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Sex-dependent increase of movement activity in the freshwater isopod *Asellus aquaticus* following adaptation to a predator-free cave habitat

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Abstract

Populations experiencing negligible predation pressure are expected to evolve higher behavioural activity. However, when sexes have different expected benefits from high activity, the adaptive shift is expected to be sex-specific. Here, we compared movement activity of one cave (lack of predation) and three adjacent surface (high and diverse predation) populations of *Asellus aquaticus*, a freshwater isopod known for its independent colonization of several caves across Europe. We predicted (i) higher activity in cave than in surface populations, with (ii) the difference being more pronounced in males as they are known for active mate searching behaviour while females are not. Activity was assessed both in the presence and absence of light. Our results supported both predictions: movement activity was higher in the cave than in the surface populations, particularly in males. Relaxed predation pressure in the cave-adapted population is most likely the main selective factor behind increased behavioural activity, but we also showed that the extent of increase is sex-specific.

Key words: adaptation, cave colonization, movement activity, predator-free environment

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Geographic interpopulation variation in behaviour is commonplace (Foster 1999; Foster and Endler 1999; Herczeg and Válimäki 2011; Michelangeli et al. 2019). Predation is a key selective pressure (Lima and Dill 1990; Endler 1991; Lima 1998), simply because avoiding or surviving predatory attempts has a direct effect on survival and the probability of future reproduction. Therefore, selection pressures stemming from predation often act against the positive selection for basic activities like foraging or mate searching. For instance, high movement activity, that increases foraging and mate finding success, in most cases also increases predation risk (Norrdahl and Orpimäki 1998; Kasumovic et al. 2007). Hence, if colonising a new habitat incorporates a decrease in predation pressure, the evolution of increased behavioural activity is expected (see Kortet et al. 2010). Concordantly, covariation between predation pressure and behavioural activity across populations has been detected in various taxa (e.g., Brown et al. 2005; Herczeg et al. 2009; Rosén et al. 2017). Further, if the benefit of increasing movement activity under low or negligible predation is sex-specific, e.g., because of sex-specific foraging or mating behaviours, the evolutionary shift should be stronger in the sex with the higher expected benefit.

So far, the latter prediction received little attention and available studies were conducted mainly on fishes. For example, it was shown recently that in the Bahamas mosquitofish *Gambusia hubbsi* behavioural lateralization (i.e., asymmetric expression of behaviours relative to the main body axis) is significantly higher at sites with high predation risk, especially so in females (Hulthén et al. 2021). Also, females of this species from low-predation sites show higher rates of foraging and food consumption, while the opposite is true in males (Pärssinen et al. 2021). Further, female guppies *Poecilia reticulata* tend to be more risk-taking (or bolder) when predator-experienced than when predator-naïve, while males behave similarly, irrespective of their previous exposure to predators (Harris et al. 2010). All three studies explain the sex-specific response to different predation contexts by sex-specific trade-offs in behaviours (e.g., risk-taking, feeding, schooling, mating) or life-histories (e.g., longevity, body size, fecundity).

Species occupying caves and surface habitats are an ideal model system for testing inter-population behavioural divergence, as caves differ remarkably from surface habitats in many environmental parameters, e.g., they are perpetually dark, generally scarce in food, and show extremely low daily and seasonal environmental fluctuations (Romero 2009; Borowsky and Cohen 2013; Culver and Pipan 2019). The oligotrophic character of caves results in simple communities with truncated trophic structure often lacking large predators (Gibert and Deharveng 2002; Bradley and Eason 2018; Culver and Pipan 2019; Manenti et al. 2020). Consequently, most cave animals do not face the trade-off between foraging and mate searching benefits on the one hand, and predation risk on the other (Poulson 1963; Hervant and Renault 2002; Salin et al. 2010). Therefore, the diminished predation in caves is expected to lead to loss of anti-predation behaviours (e.g., sheltering, Fišer et al. 2019; Horváth et al. 2021) and to increased movement activity (Carlson and Gross 2018).

Increased movement activity yields more successful foraging and mate-finding, but it also leads to higher energy expenditure and could be selected against to save energy in food scarce caves. Thus, the evolution of movement activity in cave animals faces another trade-off and is not straight forward to predict. Its direction might rather be tied to the specific combination of predation pressure, food availability, and species trophic position, than to the cave environment *per se*. For example, in detritivores predator-free and food-rich caves might select for increased movement activity, while food deprived caves with predators might select for decreased movement activity. Contrastingly, in apex predators the degree of movement activity should be

constrained mainly by food availability and quality. So far, evidence for both increased and decreased movement activity has been demonstrated in cave animals (Hüppop 2000; Hervant et al. 2001; Salin et al. 2010). In contrast to adaptive changes in movement activity of cave animals, sexual dimorphism in behavioural adaptations to cave environment was scarcely studied at best (Fišer et al. 2019; Herczeg et al. 2020, 2022). Nevertheless, this phenomenon is intuitively expected in species where certain behavioural activities (like mate searching) yield different fitness gains for the sexes.

Here, we applied the cave – surface habitat context and tested the hypothesis about the evolution of increased movement activity following colonisation of a predator free cave, with food available in presumably high quantities. Our model species was the freshwater isopod *Asellus aquaticus*. This crustacean is widespread in various aquatic surface habitats across Europe and has repeatedly colonized subterranean habitats (Verovnik and Konec 2019), where its populations exhibit cave-adapted phenotypes characterized by eye-reduction and pigment loss (Protas and Jeffery 2012; Verovnik and Konec 2019). In *A. aquaticus*, males perform intensive mate searching, which is an important component of their mating success (see Vandel 1926; Balesdent 1964; Bertin et al. 2002). Our model system comprises a cave population dwelling in the hydrothermal Molnár János Cave, a completely predator-free environment (see Balázs, 2019) and three surface populations from the cave's vicinity with complex communities, including various predators and food sources. Molecular data shows that this cave-adapted *A. aquaticus* population is genetically isolated from its geographically closest surface population for at least 60,000 years (Pérez-Moreno et al. 2017). Unlike other caves relying exclusively on low amounts of external food sources, endogenous food (mat-forming bacteria) is available in large quantity in the Molnár János Cave (Borsodi et al. 2012). We hypothesised that (i) no predation selects for higher movement activity in general and (ii) the increase in movement activity is higher in the sex that benefits more from the increase (in this case due to higher mate finding success). In our present study, we tested two predictions. First, *A. aquaticus* adapted to the Molnár János Cave show higher movement activity than its surface conspecifics and second, the difference will be more pronounced in males.

Materials and Methods

Study system

All tested populations of *Asellus aquaticus* are located in Budapest (Hungary) or its close surroundings. For a detailed description of the Molnár János Cave (MJC) see Herczeg et al. (2020). Briefly, MJC is a hypogene cave (water forming the cave does not come from the surface but from underground) filled with thermal water of 23-24 °C (Eröss et al. 2006; Bodor et al. 2015). It is predator-free and the entire ecosystem relies on endogenous, chemoautotrophic mat-forming bacteria (Eröss et al. 2006; Bodor et al. 2015; Herczeg et al. 2020, 2022). The first surface population is from the Malom Lake (ML; 47.518277° N, 19.035999° E), a small pond at the entrance of MJC formed by its outflow. Thus, the water temperature in ML is identical to that of the cave and constant all year round. However, ML is subjected to the natural surface light regime and can be seen as a typical surface habitat, including the presence of fish predators, i.e., guppies *Poecilia reticulata* that were introduced here during the 20th century (Berczik 1956) and occur in extremely high density as native fish are absent. Two other surface populations, the Dunakeszi Peat-moor (DM; 47.615613° N, 19.126392° E) and the Csömör Stream (CS; 47.593393° N, 19.121970° E), experience natural surface light regime and temperature

fluctuations of normal, non-thermal freshwaters typical to the region. These two populations have been chosen randomly among many locations in the area with similar ecological conditions that harbour *A. aquaticus* as well and probably function as one population (Pérez-Moreno et al. 2017). Both DM and CS surface populations harbour various vertebrate (unidentified fishes, amphibians, water birds, and semiaquatic mammals) and invertebrate (odonate and dytiscid larvae, erpobdellid leeches) predators.

Collecting and housing the experimental animals

Adult animals (N = 200; 25 males and 25 females per population; see also Figure 1) were collected between 16th and 17th August 2018. During this season, similar water temperature at the surface localities to the water temperature of MJC occurs naturally. Upon collection, animals were transported to the aquacultural facilities of the Eötvös Loránd University. Animals were housed individually in 90 × 25 mm (diameter × height) plastic Petri dishes with bottoms coarsened by emery paper to enable animals' normal locomotion (Fišer et al. 2019). Clean water collected at the source habitats was used to fill the Petri dishes to the half of their height and was regularly refilled when the water level dropped due to evaporation. Petri dishes were kept in light-controlled 'recording chambers' (see below) during the whole experiment. The temperature in the laboratory was 23–24 °C. Gravid females of *A. aquaticus* form a brood pouch to carry offspring (see Lafuente *et al.*, 2021) and likely display different movement activity. To avoid any bias in this respect, we only used non-gravid females. Surface populations were acclimated in a daily light cycle natural to the time of collection (16 h light: 8 h dark), while cave animals were acclimated in complete darkness (in a separate recording chamber). Both surface and cave animals were left without any disturbance and food for two days. Acclimation ensured that animals got familiar with their new artificial environment and would not show stressed behaviours later in test observations. All manipulations of cave animals were done under red light. As some mortality occurred during the first few days in the laboratory, we eventually tested 164 individuals (MJC: 10 males (M) / 16 females (F), ML: 23 M / 23 F, DM: 24 M / 23 F; CS: 22 M / 23 F).

Experimental setup

Acclimation and behavioural tests took place in four similar custom-made recording chambers (100 × 55 × 105 cm, length × width × height, respectively, see Figure S1). The chambers were equipped with two light sources: LEDs imitating daylight (colour temperature = 4500 K, colour rendering index > 90) at the top and infrared (IR) LEDs (920 nm) at the bottom. Opaque plexiglass was placed over the IR LEDs to diffuse the emitted light evenly and at the same time to serve as a surface on which Petri dishes with animals were put. IR light was switched on during all recordings. Neither light source heat up the water during the experiment. Each chamber could house a maximum of 50 Petri dishes. The chambers were closed from sides with black plastic boards, so that light did not scatter outside of the chamber. Inside each chamber, we mounted a webcam (Logitech C920 FullHD) that was technically modified to improve the quality of videos recorded in IR. OBS Studio software

(OBS Studio Contributors) was used to capture videos at 5 frames per second at HD resolution (1280 × 720 pixels).

Experimental protocol

We made two rounds of observations on 20th and 21st of August 2018. Each population was randomly divided into two groups, with sexes represented equally. Half of the animals from all populations was observed under daylight on the first day and in darkness on the second day, whereas the other half was tested in the reverse order of light conditions. Individuals from cave and surface populations were randomly positioned within the same recording chamber for the same light treatment. Recordings started at ca. 11 am and lasted 60 min. Animals were given 15 min prior recordings to get used to the change from acclimation to experimental light regimes. After recording, we restored the acclimation light regime for all animals.

We expected individuals to show increased movement activity in the presence of light, irrespective of habitat of origin. Note that the presence of light is unnatural for the cave population and can be only used to test for light avoidance (based on genetic-studies, cave-dwelling *A. aquaticus* from MJC probably retained the ability to detect light (Pérez-Moreno et al. 2018)). Similarly, dark treatment may be intuitively seen as irrelevant for the presumably diurnal surface populations. Nevertheless, results from our recent experiment (Horváth et al. unpublished) and a previous field study (Andrikovics, 1981) both indicate that *A. aquaticus* are active in diverse light conditions, with their nocturnal movement activity exceeding their diurnal movement activity. As the influence of light or its absence on these isopods' activity and behaviour in general is not fully understood, we applied a full factorial design to control for its effects.

Video analysis

We analysed 15 minutes of each video, comprised of three 5 min intervals, distributed equally along the 1 h footage: at the start (0-5 min), middle (27.5 – 32.5 min), and end (55-60 min). Animal movement (see Video S1) was tracked using the digitizing tool DLTdv (Hedrick 2008). Raw tracking data was transformed to behavioural variables using a custom written Macro in Excel. Behavioural data for each interval were first calculated separately and then averaged. For the analyses, we extracted the following five variables: total distance moved (in mm), total time moving, number of movement bouts, as well as the mean and standard deviation of the distance moved per bout (in mm) (Table S1).

Statistical analyses

Spearman rank correlations indicated strong correlations between the variables (Table S2). Therefore, we ran a Principal Component Analysis (PCA) in IBM SPSS Statistics 25.0 (SPSS Inc., Chicago, IL, USA) to calculate independent principal components (PC) that explain most of the observed variation. Result of the diagnostic Bartlett test was significant, indicating that the correlation matrices were significantly different from the identity matrices. Based on Kaiser–Guttman criterion (Kaiser 1991), only PCs with an eigenvalue greater than 1 were

retained (Tabachnick and Fidell 2014). Our PCA resulted in a single PC that explained 64.8% of the total variation (for further details see Table S3). From here on, we refer to this PC as ‘movement activity’. High values correspond to individuals that show high movement activity coupled with high variability (SD) in expression of individual activity, while low values correspond to less active individuals coupled with low variability (SD) in expression of individual activity.

To analyse effects of population, sex, and light regime on movement activity, we built a linear mixed model (LMM) using the package *lme4* (Bates et al. 2015) in R 4.1.2. (R Developmental Core Team 2021). In this model ‘movement activity’ was the dependent variable, while ‘population’ (one cave and three surface populations), ‘sex’, ‘light regime’ (light, dark), all two- and three-way interactions were added as fixed factors. To control for habituation, we added the order of trials as a fixed effect excluded from all interactions. Individual identity was fitted as random intercepts. The model's estimated marginal means were extracted using the *emmeans* R package (Lenth 2019). To test the significance of between-group differences, we performed post-hoc tests and adjusted *p*-values via the false discovery rate (FDR) method of Benjamini and Hochberg (1995).

Results

We found significant population and population \times sex \times light regime interaction effects (Table 1). Post-hoc comparisons revealed that the cave population showed higher movement activity than the surface populations, the latter being similar (Figure 1, Table S4). Further, the cave population showed male-biased sexual dimorphism in movement activity in darkness, with males being more active (see Figure 1, Table S4). We also found a significant habituation effect: irrespective of source population, individuals were less active in the second round of observations (data not shown graphically). Finally, the significant random effect showed the existence of between-individual differences in movement activity (Table 1). However, note that the latter result should not be viewed as indicative of ‘true’ animal personality as our repeated measures were performed in a short time interval, but more importantly, because daylight is mostly unnatural for the cave isopods and was applied to them only to ensure a full-factorial experimental design.

Discussion

The negative covariation between predation risk and behavioural activity across populations is well documented (e.g., Lima and Dill 1990; Lima 1998; Van Buskirk and Arioli 2002; Hettyey et al. 2015). However, how sexes with different gains from increasing behavioural activity evolve under negligible predation was rarely studied (but see Norrdahl and Orpimäki 1998; Harris et al. 2011; Hulthén et al. 2021; Pärssinen et al. 2021). Further, the above questions were even more rarely addressed within the cave *vs.* surface population comparison context, contrasting caves with negligible predation to surface habitats with high and diverse predation (Romero 1985; Uiblein and Juberthie 2000; Bradley and Eason 2018). In the present study, we compared the movement activity of male and female cave-adapted *A. aquaticus* to their surface-adapted conspecifics. Our most salient findings support our predictions and are straightforward: (i) increased movement activity in the cave population of *A. aquaticus* compared to its three similarly active surface populations and (ii) the increase in movement activity was higher in males than in females when tested in natural settings (i.e., in darkness). At first, these findings

might seem in stark contrast to the results of another study of ours (Horváth et al. unpublished) which indicated a decrease of movement activity in the MJC population compared to its close by surface populations. However, we note that in that study both experimental setup (test arenas were maze-like tracks) and design (tests were done in conditions unfamiliar to the animals) were markedly different from the present study. Thus, in each study a different aspect of movement activity was measured deliberately. Here we targeted general movement activity in familiar environment, while in the other study we focused on exploratory movement activity in novel environment.

In support to our first prediction, movement activity of cave *A. aquaticus* was substantially higher than that of the surface populations, including ML that is hydrologically connected to the cave. Predation is a major selective force for various phenotypic characteristics (Lima and Dill 1990) and is considered as a particularly important factor in the evolution of behavioural traits, such as activity, exploration, and boldness (see Kortet et al. 2010). Generally, the theory predicts a negative association between activity and predation risk (Lima 1998), which is well supported by empirical observations showing population patterns of increased behavioural activity under negligible predatory risk (e.g. Magurran and Seghers 1991; De Meester 1993; Bell 2005; Brydges et al. 2008; Herczeg et al. 2009; but see Brown et al. 2005, 2007). Old cave species or populations that display a high degree of cave-related adaptations are also expected to show a reduction of anti-predator responses (Tobler 2009). In line with this notion, Manenti et al. (2020) found that the response to predators is maintained in a recent cave colonist, i.e., the cave-dwelling populations of the Pyrenean newt *Calotriton asper*, while such behaviour is absent in a phylogenetically old species, the olm *Proteus anguinus*. As predators are missing from the herein studied MJC, it is highly plausible that increased movement activity in the cave population is the result of the lack of predation selecting against any unnecessary activity. Somewhat similarly to the results presented here, a recent study by Horváth et al. (2021) found that cave individuals from MJC shelter less (i.e., are more risk-taking) than their surface conspecifics, suggesting another behavioural adaptation to the lack of predation. A reduction of shelter-seeking has also been demonstrated for another, independently evolved cave population from Slovenia (Fišer et al. 2019). Regarding surface populations, it was suggested previously that in habitats where fast-moving and actively searching fish predators are the main threat to *A. aquaticus*, lower overall behavioural activity and slower speed might provide a selective advantage (Eroukhmanoff and Svensson 2009; Eroukhmanoff et al. 2009). Despite known differences in predator fauna between ML (high density of allochthonous guppies) and DM and CS (various vertebrate and invertebrate predators) surface populations, their movement activity was rather similar.

Alternatively, the significant increase in movement activity observed in the MJC cave population can be seen as a possible mechanism to cope with food scarcity, i.e., high movement activity increases foraging success in cave-adapted *A. aquaticus*. For example, Hervant et al. (1997) found that surface-dwelling *A. aquaticus* show increased movement activity (i.e., active food searching behaviour) during short term fasting. If food deprivation lasted longer, movement activity eventually decreased, however, refeeding triggered increased activity again. At first sight this alternative explanation seems unlikely for the MJC system as it is abundant with chemoautotrophic bacterial mats on which cave isopods feed. It was also reported that surface *A. aquaticus* feed on microbial communities growing on decaying plant material rather than plant tissue itself (Graça et al. 1993a, b, 1994a, b), and that such food sources even have a more optimal stoichiometric composition of essential chemical elements (Lürig and Matthews 2021). However, a few other studies with surface *A. aquaticus*

showed that it grows substantially slower on various microbiota than on fresh or decaying plant tissues (Marcus et al. 1978; Willoughby and Marcus 1979). Furthermore, our recent findings indicate that cave-adapted *A. aquaticus* in the MJC not just maintained the ability to feed on decaying leaf-litter, but actually this food is preferred over bacterial mats despite the latter are the sole available source of food in the cave (Herczeg et al. 2020, 2022). Although we possess no exact information regarding the nutritional content of MJC bacterial mats, it seems that they are accumulating potentially toxic chemical elements (Dobosy et al. 2016; Enyedi et al. 2019). This might present poor quality diet and, along with our previous behavioural observations, indirectly indicates that bacterial mats in this cave are rather an obstacle (surface populations unconnected to the cave avoided bacteria almost entirely), and that the MJC cave population might be somewhat food deprived after all. Thus, we cannot determine whether movement activity increased solely due to relaxed predation, or whether it was positively selected for also by the scarcity of high quality food. Nevertheless, and based on the results in hand, the lack of predation in the cave seems to be the main selective factor behind the detected pattern.

In support to our second prediction, the cave-surface divergence in movement activity was more pronounced in male than female *A. aquaticus*: while we found no behavioural sexual dimorphism in surface populations, including ML that is hydrologically connected to the cave, in cave animals tested under natural conditions (darkness), males were more active than females. Males and females often evolve differences in traits related to their sex-specific ecological niche, such as body size and shape (Kaliontzopoulou et al. 2015; Oke et al. 2019; Balázs et al. 2021) as well as resource (Gherardi 2004; Gomes-Ferreira et al. 2005) and habitat use (Morris 1984; Lindeman 2003). Despite this, the study of local adaptations mostly ignores sexual dimorphism, and at the same time, relatively few studies focussing on sex differences (or sexual selection in general) consider that environments show spatial variation (Connallon et al. 2018). However, recent empirical data shows that accounting for variability in patterns of divergence between sexes highly improves our understanding of the processes underlying adaptive divergence (e.g. Hendry et al. 2006; Riesch et al. 2013; Oke et al. 2019; Balázs et al. 2021). In the present study, we observed a marked male-biased sexual dimorphism in movement activity in the MJC cave population, i.e., cave males were considerably more active than cave females. This pattern was present when isopods were observed in darkness, but not in light. It is important to note that cave-adapted *A. aquaticus* from MJC preserved the ability to detect light (Pérez-Moreno et al. 2018) and they are known to avoid light (personal observation). Most likely, their photophobic behaviour masked the sexual dimorphism in activity in the presence of light. Male-biased movement activity is expected for *A. aquaticus* considering what we know of the reproductive biology of surface populations, which is largely retained in cave populations. Males compete for high quality females and guard them for several days before mating (Thompson and Manning 1981). Additionally, males were shown to actively search for mates, while females do not (Bertin and Cézilly 2003). Thus, the sexual dimorphism in movement activity observed for cave individuals can be (again) explained by the lack of predation. If the costs of high movement activity of males in the cave population are negligible, its mating benefits will 'freely' select for maximum activity.

However, there might be another reason behind the observed male-biased dimorphism in the cave habitat. Cave populations typically have lower densities than surface populations (Mammola et al. 2021), and spatial and temporal distribution of individuals can substantially influence individual's success of finding appropriate mates (Beauché and Richard 2013). Thus, if population density in the cave is lower than at the surface, males must be more active to find receptive females. This finds support in a study that Harris et al. (2011) conducted on surface

A. aquaticus, showing increased activity in males compared to females, but only in populations with low density. The same study might also provide an explanation for the surprising sexual monomorphism of movement activity in the herein examined surface populations, both in darkness and light. If surface populations exposed to intense predation have high densities, higher activity adds little to mate finding success and much to the risk of being predated, so males are better off similarly active as females. Again, specific combination of predation and population density seems a more likely determinant of sexual dimorphism in movement activity than the surface or cave environments *per se*.

Taken together, general movement activity was higher in the cave than in surface populations, while male-biased sexual dimorphism in movement activity was present only in the cave population. The most obvious selective factor behind this pattern seems to be the lack of predation in the cave habitat, but other factors, such as food availability and population density, likely contribute to it too. Since we studied wild-caught individuals, we cannot entirely exclude the possibility that the observed divergence is caused by phenotypic plasticity (a genotype's ability to develop or express alternative phenotypes in different environments, West-Eberhard, 2005). However, this explanation seems highly unlikely as the MJC population is genetically isolated from the adjacent surface populations, including the closest one in Malom Lake, for at least 60,000 years (Pérez-Moreno et al. 2017), and shows several morphological adaptations related to cave-life (Balázs et al. 2021). Finally, we note that our results originate from an exceptionally unique cave system (predator-free and most likely food-rich) and cannot be generalized across all cave populations of *A. aquaticus*. Most of these are subjected to different predation regimes (e.g. considerable predation pressure by the olm, *Proteus anguinus*) as well as quantity and type of food resources (e.g. temporal and spatial fluctuations of allochthonous nutrient-poor food in sinking rivers), besides they might also differ in population densities. So, it is reasonable to expect that different selection regimes in other cave systems might cause movement activity to evolve in different directions. Alternatively, we cannot exclude the possibility of stochastic events in trait evolution via strong founder effects and genetic drift during expansions of the surface populations' ranges into the subterranean environment, i.e., cave colonisation (Wessel et al. 2013; Miller et al. 2020). Further studies are needed to discover if evolution of movement activity in caves follows a common trajectory or is it diverse and rather tied to specific combinations of biotic and abiotic environmental factors, or even carries the signature of stochastic evolutionary events.

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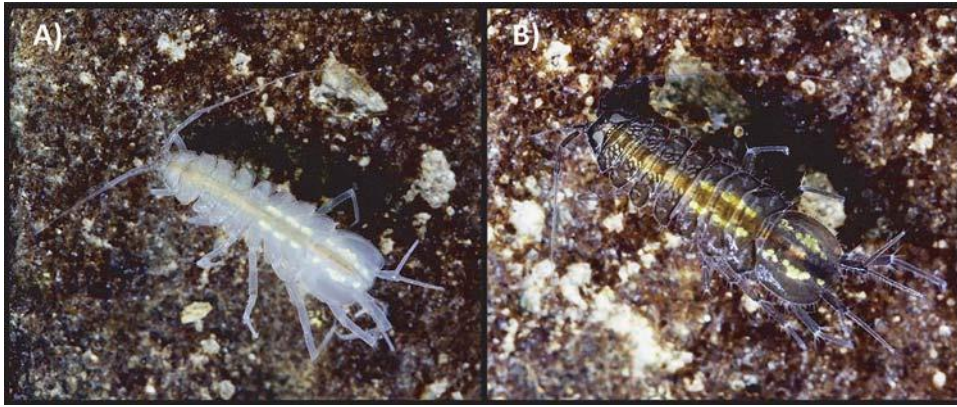


Figure 1. A typical cave A) and surface B) type *Asellus aquaticus* from this model system in their natural habitat (adapted from Balázs et al. 2021).

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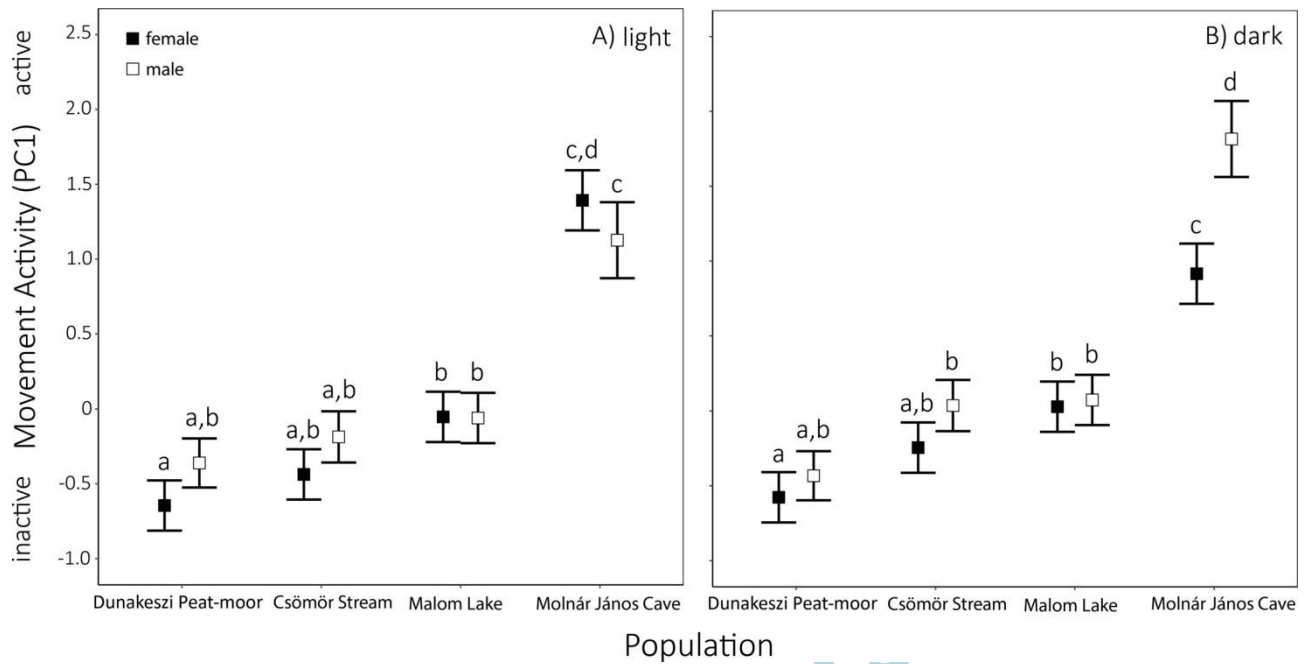


Figure 2. Movement activity of male and female *Asellus aquaticus* in A) light and B) darkness. Movement activity is the first principal component of a PCA on five behavioural variables measured from videos (see Methods); higher values on the y-axis denote higher activity. Estimated marginal means \pm standard errors are shown. Statistically significant ($P < 0.05$) between-group differences revealed by post-hoc comparisons are letter-coded (grouping factors were sex, populations, and treatments). Note that treatments marked with the same letter are not significantly different even between panels A and B (for further details see Table S4).

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Table 1. The ANOVA table of the linear mixed model on movement activity of *Asellus aquaticus*. Statistically significant effects ($P < 0.05$) are bolded.

Model term		
<i>Fixed effects</i>	<i>F</i> (df_1, df_2)	<i>P</i> -value
Population	46.43 (3; 164)	< 0.001
Sex	3.88 (1; 164)	0.05
Light regime	1.97 (1; 164)	0.16
Population × Sex	0.42 (3; 164)	0.74
Population × Light regime	0.4 (3; 164)	0.75
Sex × Light regime	3.44 (1; 164)	0.07
Population × Sex × Light regime	3.03 (3; 164)	0.03
Habituation	8.49 (1; 164)	0.004
<i>Random effect</i>	χ^2 (df)	<i>P</i> -value
Individual	17.37 (1)	< 0.001