

# The light–dark cycle of Desmoulin’s whorl snail *Vertigo moulinsiana* Dupuy 1849 (Gastropoda, Pulmonata, Vertiginidae) and its activity patterns at different temperatures

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## Abstract

*The light–dark cycle of Desmoulin’s whorl snail Vertigo moulinsiana Dupuy, 1849 (Gastropoda, Pulmonata, Vertiginidae) and its activity patterns at different temperatures.* *Vertigo moulinsiana* is a minute land snail species which requires high humidity conditions and is found in wet, temporarily inundated habitats. The species is listed in the IUCN Red List of Threatened Species under the VU (vulnerable) category and is considered a high conservation priority. It is also mentioned in Annex II of the EU Habitat Directive, which imposes the obligation to monitor the species in member countries. The monitoring of *V. moulinsiana* is based on counting individuals attached to plants in the field, and thus any results may only be properly evaluated when the behavior of the species is understood. Therefore, the aim of this study was to investigate the light–dark cycle of both adults and juveniles within the species as well as to compare activity patterns of both age groups in dark conditions in temperatures of 6°C and 21°C. Observations were carried out under laboratory conditions, at a high and constant humidity (humidity was at or nearly 100%). It was shown that juveniles were more active than adults during the day, at night, and at 6°C and 21°C. In addition, both age groups of *V. moulinsiana* were more active at 21°C than at 6°C, and their activity was higher at night than during the day. Such behavior may have an impact on monitoring results based on visual examination and should be taken into account when the data are interpreted.

Key words: Land snails, Behavior, Adults, Juveniles, Activity, Conservation

## Resumen

*El ciclo de luz y oscuridad del caracol Vertigo moulinsiana Dupuy, 1849 (Gastropoda, Pulmonata, Vertiginidae) y su patrón de actividad a diferentes temperaturas.* *Vertigo moulinsiana* es una especie de diminutos caracoles terrestres que necesitan condiciones de humedad elevada y que se encuentran en hábitats húmedos y temporalmente inundados. La especie figura en la Lista Roja de Especies Amenazadas de la UICN con la categoría VU (vulnerable) y se considera una prioridad alta de conservación. Asimismo, se menciona en el anexo II de la Directiva Hábitat de la Unión Europea, en la que se aconseja a los Estados miembros que hagan un seguimiento de la especie. Como el seguimiento de *V. moulinsiana* se basa en el conteo de los individuos adheridos a plantas en el campo, los resultados solo se podrán evaluar debidamente cuando se comprenda el comportamiento de la especie. Por consiguiente, el propósito de este estudio es analizar el ciclo de luz y oscuridad de adultos y juveniles, y comparar los patrones de actividad de ambos grupos de edad en condiciones de oscuridad a temperaturas de 6°C y 21°C. Las observaciones se realizaron en condiciones de laboratorio y con humedad elevada constante (del 100% o casi). Se observó que los juveniles eran más activos que los adultos durante el día, por la noche y tanto a 6°C como a 21°C. Además, los dos grupos de edad de *V. moulinsiana* eran más activos a 21°C que a 6°C y su actividad era más elevada por la noche que durante el día. Estos resultados indican que el comportamiento puede incidir en los resultados del seguimiento basado en el examen visual y que debería tenerse en cuenta a la hora de interpretar los datos.

Palabras clave: Caracoles terrestres, Comportamiento, Adultos, Juveniles, Actividad, Conservación

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## Introduction

Desmoulin's whorl snail *Vertigo moulinsiana* Dupuy 1849 is an Atlantic–Mediterranean species (Pokryszko, 1990) but it is distributed across most of Europe (Pokryszko, 2003; Killeen et al., 2012). This minute land snail (its shell does not exceed 2.7 mm in height) requires high and constant humidity and inhabits marshes dominated with Reed sweet grass (*Glyceria maxima*), Common reed (*Phragmites australis*) and sedges (Pokryszko, 1990; Killeen, 2003; Książkiewicz, 2010). It feeds on microorganisms related to decaying matter and growing on marsh plants (Steusloff, 1937; Bondesen, 1966). The occurrence of *V. moulinsiana* is strongly associated with local hydrological conditions (e.g. Tattersfield and McInnes, 2003; Książkiewicz et al., 2015) and it occurs in temporarily flooded areas (Killeen, 2003). The importance of the water level, however, seems to be indirect since it affects air humidity, which is important for *V. moulinsiana* (Killeen, 2003). The snail climbs up tall vegetation (mainly monocots), where it may be found in large numbers in late summer, in autumn and in winter (Killeen, 2003; Książkiewicz–Parulska and Pawlak, 2016). A part of the population, however, is always found within the litter layer (Killeen, 2003; Książkiewicz et al., 2013).

*V. moulinsiana* has been in decline throughout much of its range (Seddon, 1997). This loss of range is evident in Ireland, Germany and France but may be equally severe in other European countries although this cannot be proven due to inadequate monitoring (Killeen et al., 2012). The principal causes of the decline are the physical disturbance of habitats (Pokryszko, 2003) such as wetland drainage or changes in agricultural and land management practices (Killeen, 2003). Thus *V. moulinsiana* is highly dependent on conservation and listed in the IUCN Red List of Threatened Species in the VU category (vulnerable) (Killeen et al., 2012). Furthermore, it is included in the Annex II of the EU Habitat Directive which imposes an obligation on EU members to designate sites where this species is found as protected areas and to properly manage as well as regularly monitor its populations.

The monitoring of *V. moulinsiana* populations is traditionally based on a visual examination, i.e. counting individuals which are attached to plants (Moorkens and Killeen, 2011; Lipińska et al., 2012). To understand data collected using this method, a good knowledge of the behavior of *V. moulinsiana* in response to changing environmental conditions is needed. The previous studies on this species, however, have focused mainly on its habitat and microhabitat requirements (Cameron et al., 2003; Killeen, 2003; Tattersfield and McInnes, 2003; Jankowiak and Bernard, 2013; Książkiewicz et al., 2013) or on population dynamics (e.g. Killeen, 2003; Książkiewicz–Parulska and Ablett, 2016). Moreover, data on the activity of *Vertigo* species in general are scarce (e.g. Boag, 1985), and only a few publications on the behavior of *V. moulinsiana* in respect to changing temperature conditions have been published so far (Książkiewicz–

Parulska and Pawlak, 2017; Książkiewicz–Parulska 2017, in press). Thus, this study aims to contribute to the knowledge on the behavior of *V. moulinsiana* and investigate a light–dark cycle of adults and juveniles of the species as well as activity patterns of these age groups at temperatures of 6°C and 21°C.

## Material and methods

Individuals used for this study were acquired from a laboratory breeding group which was kept in a room temperature in a 24 hr light–dark cycle (LD 12:12). To make sure that only alive individuals were used in the experiments, I placed individual snails from the breeding container into a plastic box with high–humidity conditions. I selected only those snails which extended their body outside of the shell. Individuals were divided into adults and juveniles based on shell development. The snail was considered an adult when the aperture was fully developed (Pokryszko, 1990). In the experiments, two–whorled juveniles of *V. moulinsiana* were used. Each snail was placed in a separate 2 ml test tube and individually numbered. The experiments started 24hrs after the snails were put in the test tubes to exclude any false readings that could occur due to distress caused by the relocation of the animal.

Each tube had a ca. 0.7 mm hole on the top for oxygenation. Animals were supplied with food, i.e. the decaying leaf of a sedge harvested from a site where *V. moulinsiana* was present. At the bottom of each tube I placed cotton wool bud saturated with water, but no standing water was present in the tubes. The tubes were placed in a lockable plastic box and were sprinkled with water once a day. Relative humidity inside the tubes was at or nearly 100% and condensation formed on the sides of the tubes. A snail was only regarded as active if it was crawling, or if, although immobile, its body was extended and the tentacles fully everted (Cameron, 1970).

### Activity of adults and juveniles of *Vertigo moulinsiana* at 6°C and 21°C

The observations of activity of adults and juveniles of *V. moulinsiana* were carried out at two different temperatures: at 6°C and 21°C. The temperature of 6°C was maintained in a refrigerated room while the temperature of 21°C was in the heated room. Since data on the impact of the temperature on *V. moulinsiana* are scarce (see Książkiewicz–Parulska, in press), I chose the aforementioned temperatures since I was able to easily maintain these particular conditions at a constant level. The impact of a photoperiod was excluded from the experiment and both temperature groups were stored in dark conditions. Snails were, however, exposed to the light of a flashlight for a short time, twice a day, when the activity of the snails was recorded, e.g. at 11 a.m. and 11 p.m.

The experiment in both temperatures was carried out for 32 juveniles and 32 adults of *V. moulinsiana*. Observations were carried out for 14 days.

Table 1. Mean activity of snails during experiments: adults and juveniles at 6°C and 21°C (mean of 28 observations over 14 days) as well as activity of adults and juveniles in light and dark conditions at 21°C (mean of 20 observations over 20 days): N, individual number.

Tabla 1. Actividad media de los caracoles durante los experimentos: adultos y juveniles a 6°C y 21°C (media de 28 observaciones durante 14 días) y actividad de los adultos y juveniles en condiciones de luz y oscuridad a 21°C (media de 20 observaciones durante 20 días): N, número ide individuos.

N	Adults		Juveniles		Adults		Juveniles	
	6°C	21°C	6°C	21°C	Light	Dark	Light	Dark
1	0.08	0.04	0.08	0.82	0.05	0.1	0.45	0.2
2	0.19	0.36	0.19	0.82	0.1	0.05	0.05	0.75
3	0.04	0.07	0.08	0.18	0.1	0.45	0.5	0.7
4	0.12	0.04	0.12	0.04	0.05	0.25	0.1	0.6
5	0.12	0.04	0.12	0.46	0.1	0.65	0.45	0.65
6	0.12	0.07	0.50	0.21	0.15	0.5	0.25	0.85
7	0.04	0.07	0.08	0.79	0.1	0.4	0.05	0.85
8	0.08	0.50	0.19	0.36	0.1	0.7	0.2	0.9
9	0.04	0.14	0.04	0.50	0.1	0.15	0.15	0.55
10	0.12	0.00	0.00	0.29	0.05	0	0.05	0.1
11	0.08	0.36	0.04	0.25	0.05	0.05	0.05	0.4
12	0.00	0.25	0.12	0.96	0.05	0	0	0.2
13	0.08	0.57	0.15	0.21	0.3	0.1	0.45	0.05
14	0.15	0.04	0.27	0.68	0.35	0.1	0.55	0.2
15	0.19	0.11	0.19	0.54	0.05	0	0.1	0.25
16	0.04	0.11	0.15	0.71	0.65	0.55	0.7	0.85
17	0.04	0.04	0.00	0.79	0.3	0.6	0.35	0.95
18	0.12	0.29	0.04	0.79	0.5	0.5	0.55	0.9
19	0.19	0.54	0.04	0.29	0.15	0.35	0.1	0.45
20	0.15	0.57	0.19	0.25	0.1	0.45	0.2	1
21	0.04	0.43	0.15	0.18	0	0.2	0.05	0.4
22	0.00	0.07	0.23	0.11	0.15	0.4	0.6	0.75
23	0.27	0.00	0.23	0.04	0.25	0	0.3	0.25
24	0.12	0.07	0.31	0.18	0.15	0.15	0.2	0.2
25	0.12	0.14	0.00	0.25	0	0.4	0.1	0.55
26	0.04	0.11	0.08	0.43	0.15	0.15	0.1	0.55
27	0.08	0.21	0.08	0.21	0.15	0.05	0.15	0
28	0.19	0.18	0.46	0.18	0.25	0.55	0.15	0.85
29	0.08	0.25	0.35	0.00	0.2	0.05	0.3	0.3
30	0.08	0.21	0.04	0.46	0.05	0.3	0.15	0.75
31	0.08	0.39	0.46	0.14	0.35	0	0.45	0.1
32	0.08	0.57	0.12	0.11	0	0.25	0	0.25

The light–dark cycle of adults and juveniles of *Vertigo moulinsiana* in 21°C

The observations of the light–dark activity of adults and juveniles of *V. moulinsiana* were carried out at

21°C, in a 24–hr light–dark cycle (LD 12:12). The light conditions were acquired with a daylight lamp AQUAEL Decolight LT (6W LED). The light/ dark shifts had been set for 3 a.m. and 3 p.m., controlled with an automatic light switch. The activity of individuals was

checked at 11 a.m. in light conditions and at 11 p.m. in dark conditions. The snails were exposed to the light of a flashlight in dark conditions for a short time when the snails' activity was recorded, i.e. at 11 p.m. The experiment was carried out for 32 juveniles and 32 adults of *V. moulinsiana* and observations lasted 20 days.

#### Statistical analyses

To compare the activity of *Vertigo moulinsiana* in the case of (1) adults at 6°C and 21°C (2) juveniles at 6°C and 21°C (3) adults and juveniles in 6°C (4) adults and juveniles at 21°C (5) activity of adults and juveniles in light conditions at 21°C (6) activity of adults and juveniles in dark conditions at 21°C, I performed a one-way ANOVA test, randomized version using RndomPro 3.14 software. To compare the activity of (1) adults in light conditions and in dark conditions at 21°C and (2) juveniles in light conditions and in dark conditions at 21°C, I performed Wilcoxon matched-pairs test using Past3 software (I used non-parametric methods because of the lack of normality).

The calculations were made for the mean activity of particular individuals (i.e. total number of times that particular individual was active divided by a total number of observations; see table 1). I considered  $p < 0.05$  as the minimum level determining significance.

## Results

#### Activity of adults and juveniles of *Vertigo moulinsiana* at 6°C and 21°C

The one-way ANOVA showed that both adults and juveniles of *V. moulinsiana* were more active at 21°C than at 6°C (adults:  $F = 9.474$ ,  $P = 0.003$ , fig. 1A; juveniles  $F = 17.180$ ,  $P < 0.001$ , fig. 1B). On the other hand, juveniles were more active than adults at 6°C ( $F = 5.367$ ;  $P = 0.020$ , fig. 1C) and at 21°C ( $F = 8.109$ ;  $P = 0.006$ , fig. 1D).

#### The light–dark cycle rhythm of adults and juveniles of *Vertigo moulinsiana* in 21°C

Both juveniles and adults of *V. moulinsiana* were more active in dark conditions than in light conditions (juveniles:  $z = 4.724$ ,  $P < 0.001$ , fig. 1E; adults:  $z = 3.367$ ,  $P < 0.001$ , fig. 1F). Furthermore, juveniles of the species were more active than adults in light ( $F = 4.127$ ,  $P = 0.042$ , fig. 1G) and dark conditions ( $F = 15.240$ ,  $P < 0.001$ , fig. 1H).

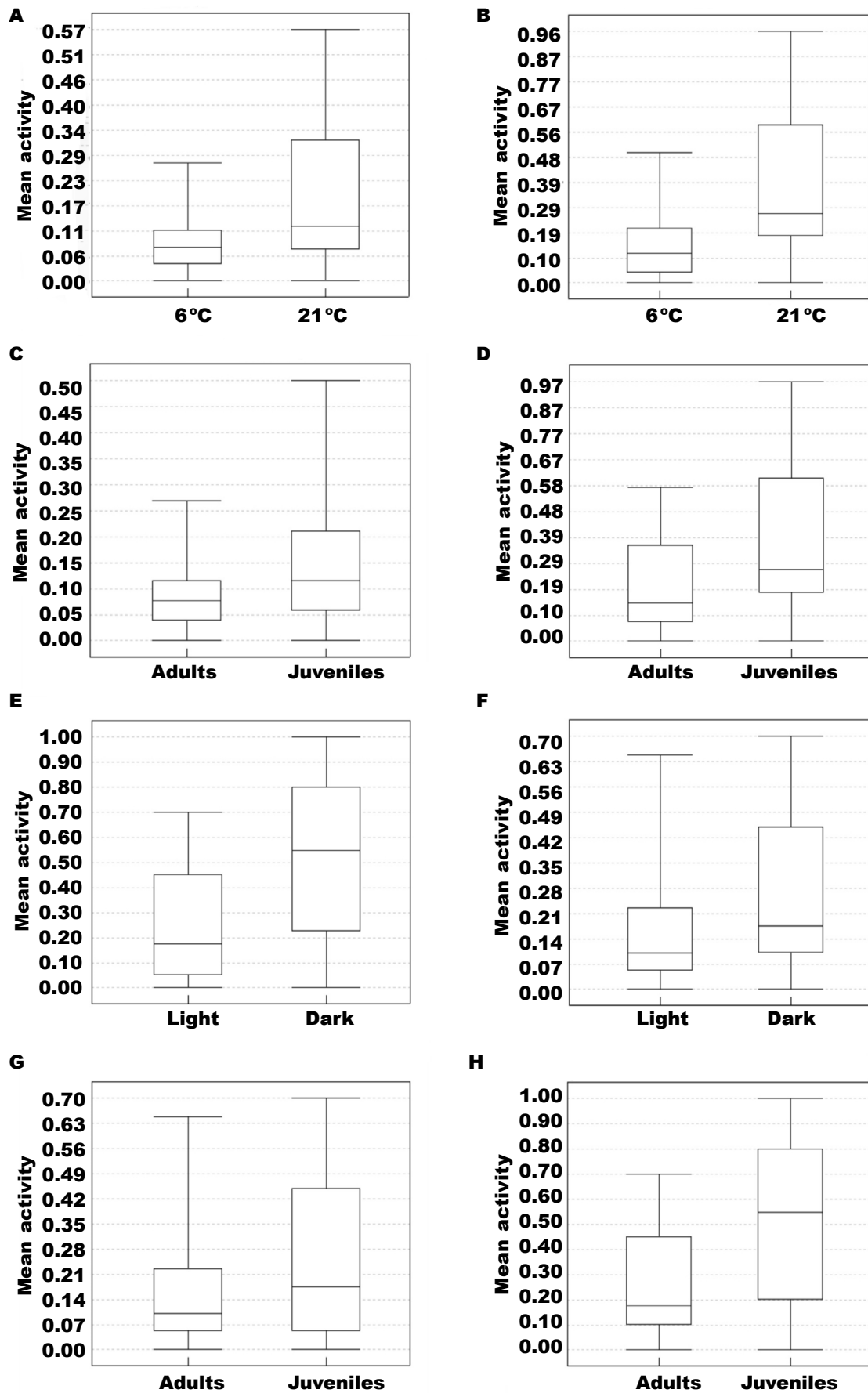
## Discussion

The activity of terrestrial snails appears to be determined partially by environmental factors (such as moisture and/or temperature), and partially by an internal rhythms (e.g. Wells, 1944; Cook, 2001; Attia, 2004). Circadian activity may be modified by different factors including temperature, wind speed, and the relative humidity of litter moisture (see Cook, 2001). In general, however, it has been shown that many of the terrestrial snails are crepuscular (e.g. the Roman snail *H. pomatia* Linnaeus 1758, see Stępczak et al., 1982), while others, such as the giant African snail *Lissachatina fulica* (Bowdich 1822) and the Bush snail *Fruticicola fruticum* (O.F. Müller 1774), are nocturnal (Butler, 1965; Kuźnik–Kowalska et al., 2013). Furthermore, Baur and Baur (1988) did not find any differences in activity between day and night in the case of the minute Dwarf snail *Punctum pygmaeum* Draparnaud 1801 when kept at a constant humidity and temperature. These authors suggested that it may be a trait characteristic for snails inhabiting the litter. This conclusion is supported by Boag (1985) who observed some litter dwelling snails, including representatives of the family Vertiginidae, namely Variable vertigo *Vertigo gouldi* (Binney 1843) and the Cross vertigo *Vertigo modesta* (Say 1824).

Although the study presented here does not give a conclusive result on the endogenous character of the circadian activity of *V. moulinsiana*, it shows that

Fig. 1. Box plot shows data on mean activity of *Vertigo moulinsiana* individuals: A, adults in temperatures of 6°C and 21°C ( $F = 9.474$ ,  $P = 0.003$ ); B, juveniles in temperatures of 6°C and 21°C ( $F = 17.180$ ,  $P < 0.001$ ); C, adults and juveniles in temperature of 6°C ( $F = 5.367$ ;  $P = 0.020$ ); D, adults and juveniles at a temperature of 21°C ( $F = 8.109$ ;  $P = 0.006$ ); E, juveniles in light and dark conditions at 21°C ( $z = 4.724$ ,  $P < 0.001$ ); F, adults in light and dark conditions at 21°C ( $z = 3.367$ ,  $P < 0.001$ ); G, juveniles and adults in light conditions at 21°C ( $F = 4.127$ ,  $P = 0.042$ ); H, juveniles and adults in dark conditions at 21°C ( $F = 15.240$ ,  $P < 0.001$ ). Middle line, mean; Box range, standard error; Whiskers, standard deviation.

Fig. 1. En los diagramas de caja se muestran los datos relativos a la actividad media de los individuos de *Vertigo moulinsiana*: A, adultos a temperaturas de 6°C y 21°C ( $F = 9,474$ ;  $P = 0,003$ ); B, juveniles a temperaturas de 6°C y 21°C ( $F = 17,180$ ;  $P < 0,001$ ); C, adultos y juveniles a una temperatura de 6°C ( $F = 5,367$ ;  $P = 0,020$ ); D, adultos y juveniles a una temperatura de 21°C ( $F = 8,109$ ;  $P = 0,006$ ); E, juveniles en condiciones de luz y oscuridad a 21°C ( $z = 4,724$ ;  $P < 0,001$ ); F, adultos en condiciones de luz y oscuridad a 21°C ( $z = 3,367$ ;  $P < 0,001$ ); G, juveniles y adultos en condiciones de luz a 21°C ( $F = 4,127$ ;  $P = 0,042$ ); H, juveniles y adultos en condiciones de oscuridad a 21°C ( $F = 15,240$ ;  $P < 0,001$ ). Línea media, promedio; Ancho de caja, error estándar; Bigotes, desviación estándar.



at a constant temperature and in conditions of high humidity, the impact of light and dark is significant for the species. Despite the fact that the life cycle of *V. moulinsiana* is integrally linked to the litter layer (place of egg laying and where the snail may overwinter; Killeen, 2003) and in some habitats, a significant part of its population may reside within the litter during the whole growing season (see Książkiewicz et al., 2013), *V. moulinsiana* may be numerous found on plants (e.g. Pokryszko, 1990; Cameron et al., 2003). In this way, individuals of this species are frequently exposed to the sunlight, which might have contributed to the development of a behavior protecting the snail against UV radiation (see Olson and Barbieri, 2014; these authors have shown that shell provides photoprotection and proved the behavioral avoidance of UV radiation in European physa *Physa acuta* Draparnaud 1805). It may explain why both adults and juveniles were less active (stayed hidden in its shells) in light conditions than in darkness. It should be also considered that such behavior may protect snails against desiccation when exposed to daylight. On the other hand, all experiments (observations of activity patterns in the case of both: light and dark conditions) were carried out in high humidity conditions where desiccation was not an issue.

Boag (1985) suggested that small, litter dwelling *Vertigo* species are most active in temperatures between 6°C and 15°C, and the proportion of observable snails diminished at higher temperatures, regardless of humidity. The experiments carried out for *V. moulinsiana*, at the high and constant humidity levels show that the highest activity of the species was noted at 11°C, lower activity at 21°C (see Książkiewicz–Parulska, in press) and the lowest at 6°C. On the other hand, the results presented here suggest that *V. moulinsiana* shows various responses to the different temperatures, regardless of humidity, as was also concluded by Boag (1985). Comparison of the activity of particular age groups showed that juveniles of *V. moulinsiana* were more active than adults in the introduced experimental conditions (i.e. when exposed to light and darkness as well as at 6°C and 21°C). The higher activity of juveniles has previously been observed for some other species of terrestrial snails. For example, juveniles of *F. fruticum* are more active than adults in the spring, summer and autumn as well as at night, irrespective of the season (Kuźnik–Kowalska et al., 2013). Also, Pollard (1974) concluded that the activity of *H. pomatia* decreases with age. Due to the fact that juveniles of *Vertigo* species (similarly to juveniles of other snail species, e.g. Cowie, 1985) are more prone to desiccation than adults (Pokryszko, 1990), it may be suspected that completing growth as soon as it possible is an advantage as it makes them more robust. Thus juveniles usually dwell in a litter (instead of climbing up plants) that ensures high humidity conditions as well as rich and easily accessible source of food (see Cowie, 1985). To confirm this suspicion, however, further studies are needed.

Our knowledge on terrestrial snail activity patterns is still scarce while extremely necessary, especially for the conservation of rare and endangered species.

In the case of the *V. moulinsiana*, the monitoring and determination of conservation activities is based on the visual assessment of population abundance (Moorkens and Killeen, 2011; Lipińska et al., 2012). This is why understanding the behavior of this species in response to changing environmental conditions is essential to properly interpret the data gathered in the field. This study shows that adults and juveniles of *V. moulinsiana* react differently to diverse temperatures and act differently in light and dark conditions. Such behavior may have an impact on the monitoring results based on visual examination and should be taken into account when the data are interpreted.

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