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Methods of laboratory maintenance of *Pterostichus melanarius* (Coleoptera, Carabidae) when studying its diet under different conditions

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We analyzed the methods of organizing studies of nutrition biology of *Pterostichus* (*Morphnosoma*) *melanarius* (Illiger, 1798), one of the commonest and most numerous species of the family Carabidae in forests and meadows of the Palearctic ecosystems. We considered the features of the maintenance of ground beetle imagoes under different conditions of laboratory experiments, noting the advantages and disadvantages. The existing methods of holding litter invertebrates used in studies were analyzed on the example of studying the trophic relations of *P. melanarius*. During a study of the range of diet and potential trophic niche of a zoophage, conducted in the absence of choice of invertebrates, it is practical to use glass Petri dishes, with sterilized moistened sand serving as a substrate. Alternatively, various types of disposable cups can be used, where (other than sand or a thin layer of sieved soil) the substrate can include foliage, moss, and stones from the locations of invertebrate capture. To prevent the invertebrates from escaping the containers, fine-mesh mosquito netting should be used, fixated with a rubber band. At the same time, determining the trophic preferences of ground beetles and the criteria by which they choose prey in the wild while having free choice of potential prey items (realized trophic niche) requires the use of plastic containers (improvised insectariums) with an optimal size of 30 × 20 cm. A fragment of trophic network is mimicked, with elements of the soil-litter mesofauna and forest litter (foliage, moss, dead tree bark) from the examined sites. The insectariums are closed with plastic covers with air holes covered with mosquito netting. Each day, all the invertebrates in the insectariums are counted to register the number of all individuals consumed by the studied ground beetle. Trophic preferences established during the modeling of trophic network should be verified by visual monitoring of relatively small plastic containers, the bottom of which is covered by a thin layer of moist sand. The primary data yielded during the study should be statistically processed by means of factorial, cluster, and correlation analyzes.

Keywords: *Pterostichus melanarius*; maintenance conditions; study of nutrition; soil-litter mesofauna; substrate modifications.

Introduction

Trophic relations among living organisms form the basis of a biogeocenosis, with a specific circulation of energy and substances (Pontegnie et al., 2005). This reflects in the composition of the forest litter and the rates of its decomposition. Detecting trophic relations and dietary preferences of epigean critters is important for determining the processes of litter breakdown. By studying the trophic ranges of aboveground invertebrates, models of energy transformation in litter biogeohorizon are developed, which aid in clarifying priorities in mitigating anthropogenic transformation of the natural ecosystems.

One of the dominant groups of epigean mesofauna is the Carabidae family, particularly the genus *Pterostichus* (Brygadyrenko, 2016a, 2016b). Among the representatives of this numerous genus, the dominant species in forest ecosystems of the Steppe Prydniprovia is *Pterostichus* (*Morphnosoma*) *melanarius* (Illiger, 1798). It is one of the most numerous Palearctic ground beetle, common almost throughout Europe (Hurka, 1996; Irmler, 2003; Avtaeva et al., 2021) and common in all oblasts of Ukraine with no exception (Faly & Brygadyrenko, 2014; Putchkov et al., 2020).

Pterostichus melanarius stands out among other species of the genus as numerically dominant in forest ecosystems of technogenically transformed territories of urban agglomerations (Luzyanin et al., 2022; Faly et al., 2025; Langraf et al., 2025). In ecosystems near industrial enterprises, this ground beetle is one of the few elements of the carabid fauna. The spread of *P. melanarius* in ecosystems of urban agglomerations indicates its high ecological flexibility and adaptation to the conditions of considerable anthropogenic loading. Detecting the peculiarities of the variability of the main morphometric characteristics of imagoes of *P. melanarius* in a population living in an urban agglomeration (Brygadyrenko & Korolev, 2015) can be a basis for multiyear monitoring of this population, as well as other background species of litter invertebrates (Rainio & Niemela, 2003). The use of the latter is expedient for designing regional programs of environmental monitoring (Lindqvist & Block, 2001) and preserve the biodiversity of forest ecosystems of the steppe zone of Ukraine.

There were found numerous living organisms whose life cycles are associated with the considered ground beetle. The intestines and the body cavity of *P. melanarius* were observed to contain larvae of parasitoid insects of the families Tachinidae (Thiele, 1977), Proctotrupidae (Critchley, 1973), Braconidae (Rivard, 1964), worms of Nematomorpha (Poinar et al., 2004) and Mermithida (Rivard, 1964), unicellular endoparasites from the subclass Gregarinasina (Baudoin, 1971; Sienkiewicz & Lipa, 2009), and parasitic fungi from the class Ascomycetes (De Kesel, 1996). Among ectoparasites, the closest relations with *P. melanarius* were observed for mites of the specialized family Podapolipidae (Regenfuss, 1968).

Pterostichus melanarius is characterized by an extensive diet (Thiele, 1977). This zoophage is able to regulate the numbers of many species of invertebrates living in soil and litter, including pests of agricultural crops (Raworth & Choi, 2001; Raworth et al., 2004). The potential prey items of *P. melanarius* are also critters living in other biogeohorizons, such as grass, shrub, and tree dwellers, most of which can damage vegetable, fruit, and berry crops. Such biological peculiarities make this ground beetle a promising agent of biological method of combating phytophages of agricultural crops.

A large percentage of the diet of *P. melanarius* is comprised of scavenging invertebrates and soil-forming invertebrates, which play an exceptionally important role in the processes of breakdown of dead animal and plant organics, as well as in the humus formation. The dominant representatives of those groups are widespread in the forest ecosystems of Europe, such as biotopes of the Samarskyi Forest in Dnipropetrovsk Oblast (Pokhylenko & Korolev, 2013), and actively participate in an array of biogeocenotic processes.

The objective of this study was to characterize the methods of laboratory maintenance of the zoophage *P. melanarius* for studying its trophic relations, conducted under different experimental designs.

Peculiarities of the diet ranges of *P. melanarius* in different ecosystems

The analysis of the intrapopulation morphological variability of *P. melanarius* and its relations with the possible individual trophic preferences of this zoophage is important for determining the adaptive characteristics of this species (Korolev & Brygadyrenko, 2014). Moreover, the studies of trophic relations and dietary preferences of *P. melanarius* are necessary to identify the reasons for its dominance in natural and anthropogenically transformed ecosystems, and forest plantings subject to a considerable technogenic loading.

An important methodological component of determining trophic relations of different groups of Coleoptera (first of all ground beetles, most of which are characteristic of zoophagy) is the maintenance of the studied invertebrates under laboratory conditions. When planning experiments dealing with dietary preferences of ground beetles, *P. melanarius* in particular, one of the main tasks is creating conditions that are as close as possible to natural. This is a crucial factor for the quality of the obtained data and the significance of the results of the experiments. One of such methods is conducting experiments in semilaboratory conditions. Specimens of a studied species, together with a certain amount of its potential prey, are placed in quite spacious containers with presorted forest litter serving as a substrate. The containers are covered with a fine-mesh net to prevent invertebrates from the environment from infiltrating them, and are dug straight into the soil at a level with the litter. The experiment lasts for several days, after which all the remaining invertebrates in the containers are counted.

Despite obvious advantages, such as this method's approximation to natural conditions, it has a number of disadvantages. The main one is conducting studies outdoors, which makes regular checks of the containers' contents impossible, which means the inability to trace the sequence in which the zoophages consume the offered prey items in real time. This complicates tracking the peculiarities of trophic preferances, creating a specific black box effect, with the option to see only the end results of the experiment.

Another drawback of this method is that the experiment becomes dependent on natural variability, as the containers are left outdoors and are subject to precipitation, excessive drying, or overheating of the substrate. Excessive amount of precipitation can make the substrate in the containers overmoist, which will ultimately affect the study results, leading to in errors. Moreover, carrying out the experiment in natural conditions makes the results of studies subject to another unpredicted external factor (anthropogenic). Conducting this type of experiment requires the presence of some shelter from prying eyes, especially if the study is performed within a settlement. On the other hand, maintenance of insects under laboratory experiments completely excludes all the factors mentioned above.

Identifying the potential trophic niche of *P. melanarius*

This paper is a generalization of the experiments performed during a multiyear study (2006–2023) of the dietary preferences and the differences between the potential and realized trophic niches of *P. melanarius*, one of the commonest ground beetle of the Ukrainian forest ecosystems.

The *P. melanarius* imagoes and soil-litter invertebrates for the study were collected using the Barber pitfall traps without a fixing device, installed in soil using a specially designed device, and also using the method of manual sorting of litter and soil. The invertebrates were collected in natural undisturbed biogeocenoses of the Samarskyi Forest (Samarivskyi District, Dnipropetrovsk Oblast), and also forest-planting ecosystems of the city of Dnipro.

During a study of the probability of this beetle to consume certain species of invertebrates under laboratory conditions, the range of diet of *P. melanarius* is evaluated in the absence of prey choice. During the studies, the imagoes of *P. melanarius* were individually held for a day in Petri dishes. As a substrate, moist sand was used, presterilized through fire so as to avoid infestation of the ground beetles with pathogenic microorganisms and fungi (Fig. 1).

Fig. 1. Maintenance of *P. melanarius* in Petri dishes without prey choice

During the experiments, Petri dishes should preferably be stored in semidarkness so that the light regime in the room is approximated to the light on forest litter, thus corresponding to natural conditions in the studied ecosystems. To achieve this goal, a rack can be used, located in a shaded place, away from direct sun rays (Fig. 2).

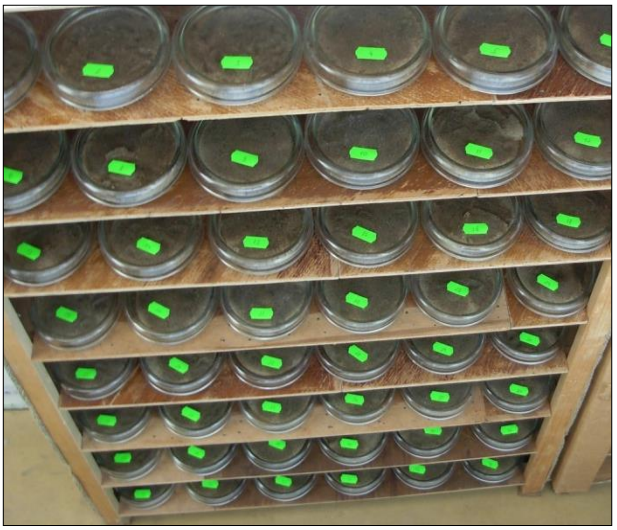

Fig. 2. A rack with Petri dishes for the maintenance of *P. melanarius*

Each specimen of the ground beetle is offered one specimen of an invertebrate belonging to the ecological group of soil, soil-surface, and grass dwellers. If, a day later, the potential prey remains unconsumed, it is replaced with specimens of a species the ground beetle consumes more actively (100% of diet), and then a specimen of a new invertebrate species is offered. To provide the specimens of the studied species with necessary amount of water, a rubber cap from a glass bottle is used, which serves as an improvised drinker (Fig. 3).

However, unnatural conditions (such as no opportunity for the potential prey to hide from the studied zoophage in Petri dishes) may lead to errors in the results, which require detection and removal. In addition, the results need to be to compared with the data yielded using other methods of laboratory study. For those reasons, instead of the abovementioned device, we propose using plastic (polypropylene) cups filled with sand to approximately one-third of their volume. Periodically, the

sand is moistened to create necessary microclimatic conditions. On top, the cup is covered with a fine-mesh mosquito net to allow fresh air in, and is fixated using a rubber band around the outside of the cup to prevent the escape of the studied invertebrates (Fig. 4).

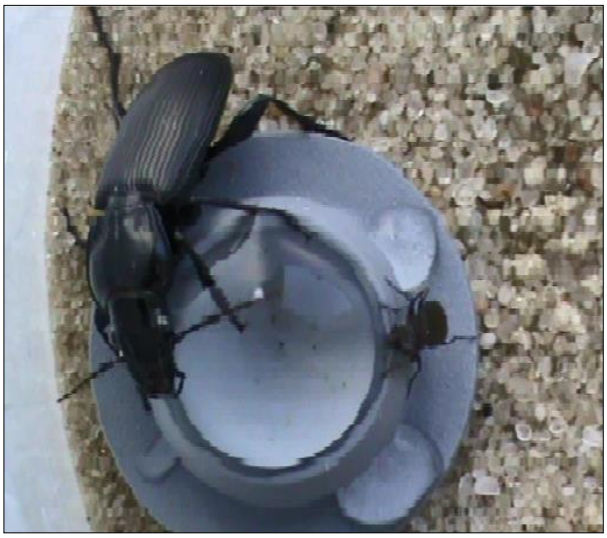

Fig. 3. Improvised drinker, a rubber cap from a glass bottle

This method of maintenance also proved effective during the studies of likelihoods of mixed phytophage ground beetles, such as *Harpalus rufipes* (De Geer, 1774), to consume certain species of invertebrates in the absence of prey for a long period of time.

Ground beetles could be kept in various transparent relatively small disposable cups. As elements of substrate (besides sand or thin layer of sieved soil) it is possible to use thoroughly sorted foliage and moss, collected in the study plots, and also stones that could be used as a shelter by small invertebrates, potential prey items (Fig. 5).

Fig. 4. Maintenance of *P. melanarius* in a plastic cup with no prey choice

Fig. 5. Maintenance of *P. melanarius* in a disposable cup without prey choice

Identifying the realized trophic niche of *P. melanarius*

In contrast to the potential trophic niche, the realized tropic niche (Korolev & Brygadyrenko, 2014) should be studied under other experimental conditions. Similarly to pantophagous invertebrates, *P. melanarius*, in a certain way, combines different species of trophic objects in its daily diet. The literature contains no reports on the methods of studying individual trophic ranges of invertebrate zoophages, and therefore we propose our own methods. To determine the trophic preferences of *P. melanarius*, detect trophic relations and dietary preferences of ground beetles with potential trophic objects, and identify the criteria by which zoophages select their prey in the wild, a fragment of a trophic network is modeled under the laboratory conditions, which includes a population of the studied species and populations of dominant representatives of soil-litter mesofauna of forest biogeocenoses, thereby creating a free choice of invertebrates. Studying the diversity of trophic ranges of ground beetles in the conditions close to natural is a challenge: Imagoes of *P. melanarius* migrate for several dozens of meters daily. A container of such a size is impossible to make, and therefore during the experiments, the imagoes of *P. melanarius* were held individually in improvised insectariums (plastic containers) sized 30 × 20 cm (Fig. 6).

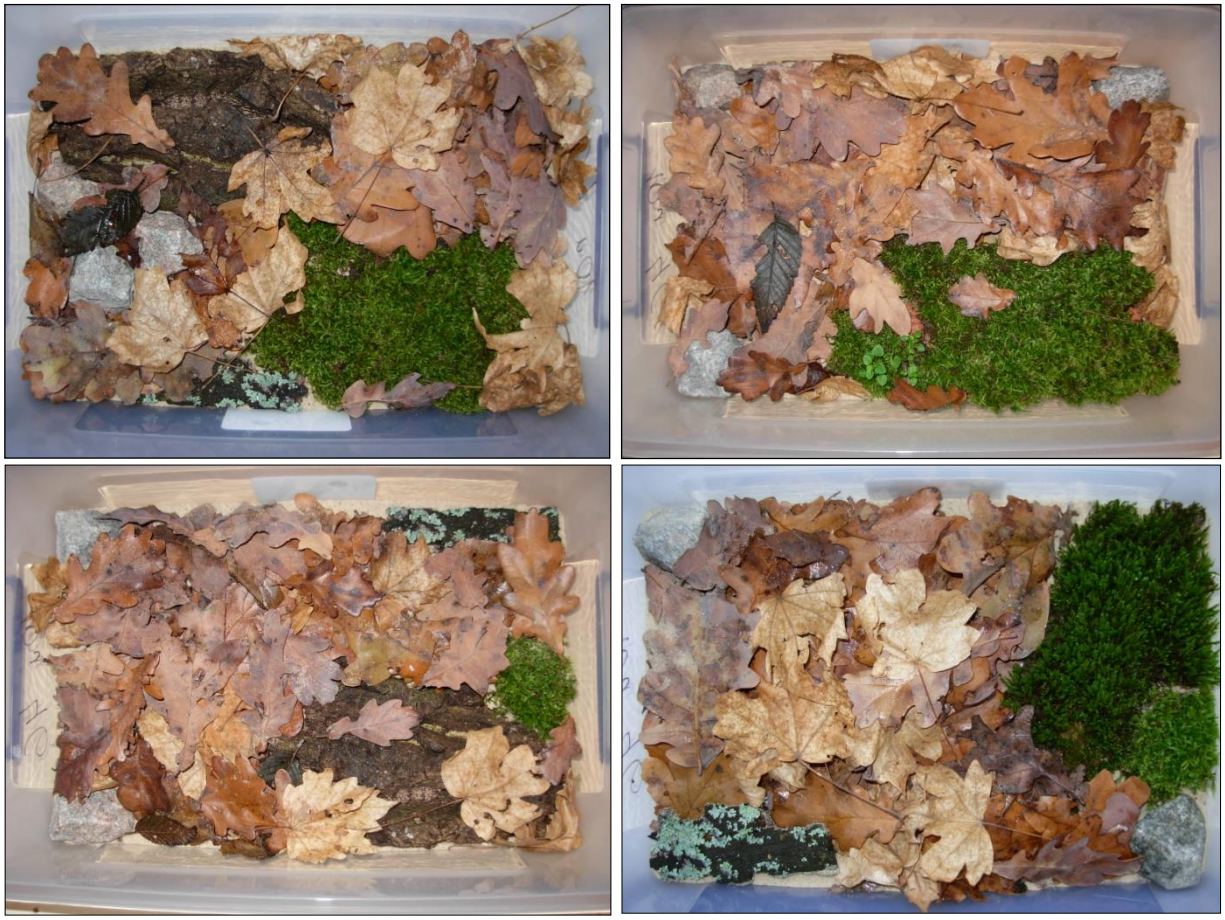

Fig. 6. Insectariums with different modifications of the substrate for the study of the trophic preferences of *P. melanarius*

On top, the insectariums were covered with plastic covers with air holes protected with mosquito netting (Fig. 7). As a substrate, we used sieved soil (4–5 cm), imitating a natural composition of forest litter (foliage, moss, dead tree bark, and stones) from the study locations in forest ecosystems where the imagoes were captured. Periodically, the substrate was moistened according to the living conditions of the studied invertebrates in nature. For several weeks, each specimen of the ground beetle was given a free choice of several specimens of different species of grass, soil surface, and soil dwellers. Also, we took into account shrub and tree dwellers, randomly occurring on the surface of soil and forest litter. An important aspect of ecosystem modeling is involving other invertebrates in the experiment that live on lower levels of the trophic network than the studied predator.

Each day, the substrate was thoroughly sorted to count all invertebrates in the insectariums and record the number of specimens they consumed. For convenience, when counting the specimens consumed by the ground beetles, the animals were transferred to small containers with respect to their trophic specialization. To prevent the invertebrates from consuming one another, the zoophages belonging to the second- and third-order consumers were kept separately. Then, the animals that remained uneaten were returned to the plastic containers. The species composition and the number of potential prey items offered to all specimens of *P. melanarius* were determined according to the actual composition and number of the soil-litter mesofauna collected in the living locations of the ground beetle populations within an area of several square meters.

To verify the results obtained during the study of trophic preferences of ground beetles under the conditions as close as possible to natural, the specimens of *P. melanarius* should to be held individually in relatively small plastic containers for a small period of time. The wall must be high enough to make the animals' escape impossible. The bottom of the containers was covered by a thin layer of moist sand so that the invertebrates offered to the zoophages had no chance of hiding. The ground beetles were given a certain selection of potential prey items, a large portion of which belonged to the invertebrates prevalent in the zoophage's diet. The dietary preferences were determined over the course of the experiment using insectariums (Fig. 7). The species of invertebrates offered to the ground beetles were combined according to the determined criteria of choice of potential prey items by the zoophages. The specimens of the studied ground beetle were visually monitored to record the behavioral aspects of choosing prey.

It is recommended that the results of the study of trophic preferences of ground beetles and the primary yielded data be processed mathematically and statistically using the methods of factorial, cluster, and correlation analyses.

Fig. 7. Maintenance of the imago of *P. melanarius* in plastic containers with a choice of prey items for the purpose of verification of the yielded results

The trials must be repeated 5–7 times, lasting for one day, with the zoophage offered 7–10 species of potential prey daily. Over a day, a *P. melanarius* imago consumes prey amounting to 100–250% of its body mass. Therefore, the biomass of its potential prey should be 500–800% of its body mass. According to the results of laboratory experiments, the trophic items were ranked by priority of consumption (the percentage of the beetle's victims), and also according to the combinations of trophic objects. If the imago of *P. melanarius* received an excessive amount of food, it began to manifest certain patterns in the consumption of trophic objects: Some specimens of the zoophage tended to repeatedly consume particular species every day, while other specimens consumed other trophic objects (Korolev & Brygadyrenko, 2014).

Conclusion

Under the conditions of laboratory maintenance, the optimal means of determining the trophic relations of ground beetles with a broad feeding range, in particular *P. melanarius*, are glass and plastic containers and various disposable cups, which can be used depending on the research objectives: Petri dishes and plastic cups are best for determining the probabilities of the species of ground beetles to consume certain invertebrate species in the absence of choice, whereas plastic containers are used to determine the trophic preferences and criteria by which the ground beetles select prey in the wild. The combination of study conditions (the absence or presence of choice of potential prey in combination with available shelter for invertebrates or the impossibility of the latter to hide from the zoophage) allows for the creation of a three-dimensional model that approximates a trophic network in the wild.

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