebrate taxa in its diet [74]. It is commonly used in behavioral experiments testing the efficacy of the antipredator signals of invertebrates [72,74–76] owing to its ability to quickly accustom itself to laboratory conditions. The birds used for the experiments were adults captured in the wild at a winter-feeding spot within the environs of the city of České Budějovice (Czech Republic). Captures were conducted from October to November 2022. Each bird was tagged with an aluminium ring with a unique code and transported in captivity. Bird was placed in a birdcage with access to freshwater and food (sunflower seeds and mealworms *Tenebrio molitor* Linnaeus, 1758). Birdcages were deposited in a room with maintained natural daylight and temperature similar to outside conditions. Altogether we used 80 individual birds. Each bird was kept in the cage one day prior to the experiment to get habituated to captivity. Birds were caged individually, to avoid any social stress. Each bird was tested only once and released back into the wild at the end of the experiment.

2.3. Experiment

Two hours before the start of the experiment, each individual was taken to the experimental cage (70 × 70 × 70 cm) which consisted of a wooden frame, wire mesh, and a front wall made of one-way glass. Inside the cage was a perch, a bowl of water, and a circular feeding tray carrying six cups (5.5 cm diameter) with a white bottom on which prey was presented. The distance between the perch and the feeding tray was 40 cm. The birds were left with only clean water and five mealworms, as had been done in previous studies [76,77], to encourage an increased interest in experimental prey but at the same time avoid inducing stress from starvation. The bird spent two hours before the experiment in the experimental cage to get accustomed to the cage and to associate the movements of the rotating tray with provisioning the food. The birds usually looked at the offered prey immediately when the tray rotated. The experimental cage was placed in a dark room, lit by a single lamp (15W) simulating daylight including UVA rays. We conducted two types of experiments. In the single-prey treatment, each prey was offered in one (middle) cup of a circular feeding tray. The moment the Great Tit started looking for food in the cups was considered the beginning of the experiment. The bird was first given a mealworm, which was used to control interest in foraging. When the bird had completely swallowed the mealworm, one isopod individual was provided. With every bird, a series of 5 trials (in order: mealworm – isopod – mealworm – isopod, etc.) were done to avoid the effect of neophobia [78]. Each trial with one isopod lasted five minutes, and the experiment continued only after mealworm consumption.

The choice tests were conducted similarly, the only difference being that the bird was presented with two types of prey at the same time. Roaches were used as second prey. Mealworms served only as an appetite indicator. Thus, a scheme of mealworm – cockroach+isopod – mealworm – cockroach+isopod (etc.) was followed. In this type of experiment, we used the prey types *Blaptica dubia* (coded as roach(+scaber) throughout the text) and *Porcellio scaber* (coded as scaber(+roach) throughout the text) and *Blaptica dubia* (coded as roach(+gestroi) throughout the text) and *Armadillidium gestroi* (coded as gestroi(+roach) throughout the text). We chose only these two species of terrestrial isopods because of their different conspicuousness of coloration and because both were sufficiently abundant for experimentation.

2.4. Data analyses

We recorded the bird behavior with the help of Behavioral Observation Research Interactive Software (BORIS). We scored multiple bird behaviors, four of them were used in further analyses, as they related to the prey. (1) attacking the prey – any manipulation with the prey, carrying the prey in the bill, (2) eating the prey – consumption of at least part of the prey body, (3) observing the prey – looking at the prey from distance, (4) discomfort behavior – cleaning bill, drinking water, flushing feathers.

Analyses were performed in R ver. 4.1.2. Firstly, we ran a generalised linear mixed effect model (GLMM, command glmer in R package lme4 [79]) to test the effect of the prey type (values: scaber, scaber(+roach), asellus, officinalis, versicolor, gestroi, gestroi(+roach), roach, roach(+scaber), roach(+gestroi)) on the occurrence of attack on the particular prey (coded binomially). As each bird experienced five consecutive trials, the bird identity was included in the model as a random factor. We used the Likelihood ratio test for binomial data to compare the models in forward stepwise selection (Chi square test). Further, we used the Fisher LSD post hoc test with Tukey correction for repeated comparisons to compare particular prey types.

We further analyzed the edibility of particular prey types by analysis of the occurrence of eating after attacking the prey. We ran a generalized linear mixed effect model (GLMM, command glmer in R package lme4) to test the effect of the prey type on the occurrence of eating (binomially coded, bird ID included as random factor). We used the Likelihood Ratio Test for binomial data to compare the models in forward stepwise selection (Chi square test). Further, we used the Fisher LSD post hoc test with Tukey correction for repeated comparisons of offered prey.

Thirdly, we analyzed the time spent observing the prey from distance to show the effect of familiarity or decision-making. In this case, we were not able to distinguish which prey the bird looked at in the choice tests. Therefore, prey scaber(+roach) and roach(+scaber) as well as gestroi(+roach) and roach(+gestroi) were coded as one prey type. As these data followed the Gaussian distribution of errors, we ran a mixed effect linear model (LMM, command lmer in R package lme4) to test the effect of the prey type on the time spent observing the prey. We used the Likelihood ratio test for normal data to compare the models in forward stepwise selection (Chi square test). Further, we used Tukey HSD post hoc test with Tukey correction for repeated comparisons to compare particular prey types.

Lastly, we analyzed how often the bird showed any form of discomfort (cleaning bill, drinking water, flushing feathers) after an attack on particular prey. We ran a generalized linear mixed effect model (GLMM, command glmer in R package lme4) to test the effect of the interaction of prey type and if the particular prey was attacked or not on the number of signs of discomfort (Poisson distribution of data). We used the Likelihood Ratio Test for Poisson data to compare the models in forward stepwise selection (Chi square test). Further, we used a post hoc test for Poisson distribution of data with Tukey correction for repeated comparisons to compare offered prey.

3. Results

3.1. Attacking of prey

The prey type significantly affected the occurrence of attacks on it (Table 1, Figure 2). Roach was attacked significantly more often than scaber ($z = 4.345$, $p < 0.001$), asellus ($z = 3.213$, $p = 0.040$), officinalis ($z = 3.408$, $p = 0.021$), versicolor ($z = 3.924$, $p = 0.003$), and gestroi (z = 4.013, p = 0.002). There was no difference in the attack occurrence among all isopod forms (z < 1.670, p > 0.798).

Roach presented together with scaber was attacked equally often as scaber presented together with roach (z = 1.779, p = 0.731). Roach presented together with gestroi was attacked equally often as gestroi presented with roach (z = 1.542, p = 0.864). Scaber was attacked equally often when presented alone or when presented with roach (z = 1.531, p = 0.869). Gestroi was attacked equally often when presented alone or when presented with roach (z = 0.434, p = 0.999). Roach was attacked significantly less often when presented with scaber than when presented alone (z = 3.498, p = 0.016). Roach was attacked a little less often when presented with gestroi than when presented alone (z = 3.018, p = 0.071).

Table 1. Effects of tested predictors on particular response behaviors of birds (always mixed effect models with bird ID included as random factor). DF refers to degrees of freedom, asterisk refers to interaction of factors.

response	predictor	data type	data included	Chi	DF	p
attacking	prey type	binomial	all	37.575	9	<<0.001
eating	prey type	binomial	only attacking	62.303	9	<<0.001
observing	prey type	gaussian	all	31.97	7	<0.001
disgust	prey type*attack	poisson	all	12.256	9	<<0.001

3.2. Eating of prey

The prey type significantly affected consumption of the already attacked prey (Table 1, Figure 2). Roach was eaten significantly more often than scaber (z = 3.001, p = 0.016), asellus (z = 2.659, p = 0.042), officinalis (z = 5.012, p < 0.001), versicolor (z = 5.123, p < 0.001), and gestroi (z = 3.568, p = 0.002). Scaber was eaten more often than officinalis (z = 2.997, p = 0.026), versicolor (z = 3.203, p = 0.009), and gestroi (z = 2.204, p = 0.049), and equally often as asellus (z = 0.982, p = 0.998). Asellus was eaten more often than officinalis (z = 3.259, p = 0.004), versicolor (z = 3.151, p = 0.008), and gestroi (z = 2.653, p = 0.039). There was no difference among eating of officinalis, versicolor, and gestroi (z < 1.090, p > 0.998).

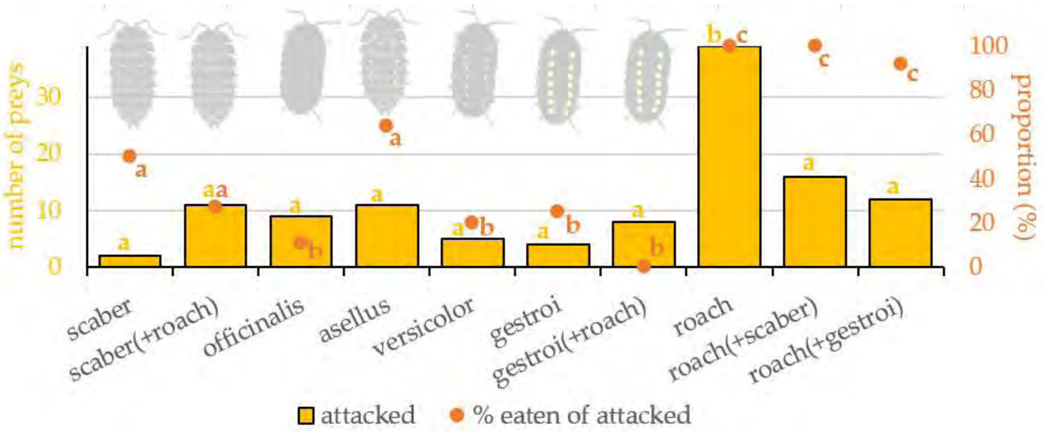

Figure 2. Number of preys of particular type attacked (yellow) and proportion of them eaten (orange) by Great Tits (*Parus major*). Tested prey types: scaber - *Porcellio scaber*, asellus - *Oniscus asellus*, officinalis - *Armadillo officinalis*, versicolor - *Armadillidium versicolor*, gestroi - *Armadillidium gestroi*, roach - *Blaptica dubia*. Combination of two preys refers to preference experiments when two prey items were presented simultaneously. Yellow and orange letters indicate significant differences.

Roach presented together with scaber was eaten more often than scaber presented together with roach (z = 4.100, p = 0.004). Roach presented together with gestroi was eaten more often than gestroi presented with roach (z = 4.993, p < 0.001). Scaber was eaten a little more often when presented alone than when presented with roach (z = 1.872, p = 0.062). Gestroi was eaten equally often when presented alone or when presented with roach (z = 1.309, p = 0.956). Roach was eaten equally often when presented with scaber, when presented with gestroi, or when presented alone (z < 0.523, p > 0.9).

3.3. Observation of prey

The prey type significantly affected the total time the bird spent observing the prey or prey combination (Table 1, Figure 3). Birds spent significantly longer observing the roaches presented in combination with scaber and gestroi than they did officinalis (roach(+scaber): z = 4.538, p < 0.001; roach(+gestroi): z = 3.902, p = 0.004) and roach (roach(+scaber): z = 4.103, p = 0.002; roach(+gestroi): z = 3.466, p = 0.017).

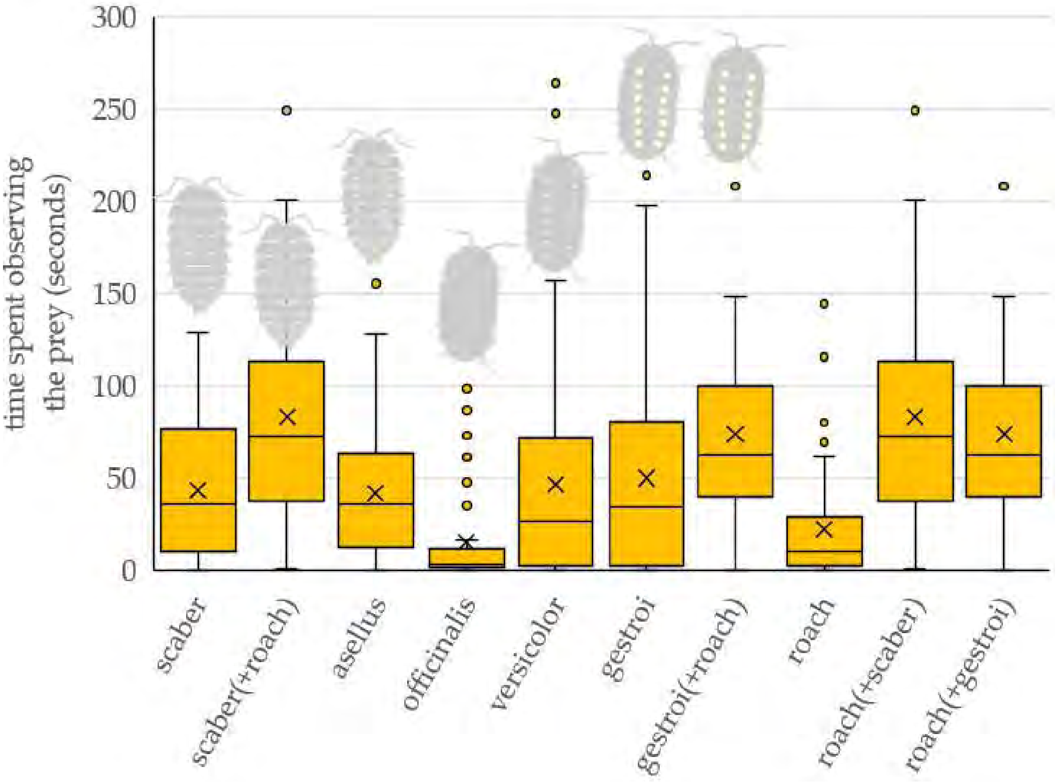

Figure 3. The total time, the bird spent observing the prey, or prey combination from distance. Tested prey types: scaber - *Porcellio scaber*, asellus - *Oniscus asellus*, officinalis - *Armadillo officinalis*, versicolor - *Armadillidium versicolor*, gestroi - *Armadillidium gestroi*, roach - *Blaptica dubia*. Combination of two prey refers to preference experiments when two prey items were presented simultaneously.

3.4. Signs of discomfort

The interaction of prey type and the occurrence of attacking the prey significantly affected the number of signs of discomfort (cleaning beak, feather flushing, drinking) the tested bird performed (Table 1, Figure 4). Birds showed discomfort most often when attacking the gestroi prey type, significantly more often than when they did not attack it (z = 5.508, p < 0.001); or significantly more often than when they attacked other prey types (scaber: z = 4.435, p < 0.001; asellus: z = 4.751, p < 0.001; officinalis: z = 4.840, p < 0.001; versicolor: z = 3.080, p < 0.001; roach: z = 5.247, p < 0.001). There was no difference in the number of signs of discomfort shown by birds when attacking and non-attacking other prey types (scaber: z = 1.257, p = 0.999; asellus: z = 3.193, p = 0.111; officinalis: z = 0.575, p = 0.999; versicolor: z = 2.854, p = 0.264; roach: z = 0.845, p = 0.999).

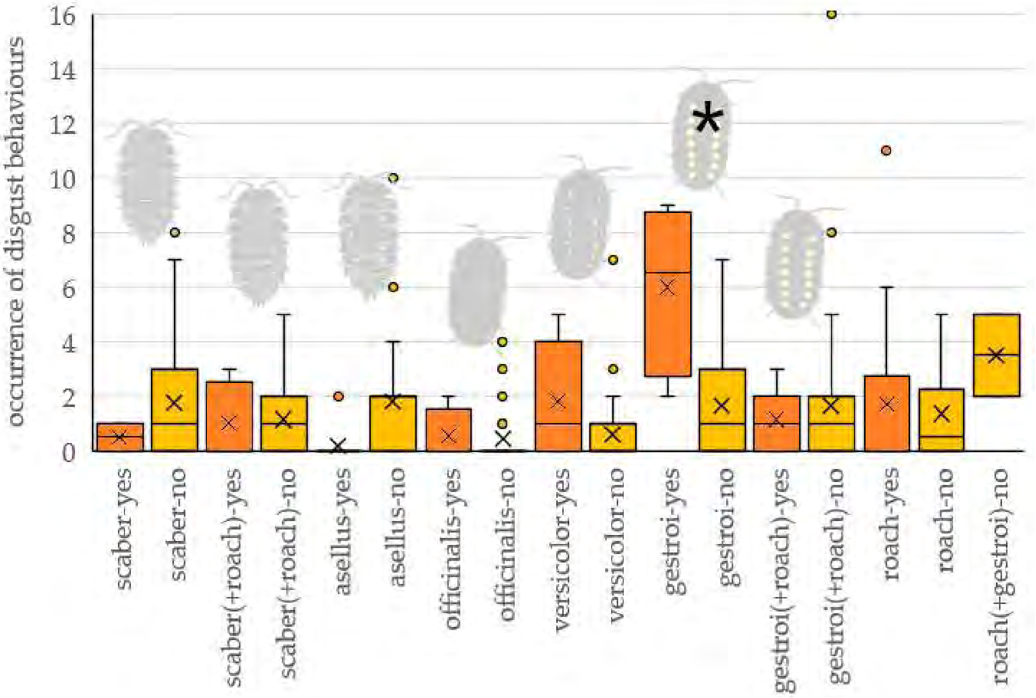

Figure 4. The number of signs of discomfort performed by tested bird attacking (yes, orange) and non-attacking (no, yellow) particular prey types. Tested prey types: scaber - *Porcellio scaber*, asellus - *Oniscus asellus*, officinalis - *Armadillo officinalis*, versicolor - *Armadillidium versicolor*, gestroi - *Armadillidium gestroi*, roach - *Blaptica dubia*. Asterisk marks significant difference in number of discomfort signs between birds attacking and nonattacking prey. Combination of two prey refers to preference experiments when two prey items were presented simultaneously.

4. Discussion

4.1. Attacking and eating prey

We found out that the type of prey significantly affected the occurrence of bird attacks. The edible roach was attacked more often than isopods. However, we did not find a statistical difference in attack occurrence among the five isopod forms. This is inconsistent with our hypotheses that no isopod species is better protected against attack and eating by birds than an edible roach. We also did not prove that isopod species with conspicuous coloration are better protected against bird attack than inconspicuous species.

The reaction to inconspicuous species of isopods differed to the reaction to the edible roach, which at first glance seems similar, differing mainly in the shape of the antennae. The use of such detailed characters in prey recognition has been demonstrated by Karlíková et al. [71]. In their study, it was tested whether Great Tits can discriminate between the edible roach (*B. dubia*) and the inedible firebug (*Pyrrhocoris apterus* (Linnaeus, 1758)) when their coloration was manipulated to be identical using paper sticker placed on their back. The study showed that some of the birds were able to recognize edible roaches and attacked only them [71]. It seems to be plausible to presume that the birds used other morphological cues related to differences between roaches and true bugs (differences in the shape of antennae or legs, body posture, etc.).

The birds tested in our study originated from the wild and probably had previous experience with common native species of woodlice (mainly *P. scaber* and *O. asellus*). Alatalo and Mappes [80] demonstrated that Great Tits initially attack all prey, but through aversion learning begin to avoid distasteful prey. Such aversive learning is particularly rapid for prey with higher levels of chemical defenses [81], which these species probably have [82,83]. The conspicuous visual appearance of *O. asellus* coloration may have increased the rate of memorization of unsuitable prey [84] and could be generalized as a color signal and used also for the protection of non-native *A. gestroi* and *A. versicolor* [85,86].

When presented alone or when presented with roach, the isopods were attacked equally often. However, when a roach was presented together with isopods it gained some level of protection. Birds probably can't distinguish between them and do not attack either of them. This is also in contrast with our hypothesis that birds prefer attacking roaches rather than isopods when encountering both simultaneously. On the other hand, the same results were found by Karlíková et al. [71]. The study showed that generally, Great Tits were not able to distinguish between inedible firebugs and edible roaches when their colorations were identical and they were presented together. The majority of birds attacked both preys, or attacked neither of them [71]. It seems that such a distinction between similar prey types is cognitively very demanding and the bird cannot solve this task fast enough. This is suggested by the longer observation times of prey in our choice experiments.

Roach was eaten more often than all isopod forms, both presented alone or together with isopods. We found out that *P. scaber* and *O. asellus* were eaten more often than other isopod species. Still, all isopods are protected better than roaches, both from being attacked and being eaten. Both of these species are native to the experimental site and it is likely that the tested birds meet with them, might have tasted them, and learned that woodlice are not very tasty. However, the optimization of decision-making strategies is influenced by a number of external factors, including current energy requirements [87]. In our laboratory arrangement, the bird may be motivated to forage on disadvantageous prey, as long as it is edible, as, unlike in the field, there is no alternative. We conducted the experiments during the autumn period, when Great Tits prepare for winter and some individuals engage in dispersal [88]. The foraging motivation of birds is generally higher before and during the migration, which may also increase their willingness to attack even an unprofitable prey.

Neophobia may also play part in the avoidance of at least some isopod forms. As we presented non-native species, at least some Great Tit individuals may have shown caution, and decided not to attack them. Neophobia can be overcome quite quickly, especially by individuals more prone to risk taking over time [89]. Obviously, five repeated encounters did not suffice to overcome the neophobia in our case.

It cannot be ruled out that the birds distinguished their prey by scent. Their sense of smell is probably powerful enough [58], but woodlice usually emit odors only after mechanical or other irritation [83]. This possibility is therefore not very likely.

4.2. *Observing prey*

When the roach was presented together with the isopod, birds spent a long time observing them from a distance. Birds spent more time observing the combination of roach and *P. scaber* and roach and *A. gestroi* than other types of prey combination. Levi [36] states that isopods use chemical defense and conspicuous coloration directly against attacks from visual predators. Apparently, the appearance of all isopod species, even the cryptic ones, evokes in birds the memory of unpleasant experiences of meeting an isopod in the wild. Birds with such experience are much more cautious and spend more time choosing their prey. This is also related to the cognitive difficulty of recognizing all the characteristics. In addition to the shape of the antennae mentioned above, the woodlice and cockroaches also differ in the nature of their movements. It is therefore advisable to wait until the prey starts to move, which can take on the order of minutes for isopods [90].

4.3. Signs of discomfort

When birds attacked *A. gestroi* they showed an increased number of discomfort signs in comparison to other prey types. This is in contrast with our hypothesis that birds do not show any disgust after attacking and eating isopods. However, we did not find any difference in the number of disgust signs shown by birds when attacking and non-attacking other types of prey. It is obvious that isopods are protected from avian predators. But the reaction after the attack is not strong. Except with *A. gestroi*, Great Tits do not show disgusted behavior like rubbing their beaks or drinking water after eating isopods, which is a common reaction to chemically protected prey. As was mentioned above, isopods' repugnatorial glands are primarily directed against invertebrates [29] or small insectivorous mammals [33,34]. It can therefore be assumed that the chemical protection is not significant regarding birds, but their aversion to isopods still occurs. We know nothing about the intensity of chemical defenses in terrestrial isopods and we have very little information on the presence of defense glands in different woodlouse species. However, if we use the number of defensive glands in each species as a proxy for estimating their effectiveness, recent research [82] suggests that conglobating species (e.g., the family Armadillidiidae) are less chemically protected than species of the family Porcellionidae or Oniscidae, which is in contrast to our finding that *A. gestroi* elicits most discomfort after the attack. Nevertheless, even in genus *Armadillidium*, there are differences in smell intensity of defensive secretions [83].

Ethical Approval: This study was conducted in accordance with the valid laws and regulations of the Czech Republic; in compliance with the Ethics Committee of the Faculty of Science, University of South Bohemia, which approved this study. Behavioral experiments on the wild birds were approved by the Ministry of Environment of the Czech Republic (no. MZP/2020/630/1544) and licenses permitting experimentation with animals no. CZ02766 and CZ01629 offered by the Ministry of Agriculture of the Czech Republic. University of South Bohemia has accredited breeding of birds in captivity (43873/2019-MZE-18134).

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Appendix VI

Ďurajková, B., Veselý, P. & Tuf, I. H.: Different responses of insectivorous birds to terrestrial isopods. (in prep.)

Different responses of insectivorous birds to terrestrial isopods.

Barbora Ďurajková[1], Petr Veselý [2], Ivan Hadrián Tuf [1]

Abstract

This study examined the feeding preferences of wild-caught avian predators for the terrestrial isopod *Armadillidium vulgare* (Common Pill Bug), testing hypotheses related to prey palatability and predator foraging specialization. Four passerine species—Great Tit (*Parus major*), European Robin (*Erithacus rubecula*), Common Blackbird (*Turdus merula*), and Eurasian Tree Sparrow (*Passer montanus*)—were presented with pill bugs and control roaches (*Blaptica dubia*) in controlled feeding trials. The results showed that while all bird species attacked and consumed both prey types, Great Tits were significantly less likely to attack pill bugs compared to roaches. The other bird species showed no significant difference in attack rates between the two prey types. Our findings suggest that pill bugs are not universally avoided as unpalatable prey by avian predators, and the reluctance of Great Tits may be linked to specific foraging habits or neophobia. Additionally, avian body size and diet specialization did not predict the rejection of pill bugs. These results highlight the complexity of predator-prey interactions and suggest that the conspicuous appearance of *A. vulgare* does not function as an effective aposematic signal in the bird species tested.

Introduction

Among arthropods, the use of chemical protection is relatively widespread. While chemical defenses in some groups like insects or arachnids are relatively well studied, in the case of crustaceans, the use of such weapons is relatively unknown. It is known that body fluids or urine of some crustaceans as spiny lobsters (Zimmer-Faust et al. 1985), crayfish (Hazlett 1994), and hermit crabs (Rittschof et al. 1992) contain chemical substances that serve as alarm cues for individuals of same species (Derby and Sorensen 2008). Similar functions have necromones released by injured or dead individuals of the terrestrial isopods (Yao et al. 2009). Moreover, the terrestrial isopods after strong stimulation release visible droplets of odorous secretion from external openings of the lobed glands along the lateral plates and uropods.

Gorvett (1956) suggested that this secretion may act as a deterrent against invertebrate predators like spiders and ants, which was confirmed in experimental studies (Deslippe et al. 1996; Paris 1963; Herold 1913; Brereton 1957). In our previous study, we showed a certain repellent effect also on Great Tits *Parus major* Linnaeus, 1758 (Ďurajková et al. unpubl.). However, there was a certain proportion of Great Tits that attacked the isopods of various species, and this variability in responses was obviously not affected by the visual appearance of the isopod species. Nevertheless, we generally considered that all isopod species are well protected against Great Tits.

Numerous studies have shown that the repellent effect of chemicals produced by specialized arthropod glands may substantially differ according to the predator. There are differences between various vertebrate predators (reptiles - Eisner 1970; Eisner et al. 2005; Sugiura 2020; Burghardt 1973; Garrett and Card 1993; Halpern 1992; Cooper 1994a,b; Larsen 2006; Byers 2015, amphibians - Dean 1980; Sugiura and Sato 2018; Matsubara and Sugiura 2017; Edgar 1971; Barlow 1998; fish - Eisner and Aneshansley 2000; Kasumyan and Marusov 2016) caused by their cognitive abilities, metabolism and ability to learn. Birds are definitely used most often as predators in similar studies, despite their poorly developed chemical senses. However, their visual sense is very well developed, enabling to perceive aposematic signals and their cognitive and memory abilities enable learning and recalling the warning signals (Speed 2000). The same ability is documented for learned avoidance of unpalatable food and cryptic prey (Zhang et al. 2024). It has been proven that birds use taste to control the intake of chemicals associated with the consumption of chemically defended prey (Skelhorn and Rowe 2006).

Despite this, we can see a significant variability in responses of various bird species to warningly signalling arthropods. Frugivorous Black-backed Orioles *Icterus abeillei* (Lesson, 1839) and granivorous Black-headed Grosbeaks *Pheucticus melanocephalus* (Swainson, 1827) consume toxic aposematic Monarch butterflies *Danaus plexippus* (Linnaeus, 1758), which are avoided by other bird species (Fink and Brower 1981). Similarly, nine passerine birds showed interspecific differences in the response to aposematic and non-aposematic variants of firebug *Pyrrhocoris apterus* (Linnaeus, 1758). While insectivorous birds such as European Robins *Erithacus rubecula* (Linnaeus, 1758), Great Tits, Eurasian Blackcaps *Sylvia atricapilla* (Linnaeus, 1758), and Eurasian Blue Tits *Cyanistes caeruleus* (Linnaeus, 1758) distinguished and avoided aposematic prey, partly granivorous finches and buntings attacked both variants without distinction (Exnerová et al. 2003). This is congruent with studies showing that partly granivorous Eurasian Tree Sparrow *Passer montanus* (Linnaeus, 1758) is willing to consume toxically defended ladybugs (Veselý et al. 2017; Aslam et al. 2019), while other species such as Great Tits or Domestic Chicken *Gallus gallus f. domestica* Linnaeus, 1758 consider them unpalatable (Aslam et al. 2019). Generally, we can conclude that granivorous species tend to be more tolerant to chemicals ingested along with the insect prey. Some birds have developed tolerance to high levels of toxins in plants (Banko et al. 2002; Ríos et al. 2012; Gunasekaran et al. 2020). Alkaloids to deter avian predators are also widely used by arthropods including insects (Numata and Ibuka 1987). Seed-eating birds are thus preadapted to be tolerant to these defence chemicals. Birds generally prefer a fast-digesting diet, while its chemical composition is secondary. This supports the hypothesis that the evolution of the digestive tract in seed-eating birds favoured the mechanical digestion of seeds over dealing with their chemical composition (Díaz 1996). Moreover, birds prioritize food availability in the habitat and high nutritional reward over other factors such as the toxicity composition of food (Banko et al. 2002; Marone et al. 2022).

On the other hand, there may be also significant difference between various species of predominately insectivorous species, where such preadaptation cannot be expected. Exnerová et al. (2003) showed Common Blackbirds *Turdus merula* Linnaeus, 1758 to be more willing to attack and eat red firebugs, while smaller insectivores like tits avoided it. The body size may importantly contribute to the ability to deal with the ingested chemicals and may thus affect the attitude to chemically defended prey. Even though the firebug is chemically protected, Common Blackbirds never showed nausea after its consumption (Exnerová et al. 2003). On the other hand, large bodied birds may also show a high aversion to chemically defended insects. Killdeer *Charadrius vociferus* Linnaeus, 1758, Common Starlings *Sturnus vulgaris* Linnaeus, 1758, and American Robins *Turdus migratorius* Linnaeus, 1766 refused to consume the chemically armoured stink bug *Cosmopepla lintneriana* Kirkaldy, 1909 (Krall et al. 1999). Similarly, young Domestic Chickens refused to eat unpalatable Colorado Potato Beetles, *Leptinotarsa decemlineata* (Say, 1824) when provided alternately with mealworms (Hough-Goldstein et al. 1993).

Another reason why predators avoid certain prey can be neophobia or the avoidance response to unknown prey (Corey 1978; Marples and Kelly 1999). Despite the neophobia seems to be a common reaction of adult birds, the intensity of expression varies greatly between individuals of the same species as well as between closely related species (Greenberg 2003). The willingness of wild birds to taste unfamiliar prey may be correlated with their physiological condition. Birds with poor state of health may be less willing to risk worsening their condition by consuming unfamiliar prey. On the other hand, a lack of food may increase interest in any available prey to prevent death from starvation (Marples et al. 1998; Barnett et al. 2007; Barnett et al. 2012). While neophobia is considered a short-term process lasting up to a few minutes (Marples and Kelly 1999), in some wild birds the reluctance to attack unknown prey can persist for weeks or months. Marples et al. (1998) in their study on wild blackbirds and robins found out that some birds are strictly conservative in their diet refusing unknown but palatable prey for a long period (Marples et al. 1998). Such long-term behaviour is called dietary conservatism (Marples and Kelly 1999) and may play an important role in the tropics where toxic prey is relatively abundant (Owen 1977). The effect of dietary conservatism on food choice was studied in Atlantic Canaries *Serinus canaria* (Linnaeus, 1758) where it was found that early contact with certain types of prey can lead to long-term feeding preferences (Doherty and Cowie 1994).

Insectivorous birds are usually voracious and the range of their prey is quite wide. Of course, they usually target the most abundant prey, which is therefore the best to find. However, often the abundance of prey does not correspond with its proportion in the bird's diet. It can then be inferred that the abundant but relatively unaccepted prey is not very palatable or suitable. Terrestrial isopods may be such an example (Nell et al. 2023). Although woodlice are found relatively frequently in many natural habitats, their contribution to the diet is rather negligible (Moore 1983; Samplonius et al. 2016; Bureš & Weidinger 2003). Novel methods can sometimes provide misleading results, and reinterpretation of, for example, molecular markers of prey can be affected by a number of factors, such as poor digestibility of prey, which overestimate their abundance in the results (Jedlicka et al. 2013). Our research aim was to examine the efficacy of chemical protection of terrestrial isopods against avian predators varying in their predominant diet and body size. In the presented study, the reaction of four native passerine birds to Common Pill Bug *Armadillidium vulgare* (Latreille, 1804) was tested.

We tested the following hypotheses:

- Wild caught predators consider terrestrial isopods as inedible (they do not eat them)
- Wild caught predators consider terrestrial isopods non-aposematic (they attack them)
- Avian predators specialized to granivory eat terrestrial isopods more.
- Large-bodied avian predators eat terrestrial isopods more.

Methods

Prey

As a model prey we used adult individuals of Common Pill Bug, *Armadillidium vulgare* (Fig. 1a). Common Pill Bug is up to 18 mm long, twice as long as wide with colouration varying from completely black to place yellow (Edney 1954). It is autochthonous in the Mediterranean region, distributed by human activities to all parts of the world (Schmalfuss 2003). Common in Czech Republic and presumed to be familiar to the wild-caught individuals of all four tested bird species. Common Pill Bug was hand-picked in an urban area of the city Olomouc, Czech Republic. Isopods were kept in 17 × 17 × 8 cm plastic boxes with a thin layer of plaster and leaf litter to maintain humidity and fed on carrot *ad libitum*.

For control baseline prey, the third larval instar of Guyana Spotted Roach *Blaptica dubia* (Serville, 1839) was used. This larval stage with brown-grey colouration carrying light and dark spots (Fig. 1b) and 10-18 mm body length is very close in appearance to Common Pill Bugs. Species have origin in Argentina, and it is novel for all four avian predators tested. The roaches were fed with carrots to minimize their ability to use the smell of volatiles derived from their food as a defensive secretion. The species is considered palatable and is commonly consumed by birds in laboratory experiments (Veselý and Fuchs 2009; Veselý et al. 2013a; Cibulková et al. 2014; Karlíková et al. 2016).

Predator

We used four species of passerine birds:

- Great Tit is a common small-bodied (mass 15-20 g) insectivorous passerine that feeds on a great variety of invertebrates (Wilkin et al. 2009). Its foraging behavior includes searching for caterpillars in the upper parts of trees (Naef-Daenzer and Keller 1999; Nakamura and Shindo 2001) as well as for ground-dwelling invertebrates among leaf litter (Smith and Dawkins 1971). During the non-breeding season plant material, especially seeds and buds are common in their diet. Great Tits can quickly adapt to laboratory conditions and are commonly used in behavioural experiments to test the antipredation signals of invertebrates (Exnerová et al. 2003; Dolenská et al. 2009; Veselý and Fuchs 2009). Great Tit can distinguish between aposematic and non-aposematic prey and avoid attacking it (Exnerová et al. 2003).
- European Robin is a small bodied (14-16 g), ground-foraging species feeding predominantly on insects and spiders, supplementing their food with seeds, fruits, or berries during the non-breeding season (Hume 2002; Ktitorov et al. 2021). European Robins are used for testing reactions toward an insect warning signal (Exnerová et al. 2003; Hotová Svádová et al. 2010; Siddall and Marples 2011). They can recognize aposematically coloured prey and attack it less often (Exnerová et al. 2003).
- The Common Blackbird is a large-bodied (85-120 g), omnivorous species feeding mainly on the ground. Its food consists of insects, earthworms, seeds, and berries (Török and Ludvig 1988). Common Blackbird was used in behavioural experiments testing birds' reactions to aposematic and non-aposematic prey (Schlee 1986; Exnerová et al. 2003). Common Blackbirds show increased tolerance to chemically protected insects like firebugs and express no sight of discomfort after their consumption (Exnerová et al. 2003).

- The Eurasian Tree Sparrow is a small-bodied (16-22 g), primarily seed and grain-eating bird whose diet also includes insects, terrestrial isopods, spiders, harvestmen, millipedes, and centipedes. It searches for the food on the ground in flocks often in the company of other bird species (Clement et al. 1993). The Eurasian Tree Sparrow is used in behavioural experiments testing the efficacy of visual and chemical signals of unpalatable insects (Veselý et al. 2017; Aslam et al. 2019). Although young sparrows show avoidance of chemically protected insects such as ladybugs, adult individuals can cope with chemicals and include ladybugs in their diet (Veselý et al. 2017).

All birds used for experiments were adults captured from October to November 2023 within the area of the town České Budějovice (Czech Republic). Birds were kept in birdcages with access to freshwater and food (Yellow Mealworms *Tenebrio molitor* Linnaeus, 1758) and sunflower seeds while their natural light and temperature regime was preserved. Each individual experienced the experiment only once before being released back into the wild. See Table 1 for a summary of experiments and traits of particular bird species.

Experiment

Two hours before starting the experiment, the birds were denied access to food which should lead to an increased interest in the experimental prey. Only a bowl of clean water and five mealworms were left in the bird cages to prevent stress from starvation (Exnerová et al. 2003; Průchová 2014).

For the experiment, wooden cages with wire mesh (70 × 70 × 70 cm) and with a front wall made of a one-way mirror were used. There was a perch, a bowl with water, and a circular feeding tray carrying six cups (5.5 cm diameter) inside every cage. The cups on the feeding tray had white bottoms which were used to present the prey. Cages were deposited in the dark room to boost up the effect of one-way mirror and were lightened up by a 15W lamp with UVA rays.

Only single-prey trials were performed since our previous study on Great Tits showed that a cockroach, if it is together with an isopod, gains protection (Ďurajková et al. unpubl.). During the experiment, the prey was presented in a single (middle) cup of the feeding tray. The experiment began when the tested predator started looking for food in cups. To control interest in the prey, the birds were first given a mealworm and after its consumption, one isopod individual was offered. To prevent neophobia (Marples and Kelly 1999), each bird experienced a series of ten trials in the following order: mealworm – isopod – mealworm – isopod, etc. One trial with one isopod lasted five minutes, and the experiment continued only after mealworm consumption. An experiment with roaches was conducted in the same way. The trials with mealworms were not analysed, they were just used to control for foraging motivation in the course of the whole session. We used only ten trials repetition to avoid satiation and loose of motivation in tested birds.

Data analyses

We scored three possible results of the prey presentation to the bird: 1 – the bird attacked the prey – took it into the bill or touched it by the bill, 2 – the bird attacked the prey and subsequently ate at least part of its body, 3 – the bird neither attacked nor ate the prey. Attacking as well as eating the prey was binomially coded. We ran two generalized linear mixed effect models with binomial error distribution of data to evaluate the effect of the interaction of factors bird species and prey species (command glmer in R package lme4). The bird identity was included as a random factor, as each bird was confronted with five prey items of a single species (isopod or roach). Likelihood ratio test for binomial data was used to compare the null and the full model (Chi squared test). To compare particular bird-prey combinations, we used Fisher LSD post hoc test (z test, command glht in R package multcomp). All analyses were conducted in R ver. 4.1.2.

Results

The attack rate during the experiments was significantly affected by the interaction of bird species and prey type (GLMM, Chi=49.9, DF=7, P<<0.001; Fig. 2). Great Tits were the only species which attacked the Common Pill Bug significantly less often than the edible roach (Z=4.218, P<0.001). In other species, there was no significant difference in attacking between the isopod and the roach prey (Erithacus: Z=0.371, P=0.999; Turdus: Z=2.504, P=0.225; Passer: Z=0.238, P=0.374), although isopods were attacked somewhat less frequently. European Robin attacked the isopod the most often, significantly more often than Great Tit (Z=4.918, P=<0.001), The difference between attack rate to isopod between European Robin and Eurasian Tree Sparrow was bordering significance (Z=2.954, P=0.074). Common Blackbird also attacked the isopod quite often, the difference from the low attack rate of Great Tits was bordering significance (Z=2.980, P=0.069). There were no differences between other species in attack rates to the isopod prey (Z values below 1.83, P values above 0.65). The roaches were commonly attacked by all bird species, which did not differ from each other in the attack rate (Z values below 1.66, P values above 0.76).

There was no effect of the interaction of bird species and prey species on the probability of eating the already attacked prey (GLMM, Chi=2.1, DF=7, P=0.978; Fig. 3). All bird species ate both roaches as well as isopods very often, once they decided to attack it.

Discussion

We tested experimentally, if birds avoid terrestrial isopods as prey depending on birds size and usual diet. With respect to the hypotheses we tested, we did not confirm that wild birds avoid eating isopods. Results show that all bird species consumed roaches as well as isopods after they decided to attack them. Only the Great Tit considered the pill bug to be abusive prey. Bird species with a higher proportion of insects in their prey also more readily accepted cockroaches, but this difference was not significant. Willingness to consume terrestrial isopod prey was unrelated to the size of the avian predator.

Common Pill Bug is highly variable in its colouration. Most adult males are a leaden black colour, while females have a series of small white or cream markings on a greyish-black body. However, the colouring of this species ranges from yellow to brown, marbled, or even red (Howard 1962). During the experiment, mainly black individuals with light markings were used. Great Tits were the only birds that attacked the isopods less often than roaches. It seems to be the only species that finds the appearance of *Armadillidium vulgare* repulsive.

Nevertheless, pill bugs are repeatedly avoided by small mammals too. The "pilot" study of food preferences of shrews was done by Brereton and Crowcroft more than seventy years ago (Crowcroft 1957). Pygmy Shrew *Sorex minutus* Linnaeus, 1766 ate 95% of offered *Philoscia*, more than half of offered *Porcellio* and *Oniscus* isopods, but none pill bug. Larger Common Shrew *Sorex araneus* Linnaeus, 1758 ate all *Philoscia* isopods, one third of offered *Porcellio* isopods and one sixth of *Oniscus*, but only 11% of pill bugs.

It is therefore clear that even amongst the terrestrial isopods, Common Pill Bug is considered to be an unpalatable prey. However, this result is based on observations on mammals. Mammals have different physiological mechanisms for processing potential unsuitable prey. For example, birds and mammals are known to have different detoxification pathways, with birds being able to get rid of some toxins more quickly and absorb less of them in the digestive tract (Guerre 2015).

In our previous work on Great Tits, we showed that edible roach was attacked more often than all isopods (Ďurajková et al. unpubl.). The ability to differentiate between pill bug and cockroach is interesting. The third larval instar of *B. dubia* is similar in appearance to isopods, both in colouration and body length, differing mainly in the shape of the antennae. Karlíková et al. (2016) showed that some of the Great Tits were able to recognize edible roaches from inedible firebugs *Pyrrhocoris apterus* when their colouration was modified to be identical using paper stickers placed on their back. Thus, it could be that the overall appearance of *A. vulgare* had an impact on the predation behaviour of the Great Tits. Nevertheless, we have no knowledge on the appearance of pill bug and cockroach under UV light. Tits are able to see reflectance in UV light and use this information to make decisions (Tanner and Richner 2008). The similarity between a pill bug and a cockroach should be tested through the eyes of a tit, not through the eyes of a human experimenter. Previous experiments with aposematic prey (e.g. Karlíková et al. 2016) have often used paper coloured stickers to manipulate prey appearance, but this may have masked natural differences in appearance that the human (mammalian) eye cannot register.

On the other hand, a pill bug and a cockroach can differ not only in appearance but also in their typical behaviour. The movement of these cockroaches is usually volatile, alternating between rapid run and immobility, while the pill bug usually moves slowly and smoothly around the dish after unrolling from their protective posture (Drahokoupilová and Tuf 2012).

The sparrows also partially refused isopods, but not significantly. Sparrows often do not react to warning signals, as was shown in a study with wild-caught Eurasian Tree Sparrows *Passer montanus* (Linnaeus, 1758) and larvae and pupae of the Harlequin Ladybird *Harmonia axyridis* (Pallas, 1773). Sparrows attacked and ate one third of the offered prey and were willing to include toxic and conspicuous ladybirds in their diet (Aslam et al. 2019). Granivory can change access to aposematic prey since insectivorous birds refuse aposematic prey, while those partly granivorous are more willing to attack conspicuous prey (Exnerová et al. 2003, Veselý et al. 2017; Aslam et al. 2019). Thus, seed-eating birds seems to be adapted to high levels of toxins (Banko et al. 2002; Ríos et al. 2012; Gunasekaran et al. 2020).

Other explanations of sparrows attitude to pill bugs may be based on previous experience of the birds. Birds that mainly consume seeds may not have personal and repeated experience of consuming aposematically coloured and slightly toxic prey presented in experimental box alone. In contrast, birds that frequently encounter brightly coloured and mildly unpalatable prey can more easily learn to avoid it. A similar explanation is given for ignoring cryptically coloured prey. A chick that has experience with inedible objects (dead leaves) is more likely to ignore cryptically coloured prey butterfly *Kallima inachus* (Boisduval, 1846) than a predator that has had no previous experience with such objects (Zhang et al. 2024).

Common Blackbirds attack and consume red firebugs more often in comparison to smaller insectivorous like tits (Exnerová et al. 2003). This is consistent with our findings that Common Blackbirds willingly attacked and ate cockroaches and isopods and neither the appearance nor any chemical protection affected it.

The reaction of the American Robin, which willingly attacked and ate the isopods, is interesting. Robins often encounter isopods in nature because they collect food on the ground, in leaf litter. At the same time, they are not granivorous, nor are they large. Previous work has shown that their response to aposematic is more similar to that of Great Tit (Turini et al. 2016). The explanation of the high willingness of robins to attack and eat the pill bug may reside in their migratory behaviour. Robin and blackbird of the bird species tested have a significant portion of the population in central and northern Europe migratory. Our experiments were conducted at the peak of migration of both these species in central Europe, where local populations start moving, associated with northern populations passing by to their winter grounds in southern Europe (Cepák 2008). This may have influenced its foraging behaviour, as the birds needed to increase their energy intake before the journey. A previous study, in which robins were much pickier (Turini et al. 2016), tested birds caught over a longer period of time. It is possible that the decision-making behaviour, the avoidance of aposematic prey, of such birds changes significantly over the course of a year (Bairlein 1990). We thus cannot disseminate the effect of migratory behaviour and body size in case of Common Blackbirds, both having similar effect. The effect of seasonality on the bird attitude to aposematic prey has been shown in Eurasian Tree Sparrows. Veselý et al. (2017) showed that sparrows were more willing to attack inedible ladybirds during the non-breeding season and thus in conditions of food shortage and more challenging weather conditions compared to summer, when the condition of tested individuals can be presumed better.

Even more surprising is the aversion of the Great Tit to *A. vulgare* (but also for other species, Ďurajková et al. unpubl.). In the case of the Great Tit, the influence of neophobia could be significant. The European Robin (and, after all, the Common Blackbird too), which knows isopods very well, readily attacks them, while the Great Tit is more of a specialist in collecting insects from twigs and leaves, and isopods may be less familiar to it. Indeed, terrestrial isopods are found in trees. However, flat-bodied species such as *Porcellio scaber* Latreille, 1804 and *Oniscus asellus* Linnaeus, 1758 predominate. Locally, pill bugs may also be abundant, but these are markedly smaller (ca 5-8 mm) and much more conspicuously coloured species such as *Armadillidium pictum* Brandt, 1833, *Armadillidium pulchellum* (Zenker, 1798) and rarely *Armadillidium versicolor* Stein, 1859 (see review in Tuf and Weissová 2022). Compared to these species, the Common Pill Bug *A. vulgare* is rarely found on trees.

Anyway, considering the Great Tit as a neophobic species is in contrast with previous studies showing their willingness to attack completely unfamiliar prey both natural (Järvi et al. 1981; Veselý et al. 2013b) and artificial (Lindström et al. 2001). Great Tits are understood as innovative species, quickly revealing novel food sources (Tryjanowski et al. 2015, 2018), including those extraordinary (Lefebvre 1995; Radzicki et al. 1999). When compared to other tit species, Great Tits show the lowest levels of neophobia and inherited prey avoidance (Exnerová et al. 2007; Adamová-Ježová et al. 2015). Their attitude to terrestrial isopods may thus represent a general attitude of small insectivorous passerines, not foraging on the ground. Further testing including broader array of species is needed to confirm this presumption.

Although the pill bug is not a particularly palatable prey, it was consumed less frequently than the more appetizing cockroach. However, this difference was not significant, except in the case of the Great Tit. Therefore, the relatively conspicuous pattern of the Common Pill Bug does not appear to serve as an advantageous aposematic coloration for the bird species tested, as it does not lead to predator avoidance. While shrews tend to reject Common Pill Bugs, insectivorous birds seem willing to consume them, possibly during specific feeding periods, such as when feeding hatchlings or before migration. Further experiments should investigate the temporal patterns of food preferences in these birds.

Conclusion

We tested the willingness of four species of songbirds to consume terrestrial millipedes that are protected by secreted chemicals. We presented the birds with pill bugs and cockroaches.

Great Tit significantly less consumed pill bugs than cockroaches. European Robin, Common Blackbird and Eurasian Tree Sparrow also consumed pill bugs less readily, but this difference was not significant. The highest willingness to consume all presented prey was exhibited by the robin, whose population was just prior to seasonal migration to the wintering grounds at the time of the experiment.

We conclude that not only species-specific traits like body size, diet and foraging habitat, but also individual conditions and motivation may importantly affect the willingness to attack and eat unprofitable or suspicious prey.

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Tables and Figures

Table 1 – Summary of ecological traits of particular tested bird species and the experiments conducted. Diet refers to the overall percentage of invertebrates in the species diet (according to del Hoyo 2021). Size refers to the average body weight of the species (according to del Hoyo 2021). Migrant refers to seasonal movement of Czech populations of tested birds between breeding and wintering grounds (Cepák 2008). N refers to the number of birds in experiments.

Species	diet	size	migrant	prey offered	N
Erithacus rubecula	90	15	yes	*Armadillidium vulgare*	14
				Blaptica dubia	10
Turdus merula	90	105	partly	*Armadillidium vulgare*	20
				Blaptica dubia	20
Passer montanus	20	17	no	*Armadillidium vulgare*	20
				Blaptica dubia	20
Parus major	70	16	no	*Armadillidium vulgare*	20
				Blaptica dubia	10

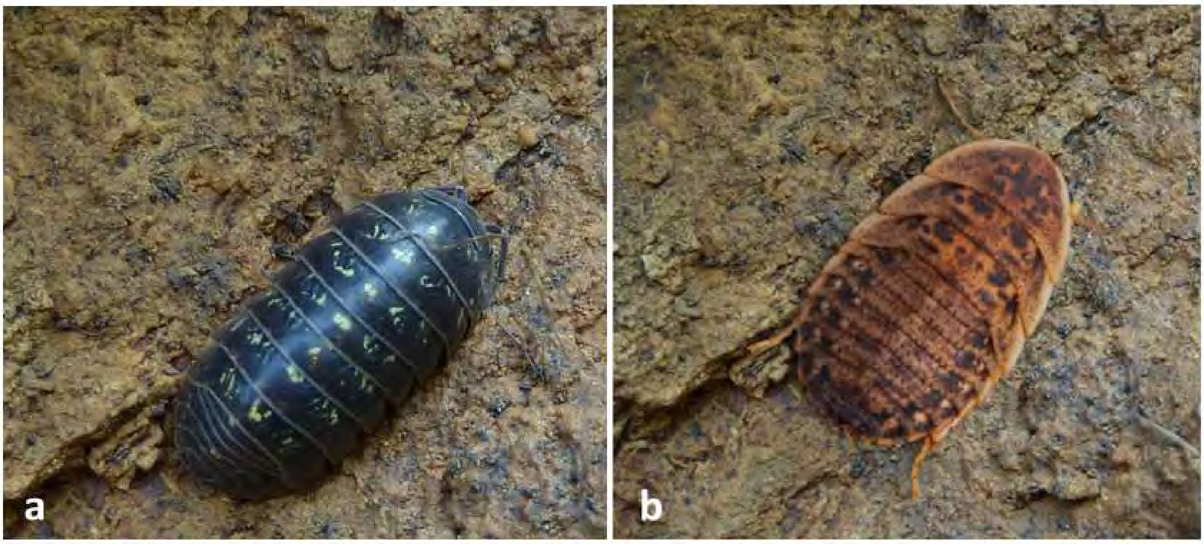

Fig. 1 – Prey presented to bird predators – a) common pill bug (*Armadillidium vulgare*), b) Guyana spotted roach (*Blaptica dubia*), the size of the individuals was 10-12 mm

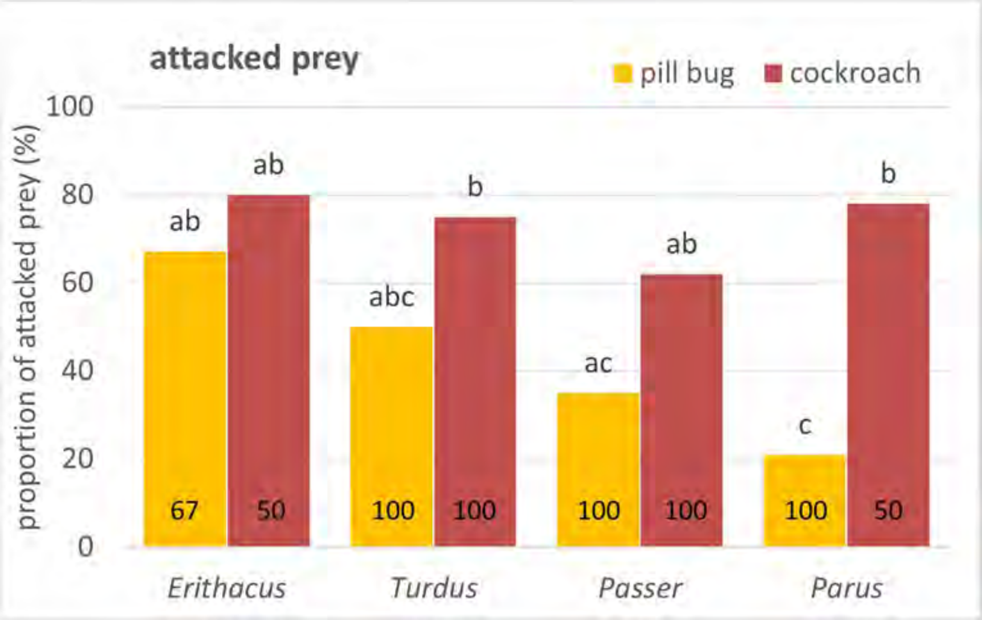

Fig. 2 – Proportion of common pill bugs (*Armadillidium vulgare* – vulgare) and Guyana spotted roaches (*Blaptica dubia* - roach) attacked by four bird species: European robin (*Erithacus rubecula* - Erithacus), Eurasian blackbird (*Turdus merula* - Turdus), Tree sparrow (*Passer montanus* - Passer) and Great tit (*Parus major* - Parus). Number at base of each column refers to the total number of prey items presented in particular bird-prey combination. Letters above columns indicate statistical significance of differences. Values with different letters are significantly different

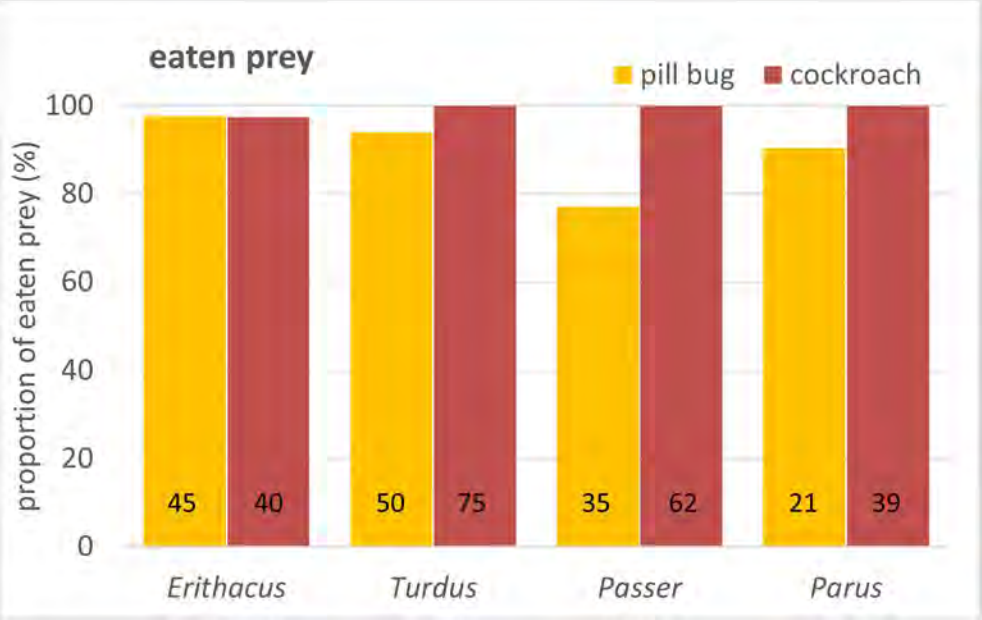

Fig. 3 – Proportion of common pill bugs (*Armadillidium vulgare* – vulgare) and Guyana spotted roaches (*Blaptica dubia* - roach) eaten by four bird species: European robin (*Erithacus rubecula* - Erithacus), Eurasian blackbird (*Turdus merula* - Turdus), Tree sparrow (*Passer montanus* - Passer) and Great tit (*Parus major* - Parus). Only trials, in which the prey was attacked are included. Number at base of each column refers to the total number of prey items attacked in particular bird-prey combination