

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/322533440>

Differences in the pattern of turn alternation between juveniles and adults of *Armadillo officinalis* Dumèril, 1816 (Isopoda, Oniscidea) in response to substrate-borne vibrations

Article in *acta ethologica* · January 2018

DOI: 10.1007/s10211-018-0282-y

CITATIONS

8

READS

327

2 authors:



Sofia Cividini

University of Ioannina

25 PUBLICATIONS 391 CITATIONS

[SEE PROFILE](#)



Giuseppe Montesanto

Università di Pisa

64 PUBLICATIONS 491 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Systematic and diversity of Terrestrial isopods (Crustacea, Isopoda) [View project](#)



Biotremology in *Armadillo officinalis* Dumèril, 1816 (Crustacea, Isopoda, Oniscidea) [View project](#)



Differences in the pattern of turn alternation between juveniles and adults of *Armadillo officinalis* Dumèril, 1816 (Isopoda, Oniscidea) in response to substrate-borne vibrations

S. Cividini¹ · G. Montesanto²

Received: 19 September 2017 / Revised: 21 December 2017 / Accepted: 4 January 2018 / Published online: 16 January 2018
© Springer-Verlag GmbH Germany, part of Springer Nature and ISPA 2018

Abstract

In this study, we focused on the relationship existing between the phenomenon of alternating turns and substrate-borne vibrations in woodlice, utilizing *Armadillo officinalis* as an experimental behavioral model. 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) non-exposed to micro-vibrations, and (iii) juveniles of *A. officinalis* 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 best model pointed out a statistically significant increased expected number of alternating turns for both groups of adult individuals, non-exposed and exposed to micro-vibrations, compared to exposed juveniles. Adults of *A. officinalis* seem to be very reactive to substrate-borne vibrations, unlike juveniles. This reactivity might be related to a defense mechanism developed as an evolutionary adaptation to the xeric environment, increasing progressively from the juvenile condition until the adult state. This feature might also fit into a complex network of inter- and intraspecific communication mediated by substrate-borne vibrations, like in insects.

Keywords Crustacea · Woodlice · Multiple T-maze · Ethology · Micro-vibrations

Introduction

Armadillo officinalis Dumèril, 1816 is a terrestrial isopod (Crustacea: Isopoda: Oniscidea) belonging to the family Armadillidae, and it is diffuse in the Mediterranean basin and on western coasts of the Black Sea (Schmalfuss 1996, 2003). This species lives in xeric environments and has mainly nocturnal habits (Vandel 1962). It is an iteroparous species (multiple reproductive cycles during lifetime), and its reproductive period depends on the geographic area: from June with a possible extension until August in France (Vandel 1962), from May to July in Sicily (Messina et al. 2011, 2012), and in October in Israel (Warburg 2013). *Armadillo*

officinalis usually lives on sand, silty-clayey substrates, and rocks, and in environments populated by different plant communities (Messina et al. 2014).

Since the last century, this species is known to be able to produce vibrations and stridulations, audible within the human spectrum of frequencies as well. Indeed, in his article, Verhoeff (1908) wrote of sounds produced by individuals of *A. officinalis* picked up in Sicily and bred in artificial conditions. At that time, nothing was known about substrate-borne vibrations as an information source for animals. After then, just an Italian local journal published a brief paper mentioning stridulation but not describing the frequencies range (Caruso and Costa 1976). Both sexes are capable of producing vibrations with 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 *Armadillo* (Schmalfuss 1996). The presence of a similar stridulatory organ was also referred by Taiti et al. (1998) in the species *Cubaris* from Nepal (*C. everesti* Vandel, 1973), and in two new still undescribed species (S. Taiti, *pers. comm.*).

So far, only a few aspects concerning the ethology of this species are known. In particular, alternating turn behavior is

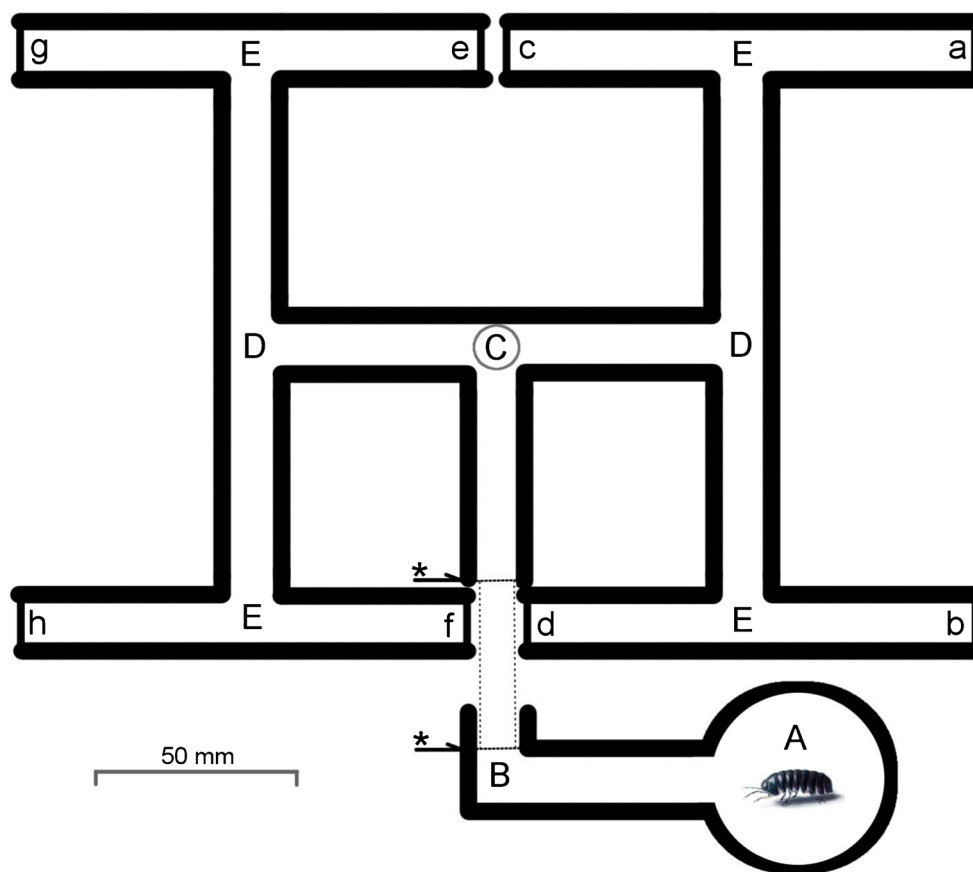
Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10211-018-0282-y>) contains supplementary material, which is available to authorized users.

✉ G. Montesanto
giuseppe.montesanto@unipi.it

¹ Independent Researcher in Biostatistics, Como, Italy

² Dipartimento di Biologia, Università degli Studi di Pisa, Pisa, Italy

Fig. 1 Outline of the test apparatus. A, position of the starting chamber; B, forced right turn; C, initial T junction; D and E, next choice of turn; a–h, final point. The number of alternating turns was recorded as follows: a = 2, b = 1, c = 1, d = 0, e = 2, f = 1, g = 3, and h = 2. The gray circle in C shows the position where the vibrational source was located on the lower surface of the T-maze



still little known, as well as the reason why this species emits vibrations. Hughes (1989) described the tendency to turn to the opposite side following an immediately preceding turn in many invertebrates. Alternating turn behavior may depend on particularly unfavorable conditions such as food deprivation (Hughes 1978), excessive disturbance at the level of substrate (Houghtaling and Kight 2006), signals from indirect predators (Hegarty and Kight 2014), or presence of predators (Carbines et al. 1992; Hughes 1967, 1978). Vibrations studied by Houghtaling and Kight (2006), however, were mainly generated inside an urban and rural environment contaminated by human activities, and, for this reason, not comparable in intensity and frequency with those generated in a natural environment. An increasing number of alternating turns was also observed in conditions of excessive light, or in dry environments (Hughes 1967), and after artificial stimulation of animals (Ono and Takagi 2006).

In a previous work (Cividini and Montesanto 2018), the authors tested adult individuals of *A. officinalis* and *Armadillidium vulgare* in a T-maze with multiple exits to assess the pattern of turn alternation in presence or absence of substrate-borne vibrations (for *A. officinalis*), and between different species at a parity of exposure. The results proved a statistically significant association between the pattern of turn alternation and both exposure to micro-

vibrations and species of the animals. In particular, *A. officinalis* non-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*. Consequently, the aim of the present study has been to provide an additional analysis of the previously collected data (Cividini and Montesanto 2018) for assessing how adults and juveniles of *A. officinalis* modulate their pattern of turn alternation when exposed to substrate-borne vibrations. Specifically, the main goal was to verify whether adults exposed to substrate-borne vibrations were able to make a higher number of alternating turns than exposed juveniles. In this way, we also investigated the capability of reacting to micro-vibrations in the two different conditions of development.

Materials and methods

Animals

Since April 2015, numerous specimens of *A. officinalis* have been collected in Sicily (37° 31' 39" N 15° 04' 20" E). These animals have been then bred in Pisa (43° 43' 07"

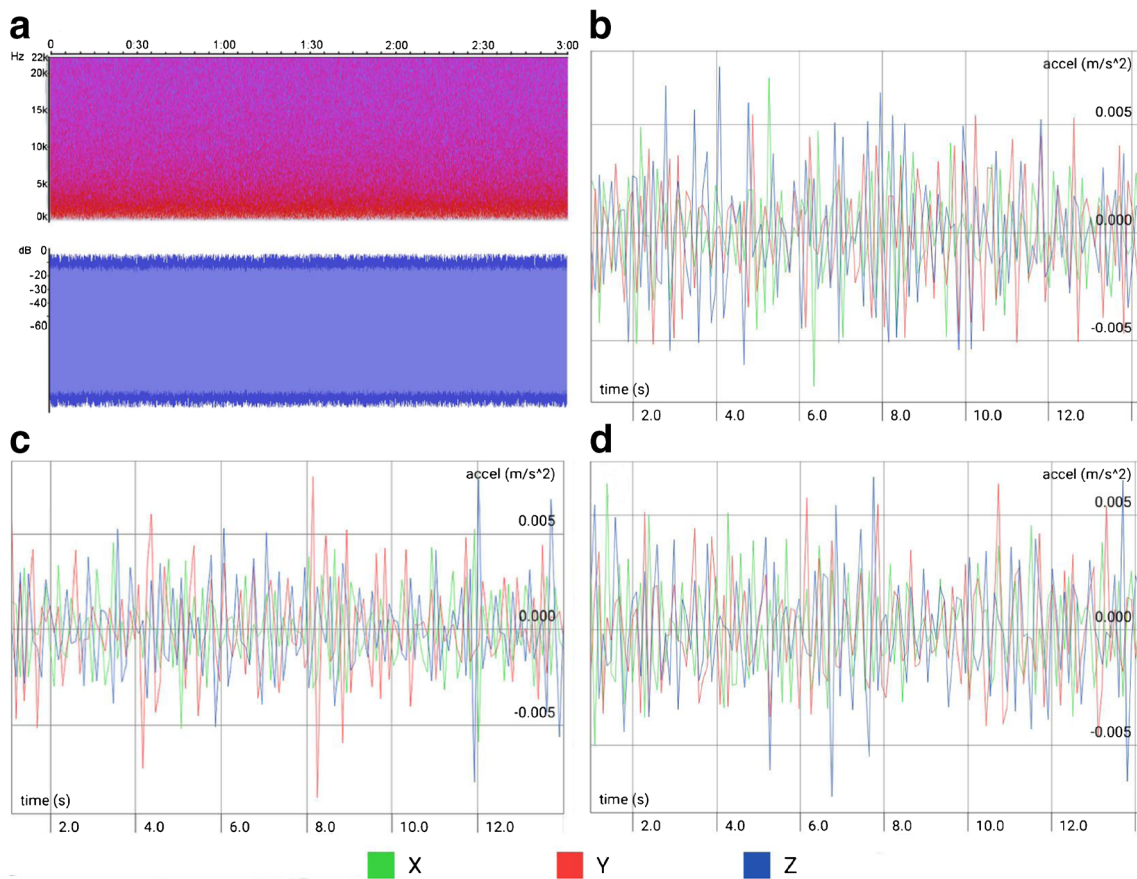


Fig. 2 Micro-vibration emitted during the experiment. **a** Spectrogram and oscillogram of the emitted sound. **b**, **c**, and **d** Micro-vibration diagrams recorded at points **b**, **c**, and (right) **d**, respectively (as in Fig. 1), with the software VIBSENSOR (see text for further details). As one

can see, intensity and frequency of the used micro-vibrations are reasonably similar and comparable at all points of the T-maze (modified from Cividini and Montesanto 2018)

N 10° 23' 45" E) inside a climate room at 20 °C with a natural photoperiod. Animals were housed in a terrarium and fed with potato tubers and plane tree leaves (Montesanto and Cividini 2017a). In this study, two random samples of 134 adult individuals and 67 juveniles (III and IV stages) of *A. officinalis* were used. All the animals were individually examined to check their bodily integrity at the beginning of the experiment. The adults of *A. officinalis* were randomized to the control group (no micro-vibration) or the exposed group (micro-vibration) in a percentage by 50% (67 animals each). A random number table and a pseudorandom sequence of 0s (control group) and 1s (exposed group) were used to this goal. It was not possible to have the control group for juveniles because of the limited number of available specimens. Gender, size, and molt cycle stage were determined for each animal (pre-molt and inter-molt stages were identified as described in Montesanto and Cividini 2017b). The size was evaluated based on the cephalothorax width (in mm), and the two groups of animals (adults and juveniles) were divided into two sub-groups (smaller and larger size) using the respective medians as the index of the central position.

Apparatus and procedure

Each animal was placed into a starting chamber with a proper exit to lead it to enter a T-maze. The T-maze (revised and modified from Hughes 1967 and Carbines et al. 1992) was built with high impact polystyrene (HIPS) panels, and its dimensions are shown in Fig. 1. Each path was about 27 cm long. Every test was carried out in a dark room, and a 40 W lamp, put at a distance of 35 cm over the T-maze, was used to get a uniform illumination. The test apparatus was covered with a glass plate, and animals were encouraged to enter with a soft brush. The first forced turn was on the right for all the animals. Each animal was tested only once. After each test, the test apparatus was cleaned with distilled water and 75% ethanol, and the paper sheet at the bottom of the T-maze was replaced.

Micro-vibrations with an intensity and frequency comparable to those an animal can find in a natural environment were produced with a moving-coil miniature earphone located on the lower surface of the test apparatus, well hid from animals' sight, and firmly fixed with hot glue (position C in Fig. 1). The micro-vibration emission lasted for all time in which each 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. The several steps for settings are indicated in the [Appendix](#).

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. The oscillogram and spectrogram of the emitted sound are shown in Fig. 2a. The micro-vibrations obtained from the emitted sound were recorded using the software VIBSENSOR, running on Android 7.0 device (Huawei P9, with the oscilloscope inside). Before starting the experiment, we tested the micro-vibrations at all crosses of every possible path in the T-maze several times. The resulting levels of acceleration were always comparable with those reported in Fig. 2b–d. This figure illustrates the vibrational diagram with comparable levels of acceleration (measured in m/s^2) recorded during a 15-s test conducted at different points of the T-maze.

The diagrams and plates in Figs. 1, 2, 3, and 4 were drawn and/or arranged with the GNU Image Manipulation Program (GIMP) (ver. 2.8.22) utilizing the methods described in Montesanto (2015, 2016).

Statistical analysis

Turn alternation pattern 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). The applied rationale for this calculation was to have enough power to use both Wilcoxon-Mann-Whitney nonparametric test and *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). 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 subsequently used to assess the power of *t* test (more details in the [Appendix](#)).

The possible association between turn alternation and the state of animals was then studied more thoroughly with Poisson regression models. A categorical variable representing the state of the animals was used in the regression models in the form of dummy variables, using juveniles

Fig. 3 **a** T-maze with all the possible paths followed by the animals tested in the experiment (*A. officinalis*). Each path is indicated with a different color from the start point until the corresponding exit. The pie charts show the percentage of the paths followed by **b** adults not exposed to micro-vibrations, **c** adults exposed to micro-vibrations, **d** juveniles exposed to micro-vibrations. It was not possible to have the control group for juveniles because of the limited number of available specimens

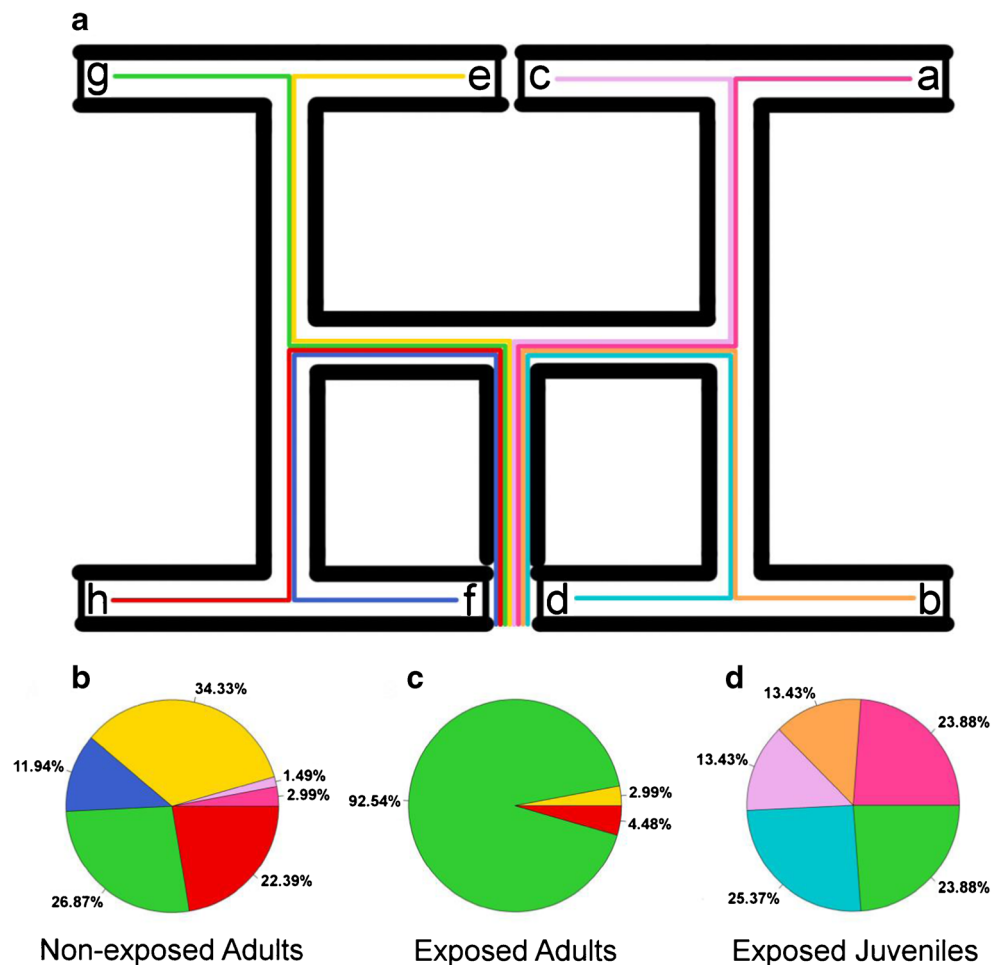
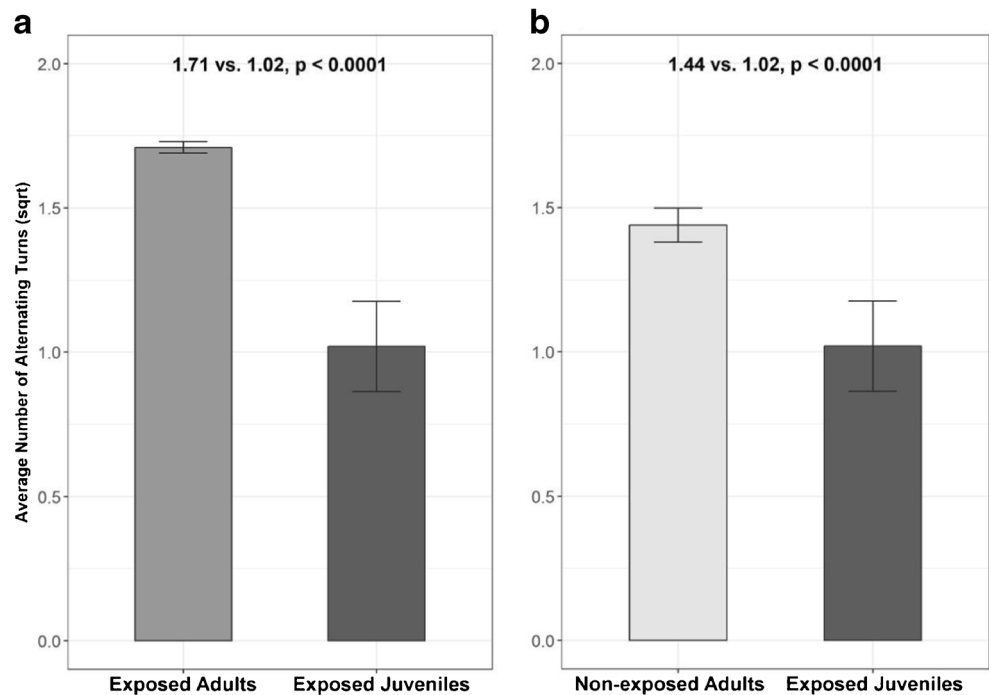


Fig. 4 *Armadillo officinalis*. Average number of alternating turns in **a** adults and juveniles, both exposed to micro-vibrations, and **b** adults non-exposed and juveniles exposed to micro-vibrations (95% confidence interval error bars). *T* test results are also shown (square-root transformed data)



exposed to micro-vibrations as the reference. Gender, size, and molt cycle stage were considered as potential confounders and included in the models as binary variables.

Data analysis was carried out with SAS 9.4 using the following procedures: *proc npar1way*, *proc ttest*, and *proc genmod* plus other standard procedures for descriptive statistics. All the graphs were created with the *ggplot2* library (Wickham, 2009), and the function *pie()* in R/RStudio (ver. 3.4.1).

Results

Table 1 summarizes number and percentage of the animals within each group, overall and stratified by gender, size, and molt cycle stage. The different paths traveled by the animals during the test are shown in Fig. 3a, and the corresponding percentages in Figs. 3b–d.

The comparisons between the average number of alternating turns in adults and juveniles, both exposed to micro-vibrations and in non-exposed adults and exposed juveniles to micro-vibrations, were carried out using the *t* test with the Satterthwaite's method for unequal variances. Data were transformed into the square root, but have remained non-normally distributed. The sample size, however, was large enough to ensure the *t* test robustness. Both *t* tests pointed out a statistically significant difference in the average number of turn alternations between adults and juveniles, both exposed to micro-vibrations ($t = 8.54$, $df = 68.18$, 1.71 ± 0.08 vs. 1.02 ± 0.65 ; $p < 0.0001$; Cohen's $d = 0.5$) (Fig. 4a) and

between adults non-exposed and juveniles exposed to micro-vibrations ($t = 5.02$, $df = 81.204$, 1.44 ± 0.22 vs. 1.02 ± 0.65 ; $p < 0.0001$; Cohen's $d = 0.5$) (Fig. 4b).

The association between turn alternation and the state of animals was investigated more thoroughly with standard Poisson regression models, with and without interactions. As all interactions were non-statistically significant, the corresponding models were not considered for a parsimony issue. The models without interactions showed very similar values of the fit statistics for the comparison of nested models (AIC criterion). Hence, we chose the full model because it is biologically more informative. Table 2 reports the results of the full model. The algorithm correctly converged, and the chi-square test on deviance ($df = 195$, $chisq = 88.85$, $p = 1$) showed a good fit of the model to data. Hence, based on the results of our best model, we can draw the following conclusions. Comparing adults non-exposed and juveniles exposed to substrate-borne vibrations, the logarithm of the expected number of alternating turns increases by 0.39 for non-exposed adults ($p = 0.003$). In other words, the expected number of alternating turns for non-exposed adults is about 1.5-times the number of alternating turns for exposed juveniles (48% increase). Comparing adults and juveniles, both exposed to substrate-borne vibrations, the logarithm of the expected number of alternating turns increases by 0.70 for exposed adults ($p < 0.0001$). The expected number of alternating turns for exposed adults is thus two times the number of alternating turns for exposed juveniles (100% increase). No statistically significant association was found with size, gender, and molt cycle stage.

Table 1 Number and percentage of the animals within each group considered in the experiment, overall and stratified by gender, size, and molt cycle stage

	Adults of <i>A. officinalis</i> not exposed to micro-vibrations <i>N</i> (%)	Adults of <i>A. officinalis</i> exposed to micro-vibrations <i>N</i> (%)	Juveniles of <i>A. officinalis</i> exposed to micro-vibrations <i>N</i> (%)
Males	24 (35.8)	23 (34.3)	28 (41.8)
Group 1	14 (20.9)	17 (25.4)	18 (26.9)
Intermolt	14 (20.9)	16 (23.9)	17 (25.4)
Premolt	0 (0)	1 (1.5)	1 (1.5)
Group 2	10 (14.9)	6 (8.9)	10 (14.9)
Intermolt	10 (14.9)	6 (8.9)	10 (14.9)
Premolt	0 (0)	0 (0)	0 (0)
Females	43 (64.2)	44 (65.7)	39 (58.2)
Group 1	20 (29.9)	16 (23.9)	16 (23.9)
Intermolt	20 (29.9)	16 (23.9)	16 (23.9)
Premolt	0 (0)	0 (0)	0 (0)
Group 2	23 (34.3)	28 (41.8)	23 (34.3)
Intermolt	22 (32.8)	27 (40.3)	20 (29.9)
Premolt	1 (1.5)	1 (1.5)	3 (4.48)
All animals	67 (100)	67 (100)	67 (100)

Group 1, smaller size; Group 2, larger size

Our results proved that adults of *A. officinalis* always perform a significantly greater number of alternating turns compared to exposed juveniles, both when exposed and when not exposed to micro-vibrations.

Furthermore, both adults non-exposed and exposed to micro-vibrations used less time, on average, to travel a distance of about 27 cm than the exposed juveniles (24.0 ± 9.9 ,

22.8 ± 7.2 , 96.4 ± 35.4 s, respectively). Consequently, the exposed adults run the path with an average speed of 71.1 cm/min versus 16.8 cm/min of the exposed juveniles. The average speed of the adults non-exposed to micro-vibrations was, however, similar to that of the exposed adults (67.6 cm/min). The differences in the time used to complete the path among the three tested groups were evaluated with the

Table 2 Adjusted standard Poisson regression model

Parameter	Estimate	Standard error	Wald 95% confidence limits		<i>p</i> value
Intercept	0.5657	0.2555	0.0649	1.0665	0.0268
State					
Adults of <i>A. officinalis</i> not exposed to micro-vibrations	0.3915	0.1320	0.1328	0.6502	0.0030
Adults of <i>A. officinalis</i> exposed to micro-vibrations	0.7037	0.1243	0.4602	0.9472	<0.0001
Juveniles of <i>A. officinalis</i> exposed to micro-vibrations	Ref.				
Size					
Group 1	-0.0046	0.0992	-0.1990	0.1897	0.9629
Group 2	Ref.				
Gender					
Males	0.0479	0.1023	-0.1527	0.2485	0.6399
Females	Ref.				
Molt cycle stage					
Intermolt	-0.2177	0.2493	-0.7064	0.2710	0.3826
Premolt	Ref.				

Analysis of maximum likelihood parameter estimates. Group 1, smaller size. Group 2, larger size

Kruskal-Wallis nonparametric test (chi-square = 130.28, $p < 0.0001$). The pairwise two-sided multiple comparison analysis was carried out using the Dwass, Steel, Critchlow-Fligner (DSCF) method (Dwass 1960; Steel 1960; Critchlow and Fligner 1991).

Statistically significant differences in the running time were found comparing non-exposed adults and exposed juveniles (mean score: 69.54 vs. 167.20, DSCF Value = 13.90, $p < 0.0001$) and adults and juveniles, both exposed to micro-vibrations (mean score: 66.26 vs. 167.20, DSCF Value = 14.02, $p < 0.0001$). Hence, both non-exposed and exposed adults are always significantly quicker than exposed juveniles.

Table 3 summarizes the descriptive statistics for the running time (in seconds) by path.

Discussion

In this study, we found evidence of the fact that adults of *A. officinalis* are able of performing a significantly greater number of alternating turns, in both presence and absence of substrate-borne vibrations, than exposed juveniles. This behavior seems to indicate a progressive improvement in the turn alternation use, passing from the juvenile state to the adult condition, as well as an increased reactivity to substrate-borne vibrations. What physiological mechanism and/or behavioral process leads to these improvements in both use of turn alternation and reactivity to micro-vibrations remains to be understood. However, the high reactivity to substrate-borne vibrations might fit into a strategy of survival developed as a form of evolutionary adaptation during the passage from water to the terrestrial environment. The existing relationship between turn alternation as a hypothetical defense mechanism and reactivity to substrate-borne vibrations (Cividini and Montesanto 2018)

might give this species a better chance to escape from predators. Adult individuals are, furthermore, always significantly quicker than juveniles.

The tendency to turn to the opposite direction following an immediately preceding turn is a particularly common behavior among invertebrates and was described for a large number of species (Hughes 1989). This feature was also observed in a unicellular organism belonging to the genus *Paramecium* (Lepley and Rice 1952; Lachman and Havlena 1962; Harvey and Bovell 2006). In many species of terrestrial isopods, turn alternation has been supposed to be a natural reaction of animals towards particularly unfavorable environmental conditions. Food deprivation (Hughes 1978), an excess of disturb at the level of substrate (Houghtaling and Kight 2006), signals coming from indirect predators (Hegarty and Kight 2014), and exposure to predators (Carbines et al. 1992; Hughes 1967, 1978) can lead an increased number of alternating turns. In a previous work (Cividini and Montesanto 2018), we proved the existence of a statistically significant association between the pattern of turn alternation and both exposure to substrate-borne vibrations and species of the animals. In particular, we found that adults of *A. officinalis* react to the presence of micro-vibrations, performing a greater number of alternating turns than in the absence of the vibrational impulse. Inside a complex network of direct and indirect communications mediated by substrate-borne vibrations, this behavior might support the hypothesis of turn alternation as a defense mechanism against adverse environmental conditions. It is our opinion that a species provided of a stridulatory organ and, therefore, able to emit vibrations like *A. officinalis* might also be able to manage substrate-borne vibrations more efficiently than other species and for multiple aims, for instance, in the ambit of a defense strategy and/or as a means of intra- and interspecific communication, like in insects.

Table 3 Descriptive statistics for the running time (in seconds) by path. Number of alternating turns: a = 2, b = 1, c = 1, d = 0, e = 2, f = 1, g = 3, h = 2

State	Exit	N	Mean	SD	Min	Max
Adults of <i>A. officinalis</i> not exposed to micro-vibrations	a	2	25.7	9.6	18.9	32.5
	c	1	87.2	–	87.2	87.2
	e	23	22.2	6.5	14.5	36.3
	f	8	21.6	3.1	17.0	26.8
	g	18	22.5	5.8	12.3	33.3
	h	15	25.3	6.7	18.5	45.7
Adults of <i>A. officinalis</i> exposed to micro-vibrations	e	2	19.4	7.1	14.4	24.4
	g	62	23.0	7.3	12.6	67.6
	h	3	20.0	5.4	16.5	26.1
Juveniles of <i>A. officinalis</i> exposed to micro-vibrations	a	16	95.5	34.3	36.2	150.3
	b	9	99.1	44.4	44.7	152.5
	c	9	87.5	32.6	37.4	128.2
	d	17	107.0	31.7	48.8	154.9
	g	16	89.3	37.5	34.1	156.1

As reported by Hill and Wessel (2016), substrate-borne vibrations might be interpreted by an unintended vibrational receiver as a coming danger. For instance, the arrival of a predator which, pressing its body on a solid substrate, produces substrate-borne vibrations leading the potential prey to escape.

Sensory receptors might be involved in the high reactivity of *A. officinalis* to substrate-borne vibrations, although no specific receptor with these characteristics has been so far described in terrestrial isopods, unlike other invertebrates (Barth 1982; Hutchings and Lewis 1983; Kalmring 1985; Popper et al. 2001; Devetak et al. 2004). No homology may, however, be present with the already known receivers both within and among taxa (Hill 2009), although analogies due to a process of evolutionary convergence cannot be excluded. In this context, tactile receptors might play an important role being also sensitive to substrate-borne vibrations. Evidence in this regard was found, for instance, in *Apis mellifera carnica* (Hymenoptera, Apidae) whose legs are provided with special receptors able to detect micro-vibrations propagating on the combs of its nests (Sandeman et al. 1996).

Communication via substrate-borne vibrations in insects has acquired more and more importance compared to what was previously thought (Virant-Doberlet and Čokl 2004) because it seems to be involved in a wide range of adaptive roles in animals (Hill 2001, 2009), for instance, mating, parental cares, foraging, competition, and danger perception. Signaling mediated by substrate-borne vibrations is widely used in both invertebrates (Virant-Doberlet and Čokl 2004; Cocco and Rodriguez 2005) and vertebrates (Hill 2008).

Terrestrial isopods are frequently preyed by several species of lizards (Carrettero 2004; Castilla et al. 2008), but no study in the literature has so far proved that the emission of stridulations during the predatory act may be useful to this species to escape from a predator. The first hypothesis on the possibility of an intraspecific communication in *A. officinalis* goes back to Caruso and Costa (1976), but without any explanation supporting this speculation.

Vibrational communication might also be involved in favoring aggregation phenomena, already known for this species and, in general, in terrestrial isopods as a way to prevent dehydration (Warburg and Berkovitz 1978, Sibly 1983, Hornung 2011, Broly et al. 2012). Aggregation, along with turn alternation, might constitute another defense strategy in case of predation (Broly et al. 2013). The Allee effect on group size and survival rate is well known for *Porcellio scaber*, too (Brockett and Hassall 2005).

We think that this study has contributed to clarifying some interesting, novel aspects of still little known behavioral processes in woodlice, proving the existence of a different capability of modulating turn alternation between adults and juveniles of *A. officinalis*, both before and following exposure to substrate-borne vibrations. This behavior apparently does not seem to depend on sex, size, and molt cycle stage of animals. Adult individuals seem to have a higher reactivity to substrate-

borne vibrations than juveniles, reacting with a greater number of alternating turns, and more quickly. To our knowledge, no study in the literature has studied until now the different sensitivity to substrate-borne vibrations among adult individuals and juveniles, in neither insects nor other invertebrates.

The high ability to perceive vibrations might also be involved in the use of substrate-borne vibrations as a means of communication to be fitted into a wider and more complex network of inter- and intraspecies communications, like in insects.

Additional research is needed to understand better so complex phenomena, which might also have important implications in a context of applied ecology (e.g., Agodi et al. 2015). Indeed, *A. officinalis* represents an excellent experimental model to investigate the reasons behind these behavioral processes mediated by substrate-borne vibrations.

Acknowledgements Special thanks to Dr. Clara P. Amorim (Associate Editor) and the anonymous reviewers who checked over our manuscript for their valuable suggestions.

Compliance with ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no conflict of interest.

Appendix

Settings of the parameters used in G*Power 3.1 for the sample size calculation and power analysis

Wilcoxon-Mann-Whitney nonparametric test (two groups)

Analysis: A priori (Compute required sample size)

Options: A.R.E. method

Input:

Tail(s) = Two

Parent distribution = Normal

Effect size $d = 0.5$

α err prob = 0.05

Power ($1 - \beta$ err prob) = 0.80

Allocation ratio $N_2/N_1 = 1$

T test: Difference between two independent means (two groups)

Analysis: Compromise (Compute implied α and power)

Input:

Tail(s) = Two

Effect size $d = 0.5$

β/α ratio = 4

Sample size group 1 = 67

Sample size group 2 = 67

Settings to generate the micro-vibrations used in the experiment with the software Audacity (ver. 1.2.4)

The option *Noise* was selected starting from the menu *Generate*.

In the window named *Noise Generator*, the following parameters were set:

- *Noise type*: Brownian
- *Amplitude*: 1
- *Duration*: 3 min

The sound was normalized at -4.0 dB with the command *Normalize*, in the *Effect* menu.

References

- Agodi A, Oliveri Conti G, Barchitta M, Quattrocchi A, Lombardo BM, Montesanto G, Messina G, Fiore M, Ferrante M (2015) Validation of *Armadillo officinalis* Dumèril, 1816 (Crustacea, Isopoda, Oniscidea) as a bioindicator: in vivo study of air benzene exposure. *Ecotox Environ Safe* 114:171–178. <https://doi.org/10.1016/j.ecoenv.2015.01.011>
- Barth FG (1982) Spiders and vibratory signals: sensory reception and behavioral significance. In: Witt PN, Rovner JS (eds) *Spider communication*. Princeton University Press, Princeton, pp 67–122
- Brockett B, Hassall M (2005) The existence of an Allee effect in populations of *Porcellio scaber* (Isopoda: Oniscidea). *Eur J Soil Biol* 41(3–4):123–127. <https://doi.org/10.1016/j.ejsobi.2005.09.004>
- Broly P, Mullier R, Deneubourg J-L, Devigne C (2012) Aggregation in woodlice: social interaction and density effects. *ZooKeys* 176:133–144
- Broly P, Deneubourg JL, Devigne C (2013) Benefits of aggregation in woodlice: a factor in the terrestrialization process? *Insect Soc* 60(4):419–435. <https://doi.org/10.1007/s00040-013-0313-7>
- Carbines GD, Dennis RM, Jackson RR (1992) Increased turn alternation by woodlice (*Porcellio scaber*) in response to a predatory spider, *Dysdera crocata*. *Int J Comp Psychol* 5:138–144
- Carretero MA (2004) From set menu to *a la carte*. Linking issues in trophic ecology of Mediterranean lacertids. *Ital J Zool* 71(sup2):121–133. <https://doi.org/10.1080/11250000409356621>
- Caruso D, Costa G (1976) L'apparato stridulatore e l'emissione di suoni in *Armadillo officinalis* Dumèril (Crustacea, Isopoda, Oniscidea). *Animal* 3:17–27
- Castilla AM, Vanhooydonck B, Catenazzi A (2008) Feeding behaviour of the Columbrete lizard *Podarcis atrata*, in relation to Isopoda (Crustacea) species: *Ligia italica* and *Armadillo officinalis*. *Belg J Zool* 138:146–148
- Cividini S, Montesanto G (2018) Changes in turn alternation pattern in response to substrate-borne vibrations in terrestrial isopods. *Behav Process* 146:27–33. <https://doi.org/10.1016/j.beproc.2017.11.005>
- Cocroft RB, Rodriguez RL (2005) The behavioral ecology of insect vibrational communication. *Bioscience* 55(4):323–334.
- Critchlow DE, Fligner MA (1991) On distribution-free multiple comparisons in the one-way analysis of variance. *Commun Stat Theory Methods* 20:127–139
- Devetak D, Pabst MA, Lipovšek Delacord A (2004) Leg chordotonal organs and campaniform sensilla in *Chrysoperla* Steinmann 1964 (Neuroptera): structure and function. *Denisia* 13:163–171
- Dwass M (1960) Some k-sample rank-order tests. In: Olkin I, Ghurye SG, Hoëffding W, Madow WG, Mann HB (eds) *Contributions to probability and statistics*. Stanford University Press, Stanford, pp 198–202
- Faul F, Erdfelder E, Buchner A, Lang A-G (2009) Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods* 41(4):1149–1160. <https://doi.org/10.3758/BRM.41.4.1149>
- Harvey AW, Bovell NKA (2006) Spontaneous alternation behavior in *Paramecium*. *Learn Behav* 34(4):361–365. <https://doi.org/10.3758/BF03193200>
- Hegarty KG, Kight SL (2014) Do predator cues influence turn alternation behavior in terrestrial isopods *Porcellio laevis* Latreille and *Armadillidium vulgare* Latreille? *Behav Process* 106:168–171. <https://doi.org/10.1016/j.beproc.2014.06.005>
- Hill PSM (2001) Vibration and animal communication: a review. *Am Zool* 41:1135–1142
- Hill PSM (2008) *Vibrational communication in animals*. Harvard University Press, Harvard
- Hill PSM (2009) How do animals use substrate-borne vibrations as an information source? *Naturwissenschaften* 96(12):1355–1371. <https://doi.org/10.1007/s00114-009-0588-8>
- Hill PSM, Wessel A (2016) Biotremology. *Curr Biol* 26:R181–R191
- Hornung E (2011) Evolutionary adaptation of oniscidean isopods to terrestrial life: structure, physiology and behavior. *Terr Arthropod Rev* 4(2):95–130. <https://doi.org/10.1163/187498311X576262>
- Houghtaling K, Kight SL (2006) Turn alternation in response to substrate vibration by terrestrial isopods, *Porcellio laevis* (Isopoda: Oniscidea) from rural and urban habitats in New Jersey, USA. *Entomol News* 117(2):149–154.
- Hughes RN (1967) Turn alternation in woodlice (*Porcellio scaber*). *Anim Behav* 15(2-3):282–286. [https://doi.org/10.1016/0003-3472\(67\)90013-9](https://doi.org/10.1016/0003-3472(67)90013-9)
- Hughes RN (1978) Effects of blinding, antennectomy, food deprivation, and simulated natural conditions on alternation in woodlice (*Porcellio scaber*). *J Biol Psychol* 20:35–40
- Hughes RN (1989) Phylogenetic comparisons. In: Dember WN, Richman CL (eds) *Spontaneous alternation behavior*. Springer, New York, pp 39–57. https://doi.org/10.1007/978-1-4613-8879-1_3
- Hutchings M, Lewis B (1983) Insect sound and vibration receptors. In: Lewis B (ed) *Bioacoustics: a comparative approach*. Academic Press, London, pp 181–205
- Kalmring K (1985) Vibrational communication in insects (reception and integration of vibratory information). In: Kalmring K, Elsner N (eds) *Acoustic and vibrational communication in insects*. Paul Parey, Berlin, pp 127–134
- Lachman SJ, Havlena JM (1962) Reactive inhibition in the paramecium. *J Comp Physiol Psychol* 55(6):972–973. <https://doi.org/10.1037/h0049191>
- Lehmann E (1975) *Nonparametrics: statistical methods based on ranks*. McGraw-Hill, New York
- Lepley WM, Rice GE (1952) Behavior variability in paramecia as a function of guided act sequences. *J Comp Physiol Psychol* 45(3):283–286. <https://doi.org/10.1037/h0056313>
- Messina G, Montesanto G, Pezzino E, Caruso D, Lombardo BM (2011) Diversity of terrestrial isopods in a protected area characterized by salty coastal ponds (Vendicari, Sicily). *J Nat Hist* 45(35-36):2145–2158. <https://doi.org/10.1080/00222933.2011.587899>
- Messina G, Pezzino E, Montesanto G, Caruso D, Lombardo BM (2012) The diversity of terrestrial isopods in the natural reserve “Saline di Trapani e Paceco” (Crustacea, Isopoda, Oniscidea) in northwestern Sicily. *Zookeys* 176:215–230
- Messina G, Montesanto G, Pezzino E, Sciandrello S, Caruso D, Lombardo BM (2014) Plant communities preferences of terrestrial

- crustaceans (Isopoda: Oniscidea) in a protected coastal area of southeastern Sicily (Italy). *Biologia* 69:354–362
- Montesanto G (2015) A fast GNU method to draw accurate scientific illustrations for taxonomy. *ZooKeys* 515(515):191–206. <https://doi.org/10.3897/zookeys.515.9459>
- Montesanto G (2016) Drawing setae: a GNU way for digital scientific illustrations. *Nauplius* 24:e2016017
- Montesanto G, Cividini S (2017a) A crossover design to assess feeding preferences in terrestrial isopods: a case study in a Mediterranean species. *Biologia* 72:194–203
- Montesanto G, Cividini S (2017b) The moult cycle of the terrestrial isopod *Armadillo officinalis* Duméril, 1816 (Crustacea: Isopoda: Oniscidea). *Acta Zoologica* (early preview). <https://doi.org/10.1111/azo.12210>
- Ono T, Takagi Y (2006) Turn alternation of the pill bug *Armadillidium vulgare* and its adaptive significance. *Jpn J Appl Entomol Zool* 50(4):325–330. <https://doi.org/10.1303/jjaez.2006.325>
- Popper AN, Salmon N, Horch KW (2001) Acoustic detection and communication by decapod crustaceans. *J Comp Physiol A* 187(2):83–89. <https://doi.org/10.1007/s003590100184>
- Sandeman DC, Tautz J, Lindauer M (1996) Transmission of vibration across honeycombs and its detection by bee legs receptors. *J Exp Biol* 199(Pt 12):2585–2594
- Schmalzfuss H (1996) The terrestrial isopod genus *Armadillo* in western Asia (Oniscidea: Armadillidae) with descriptions of five new species. *Stutt Beitr Natur A* 544:1–43
- Schmalzfuss H (2003) World catalog of terrestrial isopods (Isopoda: Oniscidea). *Stutt Beitr Natur A* 654:1–341 Revised version available from: http://www.oniscidea-catalog.naturkundemuseum-bw.de/Cat_terr_isop.pdf
- Sibly RM (1983) Optimal group size is unstable. *Anim Behav* 31(3):947–948. [https://doi.org/10.1016/S0003-3472\(83\)80250-4](https://doi.org/10.1016/S0003-3472(83)80250-4)
- Steel RGD (1960) A rank sum test for comparing all pairs of treatments. *Technometrics* 2(2):197–207. <https://doi.org/10.1080/00401706.1960.10489894>
- Taiti S, Paoli P, Ferrara F (1998) Morphology, biogeography, and ecology of the family Armadillidae (Crustacea, Oniscidea). *Israel J Zool* 44:291–301
- Vandel A (1962) Faune de France: 66. Isopodes terrestres (deuxième partie). Le Chevalier, Paris
- Verhoeff KW (1908) Über Isopoden: 15. Aufsatz. *Archiv Biontol* 2:335–387
- Virant-Doberlet M, Čokl A (2004) Vibrational communication in insects. *Neotrop Entomol* 33(2):121–134. <https://doi.org/10.1590/S1519-566X2004000200001>
- Warburg MR (2013) Post-parturial reproduction in terrestrial isopods: a partial review. *Invertebr Reprod Dev* 57(1):10–26. <https://doi.org/10.1080/07924259.2011.633620>
- Warburg MR, Berkovitz K (1978) Hygroreacation of normal and dessicated *Armadillo officinalis* isopods. *Entomol Exp Appl* 2:55–64
- Wickham H (2009) *ggplot2: elegant graphics for data analysis*. Springer-Verlag, New York. <https://doi.org/10.1007/978-0-387-98141-3>