

# Changes in turn alternation pattern in response to substrate-borne vibrations in terrestrial isopods



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## ARTICLE INFO

### Keywords:

Crustacea  
Oniscidea  
*Armadillo officinalis*  
Multiple t-maze  
Turn alternation

## ABSTRACT

The present study focuses on the relationship existing between the phenomenon of turn alternation and substrate-borne vibrations in woodlice. *Armadillo officinalis* was utilized as a behavioral model in comparison to *Armadillidium vulgare* so as to assess its capability of perceiving external vibrations too. A T-maze with multiple exits was used to collect information on the pattern of turn alternation in i) adult individuals of *A. officinalis* exposed, and ii) not exposed to micro-vibrations, and iii) adult individuals of *A. vulgare* exposed to micro-vibrations. Turn alternation was assessed as the number of times that an animal turned on the opposite side in the T-maze. Our results showed a statistically significant association between turn alternation pattern and both exposure to micro-vibrations and species of the animals. According to our best-fitting model, *A. officinalis* not exposed and *A. vulgare* exposed to substrate-borne vibrations have 97% and 98% lower odds, respectively of being in a higher category of turn alternations compared to a lower category than exposed individuals of *A. officinalis*. *A. officinalis* seems to be very reactive to substrate-borne vibrations, unlike *A. vulgare*. This different reactivity might be related to a more complex defense mechanism developed as an evolutionary adaptation to the xeric environment, and/or to a means of communication mediated by substrate-borne vibrations, like in insects.

## 1. Introduction

*Armadillo officinalis* Duméril, 1816 is a species of terrestrial isopod (Crustacea: Isopoda: Oniscidea) belonging to the family Armadillidae, and it is widespread in the Mediterranean basin and on western coasts of the Black Sea (Schmalfuss, 1996, 2003). This species has adapted to live in the xeric environment, has mainly nocturnal habits (Vandel, 1962), and can reproduce several times during its life (iteroparous). Its reproductive period depends on the geographic area: in France from June with a possible extension until August (Vandel 1962), in Sicily from May to July (Messina et al., 2011, 2012), and in Israel in October (Warburg, 2013). *A. officinalis* usually lives on several kinds of substrates such as sand, silty-clayey substrates, and rocks, as well as in environments populated by different plant communities (Messina et al., 2014). In their article, Agodi et al. (2015) have proposed the use of this species as a potential biomarker of benzene. Recently, we studied the food preferences of *A. officinalis* in artificial breeding conditions (Montesanto and Cividini, 2017a) and its molt cycle in details (Montesanto and Cividini, 2017b).

This species is also capable of producing vibrations, and this

characteristic is known since the last century. Verhoeff (1908) reported having heard sounds produced by individuals of *A. officinalis* picked up in Sicily, and bred in artificial condition. In both sexes, vibrations are produced by a ledge of scales situated on the propodus of the fourth and fifth pereopod (Caruso and Costa, 1976; Taiti et al., 1998). This feature is present in all species belonging to the genus *Armadillo* (Schmalfuss, 1996). Taiti et al. (1998) reported the presence of a similar stridulatory organ in a species of the genus *Cubaris* from Nepal, as well.

A few aspects regarding the general ethology of this species are known so far. In particular, alternating turn behavior has never been investigated in detail, as well as the reason why this species can emit vibrations. As reported by Carbines et al. (1992), it is not well known yet which particularly unfavorable conditions might most influence the rate of turn alternation in terrestrial isopods, and, above all, in which way. Although in the last years, many published articles studied the behavior of turn alternation in terrestrial isopods under different conditions, it is not clear yet what physiological mechanism actually leads to this phenomenon (see also the discussion section for further references).

Some personal observations of one of the authors (GM) led to the

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idea of this study. The aim of this experiment has been of investigating if substrate-borne vibrations lead *A. officinalis* to modify its behavioral pattern of turn alternation, evaluating more thoroughly its capability to perceive external vibrations. Our first study goal was thus to verify whether exposure to substrate-borne vibrations led *A. officinalis* to make a higher number of alternating turns than in the absence of a vibrational stimulus. Our second goal was to assess whether, for an equal level of exposure to substrate-borne vibrations, *A. officinalis* was able to carry out a higher number of alternating turns compared to *Armadillidium vulgare*, a different species of terrestrial isopod not able to emit vibrations.

## 2. Materials and methods

### 2.1. Subjects

In this study, two random samples of 134 adult individuals of *Armadillo officinalis* and 67 adult individuals of *Armadillidium vulgare* were used. Since April 2015 numerous specimens of these two species have been collected in Sicily (37°31'39" N; 15°04'20" E) and then bred in Pisa (43°43'07" N; 10°23'45" E), in a climate room at 20 °C and with a natural photoperiod. Before starting the test, all the animals were examined to check their bodily integrity. The individuals of *A. officinalis* were randomized to the control group (no micro-vibration) or the exposure group (micro-vibration) in a percentage by 50% (67 animals each). To this aim, a random number table and a pseudorandom sequence of 0s (control group) and 1s (exposure group) were used. All the individuals of *A. vulgare* were only tested in the presence of micro-vibrations and compared to the exposed and not exposed individuals of *A. officinalis*. For each group of animals, gender and size were determined, as well as the molt cycle stage (pre-molt and intermolt stages were identified as described in Montesanto and Cividini, 2017b). For both species, the size was evaluated based on the cephalothorax width (in mm), and each group of animals was divided into two sub-groups (smaller and larger size) using the corresponding medians.

### 2.2. Apparatus and procedure

Animals were individually put in a starting chamber with a proper exit to lead them to enter a T-maze, making the first forced turn on the right. Each animal was tested only once. The T-maze's structure (revised and modified from Hughes, 1967 and Carbines et al., 1992) was built with high impact polystyrene (HIPS) panels. The dimensions are

shown in Fig. 1. Overall, each path was about 27-cm long. The experiment was carried out in a dark room, with a uniform illumination got with a 40 W lamp placed at a distance of 35 cm over the T-maze to avoid shadow zones. The test apparatus was covered with a glass plate, and the animals were encouraged to enter with a soft brush. The paper sheet at the bottom of the T-maze was replaced after each test, and the test apparatus was cleaned with distilled water and 75% ethanol.

Micro-vibrations were produced with a moving-coil miniature ear-phone located on the lower surface of the test apparatus (position C in Fig. 1) and were emitted for all time in which the animal moved inside the T-maze. The segments containing the acoustic recording needed to generate the micro-vibrations were produced using the software Audacity ver. 1.2.4 (available at <http://audacity.sourceforge.net>), as indicated in Fig. 2A–B. The audio file was carefully inspected, checked for the clipping, and saved in a WAV digital format (16-bit amplitude resolution). The temporal and spectral features of the signals were measured. Fig. 2C shows the oscillogram and spectrogram of the emitted sound. The micro-vibrations obtained from the emitted sound were recorded with the software VIBSENSOR (Now Instruments, available at [www.now-instruments.com](http://www.now-instruments.com)), running on Android 6.0 device. Fig. 2D–F illustrates the vibrational diagram recorded during a 15-s test conducted at different points of the test apparatus, and they show that the intensity of the generated micro-vibrations has identical levels of acceleration ( $m/s^2$ ) at all the points of the T-maze.

The diagrams and plates in Fig. 1–4 were drawn and/or arranged using the GNU Image Manipulation Program (GIMP) (ver. 2.8.22) with the methods described in Montesanto (2015, 2016).

### 2.3. Statistical analyses

The pattern of turn alternation was assessed as the number of times that an animal turned on the opposite side in a predefined path (Fig. 1).

The sample size was calculated with G\*Power 3.1 (Faul et al., 2009), so as to have enough power to use either the Wilcoxon-Mann-Whitney nonparametric test or the *t*-test for two independent samples. G\*Power implements two different methods for assessing the power of the WMW-test (A.R.E.-method and Lehmann method). For this calculation, we used the asymptotic relative efficiency (A.R.E.) method, which relates normal approximations to the power of the *t*-test and the Wilcoxon test for a specified *F* (Lehmann, 1975, Eq. 2.42 and Eq. 2.29, respectively). The *compromise power analysis* was used for assessing the power of *t*-test. The possible associations between alternating turn behavior and i) substrate-borne vibrations, and ii) the species of the

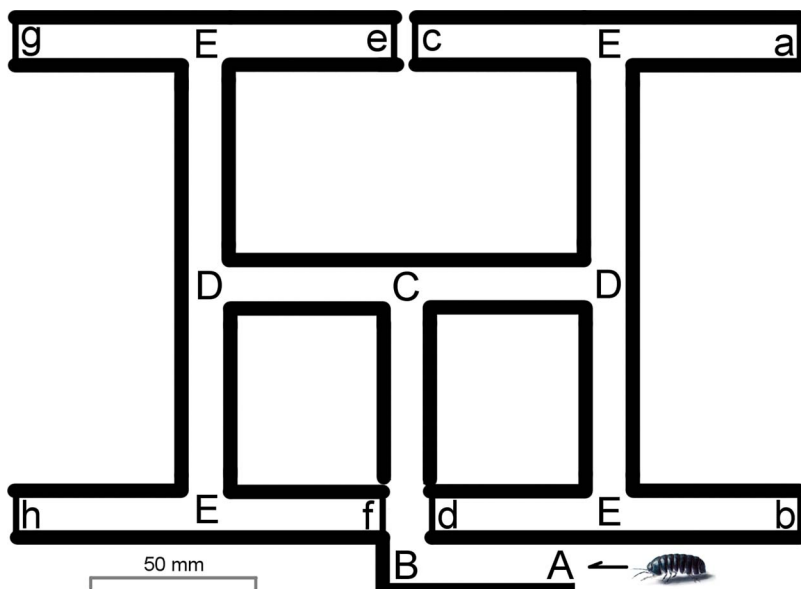


Fig. 1. Outline of the test apparatus. A, position of the starting chamber; B, forced right turn; C, initial T junction; D-E, next choice of turn; a-h, final point. The number of turn alternations was recorded as follows: a = 2, b = 1, c = 1, d = 0, e = 2, f = 1, g = 3, h = 2.

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